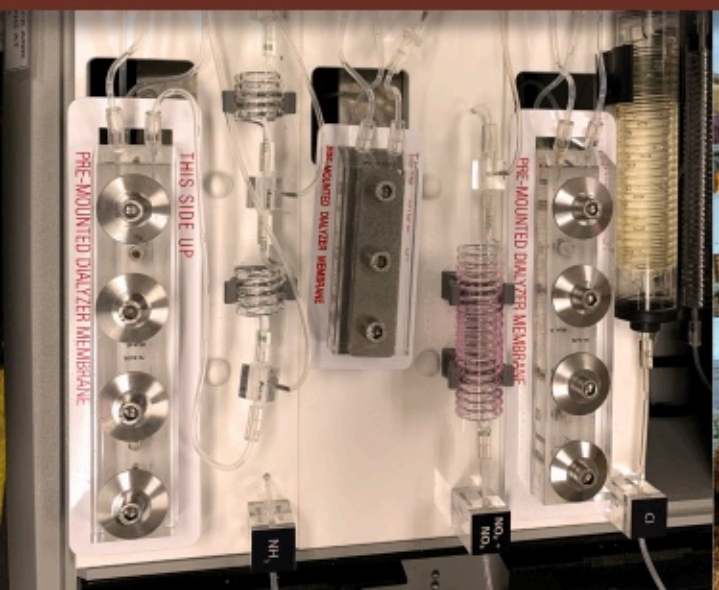
Recommended Methods of Manure Analysis

SECOND EDITION

Melissa L. Wilson & Scott Cortus, editors



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SECOND EDITION

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Preface

Animal manure can be a useful nutrient source for plants, but it is an inconsistent product that requires thorough testing to determine nutrient content. Efforts to standardize testing methods began in 1996 with a U. S. multi-regional group, and led to the first edition of the manure testing manual *Recommended Methods for Manure Analysis*, published by University of Wisconsin-Madison Division of Extension (formerly Cooperative Extension) in 2003. During this same time frame, the Manure Analysis Proficiency (MAP) Program, the first national laboratory proficiency testing program of its kind for manure, was started by the Minnesota Department of Agriculture.

During the past two decades, the U. S. Department of Agriculture's Natural Resources Conservation Service (USDA-NRCS) began requiring that anyone writing a comprehensive nutrient management plan (NRCS 590 standard) and applying for federally-funded conservation money for agricultural operations use MAP-certified labs or other NRCS-approved programs for manure analysis. As fertilizer prices have risen and environmental issues with over-application have been increasingly reported in the media, farmers and crop consultants, including those not participating in USDA-NRCS conservation programs, have also begun to recognize the importance of properly crediting manure for nutrients. This has led to an increase in demand for manure testing over time.

With increasing laboratory workloads, and labs receiving more and more manure samples on an annual basis, the methodology and equipment for manure analyses have evolved. Besides analyses for macronutrients like nitrogen, phosphorus, and potassium, there is increasing interest in analysis of secondary and micronutrients in manure. To meet the needs of manure-testing laboratories wanting updated, standardized methodology, a new multi-regional working group met in April 2019 in Minneapolis, Minnesota, with the goal of revising and adding methods to the 2003 manure analysis handbook. *Recommended Methods for Manure Analysis, 2nd edition*, is a result of that effort, and is to be used as a reference for sampling and testing animal manure.

— Melissa L. Wilson

Acknowledgements

We would like to acknowledge the original editor and authors of the first version of *Recommended Methods of Manure Analysis*, published by the University of Wisconsin-Madison Division of Extension. Their hard work made the second edition possible.

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This revised manual is the result of the integrated efforts of many people. We would especially like to thank Larry Gunderson at the Minnesota Department of Agriculture for his support and work on the Manure Analysis Proficiency (MAP) Program. Data from the MAP program were used to determine which methods are most widely used across the country. We are also indebted to the commercial labs and expert reviewers that participated in reviewing this handbook to make it a more practical tool.

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*Please note that the organizations involved with revisions of this manual were selected because of their longstanding activity in manure analysis or their work in the manure application industry. This is not intended to be an endorsement of any particular service these organizations may provide.

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Introduction

AUTHORS: Melissa L. Wilson and Jeff Porter

Animal manure contains all the essential nutrients for crop production and can be a beneficial resource for agricultural lands. Ideally, it is applied to crop fields based on a nutrient management plan, which accounts for crop needs, nutrients already present in the soil, and those present in the manure. However, nutrient composition in manure can vary greatly across animal types and across storage and handling systems. It can also be influenced by animal genetics and diet, bedding, and how the manure is applied to agricultural fields. Manure properties can thus vary significantly across time and space, even within a single manure pit or pile. This variability presents challenges for determining the amount and form of nutrients in manure, as well as for sampling and analysis.

Nutrient management plans are becoming a best management practice (BMP) around the country and are routinely required for regulatory or financial support programs. Consequently, it is important to understand the nutrient characteristics of various manure types. One method for estimating nutrient content is to look up "book" or average values for total nitrogen (N), phosphorus (P, usually reported as P_2O_5), and potassium (K, usually reported as K_2O). The MidWest Plan Service (MWPS) is a common source of book values (Lorimor et al., 2004),

Table 1. Comparison of analyzed semi-solid and solid manure nutrient concentrations to the MidWest Plan Service (MWPS) "book" values.

		Upper Midwest*		Mid-Atlantic†		Southeast‡		West¶		MWPS
Animal Type	Nutrient	Median	Range§	Median	Range	Median	Range	Median	Range	Average
		pounds / ton								
Dairy	N	12	9–16	10	8–12	9	6–16	16	6–26	9
	NH_4-N	1	1–3	2	1–3	—	—	—	—	2
	P_2O_5	6	4–9	4	3–6	5	3–9	11	4–19	4
	K_2O	13	7–20	9	6–15	8	5–13	21	6–38	7
Beef	N	16	12–21	12	8–15	13	8–19	22	16–31	9
	NH_4-N	1	0–3	0.7	0.2–1.4	—	—	3	1–5	3
	P_2O_5	10	7–14	6	4–8	8	4–13	21	14–29	5
	K_2O	14	11–20	12	6–20	12	6–20	25	17–34	9
Poultry	N	51	40–60	52	42–62	53	42–64	53	40–63	42
	NH_4-N	11	7–15	8	6–12	—	—	7	4–10	10
	P_2O_5	43	34–56	42	32–59	36	26–49	54	41–67	48
	K_2O	37	29–45	40	28–50	46	37–55	48	36–59	30

* Data from AGVISE, Minnesota Valley Testing, and Stearns DHIA Laboratories from 2012–2018 (n = 2082, 4422, and 5331 for dairy, beef, and poultry, respectively).

† Data from Penn State Analytical Lab from 2012–2018 (n = 594, 357, and 1020 for dairy, beef, and poultry, respectively).

‡ Data from North Carolina Department of Agriculture from 2010–2019 (n = 729, 229, and 5840 for dairy, beef, and poultry, respectively).

¶ Dairy data from JM Lord Laboratory from 2012–2018 and ServiTech Laboratories from 2008–2020 (n = 3623), beef and poultry data from ServiTech Laboratories 2008–2020 (n = 4359 and 1076 for beef and poultry, respectively).

§ Range is the middle 50% of all samples analyzed (the inner-quartile range).

as is the American Society of Agricultural and Biological Engineers (ASABE, 2005). However, testing manure on a regular basis is more likely to give site-specific results on how animal management and storage factors affect nutrient content. This also allows more flexibility in testing for other nutrients for which book values may not be available, including sulfur, calcium, and magnesium. Tables 1 and 2 illustrate the differences between actual manure tested in select regions of the country and the MWPS book values. Table 3 compares lagoon manure characteristics in California dairies and southeastern U. S. swine facilities to the ASABE book values.

Table 2. Comparison of analyzed liquid and slurry manure nutrient concentrations to the MWPS "book" values.

Animal Type	Nutrient	Upper Midwest* Median	Upper Midwest* Range‡	Mid-Atlantic† Median	Mid-Atlantic† Range	MWPS Average
		pounds / 1000 US gallons				
Dairy	Total N	20	15–25	24	18–28	31
	NH_4-N	10	7–14	11	8–13	6
	P_2O_5	9	6–11	9	6–11	15
	K_2O	21	17–26	20	15–27	22
Beef	Total N	34	8–58	19	7–27	29
	NH_4-N	11	0–32	4	1–7	8
	P_2O_5	18	4–36	9	2–17	18
	K_2O	26	11–34	13	5–18	26
Swine	Total N	45	27–60	28	19–40	31
	NH_4-N	24	15–37	18	12–25	20
	P_2O_5	21	11–30	10	4–24	26
	K_2O	31	17–40	17	12–27	24

* Data from AGVISE, Minnesota Valley Testing, and Stearns DHIA Laboratories from 2012–2018 (n = 9606, 2352, and 40476 for dairy, beef, and swine, respectively).

† Data from Penn State Analytical Lab from 2012–2018 (n = 528, 12, and 369 for dairy, beef, and swine, respectively).

‡ Range is the middle 50% of all samples analyzed (the inner-quartile range).

What's in this Handbook?

Due to the inherent variability of manure, there is a need for standardized methodology for sampling and analyzing for various manure constituents. This handbook can serve as a reference to meet these goals. The first unit delves into sampling methods in the field for collecting manure in liquid and solid handling systems, and covers how to collect a representative subsample in the laboratory prior to analysis. Both of these are critical for providing confidence in results that represent what is being land applied. The second unit provides details for laboratory procedures. The third unit gives a brief overview of laboratory quality management and the Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) program, covers how to evaluate laboratory performance, and discusses the reporting of analytical results. The appendices include definitions of common terminology and conversion factors for typical calculations in manure laboratory procedures.

The handbook's second unit, on laboratory procedures, outlines the most commonly used manure analysis methodology in the U. S. Where available, data from the MAP program (typically for total N and P) were used to determine the methods being used in participating laboratories and which are the most accurate and precise. As an example, phosphorus, potassium, and metals were rarely measured through atomic absorption spectroscopy (AAS) in recent sample exchanges, so the methodology was dropped from this edition. On the other hand, there has been increased interest in several constituents that were not previously included. As a result, methods for analyzing nitrate, water extractable phosphorus, and chloride have been added.

The methods included in this manual are for measuring nutrients and other agriculturally relevant constituents that are important for agricultural nutrient management planning. They are not inclusive of all tests that might be conducted on manure, including pathogens and contaminants of emerging concern. These might include antibiotic residues, heavy metals, and per- and polyfluoroalkyl substances (PFAS).

Please note that while commercial products or manufacturer's names may be referenced throughout this handbook, this does not constitute an endorsement by the authors or their employers. These references are provided only to give an indication of the relative type of equipment, chemicals, or supplies that may be used.

Manure Classification

Along with standardized methodology for manure analysis, it is important to have a standardized language. This starts with how manure is classified. Is it a liquid, solid, or something in between? The MWPS defines manure by its solids content. The standard definitions that will be used in this book moving forward are found in Table 4.

Understanding how clients handle and apply manure — usually determined by the solids content — is important for

Table 3. Comparison of analyzed liquid manure nutrient concentrations from lagoons to the American Society of Agricultural and Biological Engineers (ASABE) "book" values.

Animal Type	Nutrient	West* Median	West* Range‡	Southeast† Median	Southeast† Range	MWPS Average
		pounds / 1000 US gallons				
Dairy	Total N	3	2–6	--	--	6
	NH_4-N	1	1–3	--	--	7
	P_2O_5	0.8	0–1	--	--	3
	K_2O	4	2–7	--	--	11
Swine	Total N	--	--	3	2–4	5
	NH_4-N	--	--	--	--	4
	P_2O_5	--	--	0.7	0.5–1	4
	K_2O	--	--	5	4–7	7

* Data from JM Lord Laboratory from 2012–2018 and ServiTech Laboratories from 2008–2020 (n = 3162).

† Data from North Carolina Department of Agriculture and Consumer Sciences from 2013–2018 (n = 44691).

‡ Range is the middle 50% of all samples analyzed (the inner-quartile range).

Table 4. Manure sample classification (adapted from Lorimor et al., 2004).

Designation	Estimated Total Solids Content (%) as Received
Liquid	1–4%
Slurry	4–10%
Semi-Solid	10–20%
Solid	> 20%

reporting nutrients. Liquid and slurry manures, for example, are typically pumped, and are therefore measured in gallons. Semi-solid and solid manures are often applied based on the tonnage of material. The nutrient content of a liquid or slurry is thus typically reported in pounds per 1,000 gallons, while semi-solid or solid manure is typically reported in pounds per ton. Since most laboratories measure nutrients as a percentage, using a standard conversion factor is important for getting consistent results. Typical conversion factors used in the manure analysis industry can be found in Appendix A.

It should be noted that other organizations may utilize slightly different definitions for manure liquids, slurries, semi-solids, and solids. For instance, the United States Department of Agriculture's Natural Resources Conservation Service (USDA-NRCS) defines manure based on solids content within certain species (Figure 1). This is likely due to the importance placed on storage and handling characteristics — rather than nutrient content — by these organizations.

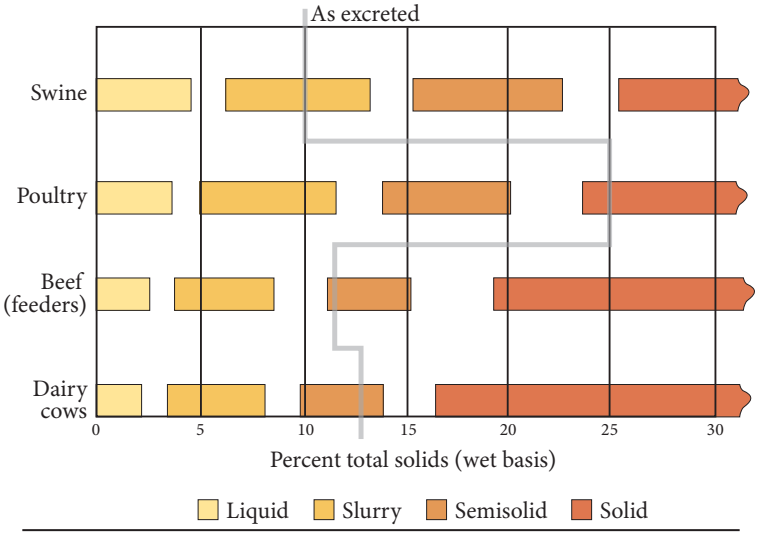

Figure 1. Manure definitions used by the USDA-NRCS. These vary by species and solids content of the manure. The gray line indicates the average total solids content of as-excreted manure from the various types of livestock.

References

American Society of Agricultural Engineers. (2005). ASAE D384.2 Manure production and characteristics. https://efotg.sc.egov.usda.gov/references/Public/NE/ASABE_Standard_D384.2.pdf.

Lorimor, J., Powers, W., and Sutton, A. (2004). Manure characteristics. MidWest Plan Service.

Hoop house manure storage facility at the North Carolina Agricultural and Technical State University Research Farm. Photo by Tibor Horvath (USDA-NRCS).

Sampling Procedures

S-1.0
Manure Sampling in the Field

AUTHOR: Robert J. Meinen

Introduction

Manure analysis is a critically important component of two intertwined and overarching goals of nutrient management planning. First, analysis identifies nitrogen, phosphorus, and potassium contents in manure that are utilized in conjunction with projected crop yield and soil analysis data to develop application rates to meet agronomic and economic farm goals. Second, analysis aids in protecting the environment from the inadvertent release of nitrogen and phosphorus when manure is applied to agronomic lands. Agencies charged with protecting the environment against agricultural nutrients often require agricultural operators to obtain routine manure analysis. Additional analysis can provide further details, such pH, micronutrients, and dry matter content, to assist farm decision-making. Good manure sampling techniques are imperative for ensuring that analysis accurately reflects the bulk of manure that will be utilized at a farm. Manure sampling is thus a best management practice that offers a level of environmental protection.

The chapter explores three primary steps of manure sample collection: 1) determining sampling protocol, 2) collection of manure samples, and 3) shipment of samples to the laboratory.

Considerations for Determining Sample Protocol

Manure is a complex mixture of organic and inorganic compounds, with nutrients found in a variety of forms that change over time. Within any manure storage system, many variables can influence manure nutrient content, including animal species, feed ration, bedding material, water utilization, manure storage type, manure storage management, and time of year (Muck et al, 1984; Rieck-Hinz et al., 1996). Manure that contains 20% or more dry matter is often considered to be solid. Manure with lower solid content is considered liquid (< 4% dry matter), slurry (4–10% dry matter), or semi-solid (10–20% dry matter) (Lorimor et al., 2004). Obtaining a truly representative sample from any manure storage is challenging. Spatial and temporal variations result in heterogeneous nutrient content.

In liquid systems, stratification can cause variation on a macro-scale (Dou et al., 2001). Floating solids are commonly referred to as a crust. Settled solids accumulate at the bottom of a liquid manure storage. Stratified sections of manure are expected to have different nutrient concentrations than the bulk manure found in the interior of the storage. Phosphorus content, for example, can vary greatly from top to bottom of a storage facility; the greatest concentration is often found in settled solids. Potassium, which has high solubility, may have the greatest concentration in manure with lowest dry matter. Nitrogen may be more evenly distributed, since it is present in soluble and insoluble forms (Lorimor et al., 2004).

Figure S-1.0.1 and Table S-1.0.1 show nutrient and dry matter concentrations from an unagitated liquid manure storage at a sow farm where manure was removed from the bottom of the storage. The unpublished data (Meinen, 2011) demonstrated that higher levels of dry matter contained greater levels of P in storage. During the unloading process, before the liquid portions could easily flow over remaining solids and into the pipe, manure was pumped from the bottom of the storage, where the initial loads were very thick. The slight increase of dry matter at load 60 occurred after overnight inactivity. After the liquid portion was removed, the stratified slurry manure moved to the pipe beginning around load 120. Investigation of the data estimated that 39% of total P was found in just 12% of the manure volume from the initial (loads 1–5) and final (loads 161–180) loads. The loads with lowest dry matter content (loads 5–100) represented 58% of manure volume, but contained just 13% of total P. Samples collected during loads 20 and 160, for example, would generate very different phosphorus application rates. Variation at this storage may have been reduced with agitation, since agitation of liquid manures reduces stratification and enhances homogeneity (Muck et al., 1984, Dou et al., 2001).

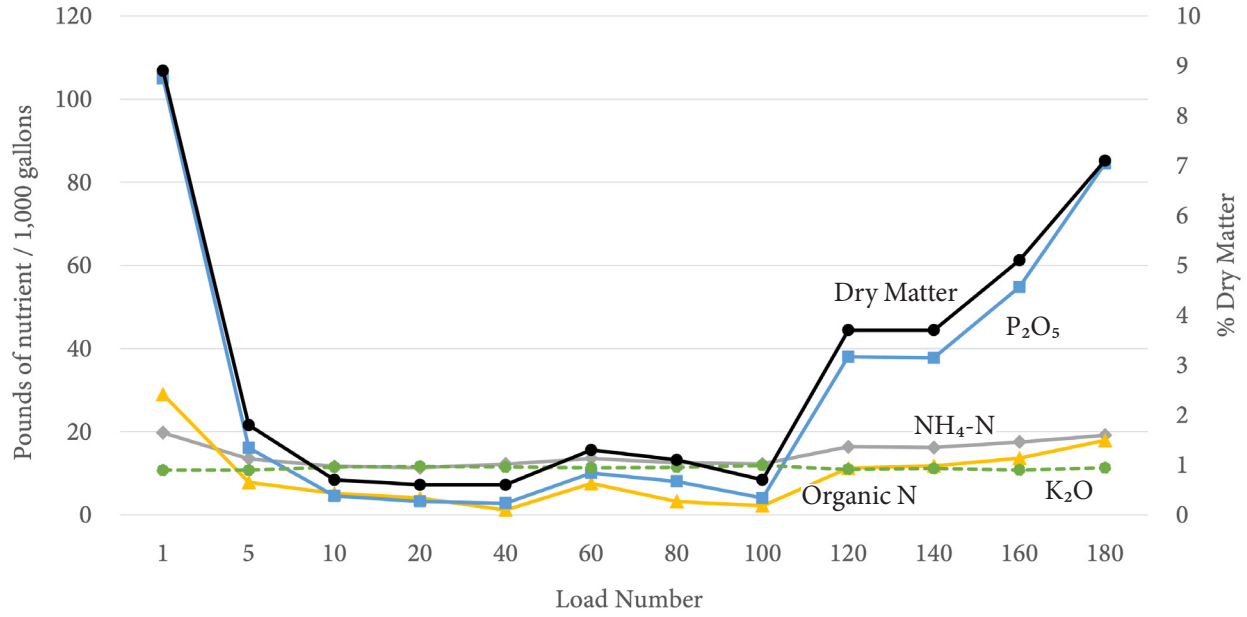

Figure S-1.0.1. Nutrient content (pounds per 1,000 US gallons) and dry matter content (%) of manure sampled from an outdoor manure storage basin at a sow farm as manure spreaders were loaded for transport to land application locations. Total N was removed to prevent clutter in the graph. Note that Load Numbers on the x-axis are unevenly spaced.

Composite Sampling. Since a very small amount of manure is analyzed to represent a larger manure volume, a meticulous manure sampling protocol is crucial. A non-representative sample may cause over-application of nutrients, increasing environmental risk and concentrating nutrients that could otherwise be utilized on additional cropland. Conversely, under-application could hinder expected crop yield or prompt unnecessary purchase of supplemental commercial fertilizer. Most manure sampling protocols recommend obtaining a number of samples that are mixed together into a single composite sample, from which a single subsample is analyzed.

Table S-1.0.1. Manure analysis results taken from loads of manure removed from the sow farm detailed in Figure S-1.0.1. Total N is included in the table.

Load Number	Dry Matter (%)	*(lbs/1000 US gallons)* Total N	NH_4-N	Organic N	P_2O_5	K_2O
1	8.9	19.75	29.01	104.91	10.72	10.72
5	1.8	13.51	7.78	16.13	10.73	10.73
10	0.7	11.64	5.15	4.55	11.48	11.48
20	0.6	11.30	4.05	3.22	11.61	11.61
40	0.6	12.20	1.17	2.79	11.47	11.47
60	1.3	13.59	7.55	10.08	11.30	11.30
80	1.1	12.50	3.23	8.02	11.45	11.45
100	0.7	12.23	2.19	4.04	11.87	11.87
120	3.7	16.37	11.22	38.04	10.94	10.94
140	3.7	16.21	11.80	37.76	11.15	11.15
160	5.1	17.51	13.61	54.79	10.75	10.75
180	7.1	19.16	17.89	84.50	11.31	11.31

Timing. Annual manure sampling manure is recommended, and may be required to meet nutrient and manure management planning requirements. A number of situations warrant more frequent analysis—for example, if manure is applied in different seasons or if farm management seasonally impacts manure characteristics. Since manure nutrient composition changes temporally, the ideal sampling time is as close to application timing as possible. Earlier sampling is often necessary, however, when nutrient management planning approval processes require that application rates are determined well ahead of field operations. Many agencies allow historic sampling averages to be utilized for manure application rate calculations. Multiple samples and establishment of historic analysis may improve nutrient utilization.

Manure sampling is conducted either prior to or during manure loadout. The prior-to method involves sampling in the weeks prior to field application so analysis results can be used to determine application rates for the upcoming crop. The during-loadout method involves sampling manure as it is removed from storage to the field. Sampling at regular intervals during manure removal is often most practical, and a favorable way to obtain a representative composite sample. During-loadout samples can be used to determine application rates for the following growing season or included with averaged historic values from the same manure storage, as allowed by local planning guidance. If a significant management change occurs that results in noticeable change from the historical average, it is appropriate to begin a new averaging period. Some advantages and disadvantages of sample timing are outlined in Table 2-1.0.2.

Andersen (2014) evaluated manure testing for swine producers using an economic model that demonstrated the importance of manure analysis in helping producers make informed decisions and maximize manure's monetary value. Results indicated that sampling before manure application was best if applying manure at an N-based rate, while sampling during application was best if using a P-based rate. The dynamic characteristics of N, such as ammonia volatilization and first-year availability for crop uptake, were large contributors to this conclusion.

Advantages of both sampling methods can be realized by using precision application equipment that allows immediate in-field nutrient assessment as manure is being applied. When coupled with computer-driven variable-rate application, this in-field analysis may become the gold standard for matching manure nutrient content to in-field crop nutrient uptake. Such systems utilize integrated geographic information systems (GIS) to pair spatial variations of soil, historic crop yields, and current field conditions with variations in manure nutrient content to automatically match manure application rates to expected crop uptake needs in real time. As these on-the-go technologies advance, agencies are likely to require concurrent manure sampling verification of system accuracy and calibration, which may mean collection of a single non-composited sample in the field.

Collecting Composite Sample Prior to Application

Liquid Manure. When sampling liquid manure prior to application, it is rarely practical to agitate manure. But if liquid agitation does not occur, some degree of certainty is forfeited and true representation is difficult. The recommendation in this scenario is to obtain samples from multiple storage depths. Sampling from the very top and very bottom of the storage should be avoided, as stratification may cause samples from these regions to adversely influence accuracy of the composite samples. Collect samples from multiple locations, but avoid areas that appear atypical compared to the bulk of manure volume. Anaerobic lagoons may require separate sample collection for liquid and sludge portions. More samples of equal volume are always better.

There are a number of commercially available liquid samplers. A tube sampler has a valve or cork so it can be placed into liquid manure to collect samples from multiple depths. Inserting a tube with depth markings and with the valve closed allows the worker to know exactly which depth the sample will come from when the valve is opened. This process can be conducted at multiple depths and locations to obtain a representative sample. Alternatively, careful insertion of the tube with the valve open will collect an entire column of manure from the surface to the depth of insertion. Another type of collector contains a cup or bucket with a lid or valve that prevents manure entry until the submerged apparatus is opened at a desired depth. If sampling from beneath a slatted floor, a sampling tube can be inserted through the slats.

Solid Manure. Samples should be collected from at least 16 inches below the surface and away from the outer edges, as volatilization and drying will prevent manure in these regions from representing the bulk volume (Miller et al., 2019). It may be necessary to dig into the pile with a shovel, skid-steer, or tractor to expose profile walls of a pile where interior grab samples can easily be obtained. Dutch augers can be utilized to extract samples from deep in a static manure pile. A long soil probe can be used to pull a core sample from the deepest portion of the material, and a drilling core sampler or probe, such as a hay sampler, can be helpful if bedding material such as straw makes it difficult to push a soil probe deep into the pile. More samples of equal volume are always better. Material that appears inconsistent with bulk manure should be discarded.

Not all sampling is conducted from storage. When sampling within a poultry building or bedpack area, at least 10 subsamples are recommended. If heterogeneity is apparent at certain proportions, strive to match the proportions of samples taken from each different area. For feedlots and barnyards, use a shovel to completely collect manure from a small area of the ground or concrete. Mix (homogenize) the small amount of manure thoroughly, then acquire a sample from the mixed manure and add it to the composite mixture. Repeat this process 10 or more times to obtain a composite sample for analysis.

Collecting Composite Sample During Application

Liquid Manure. Consistent sampling during emptying of liquid manure storage or during application is preferable for storages with or without agitation. Samples can be collected at the manure pump or spreader tank loading point,

Table 2-1.0.2. Comparison of advantages and disadvantages of sampling manure prior to or during manure application.

		Advantages	Disadvantages
Liquid Manre	Sample Prior to Loadout	Results can be used to plan for the upcoming crop.	Difficult to obtain representative sample because of heterogeneous nature of manure in storage and difficulty in accessing manure in the interior of the storage.
		May provide most economic option for N-based application rates.	Lack of agitation means results may not be representative.
		Can provide addition to historic manure analysis records.	May only reach limited volume of manure, even with tools designed for sample collection.
		Results can be used for export decisions and marketing. May be required by agencies in manure export situations.	Difficult to collect. Large equipment or agitation may be required to get representative sample. May not be practical to operate agitation equipment at the time of collection.
			Gas safety concern, especially during agitation or within confined spaces.
			Falling into storage.
	Sample During Loadout	Most convenient and practical collection timing. Agitation often occurs simultaneously with manure removal.	Not possible to use test results from sampling period for current year's planning.
		May provide most economic option for P-based application rates.	If necessary to climb to top of liquid spreader tank, there is risk of fall and gas concern.
		Can provide addition to historic manure analysis records.	Gas safety concern, especially during agitation or within confined spaces.
		In-field collection may be more practical in irrigation or dragline scenarios.	
		Subsample mixture is more homogenous, especially if agitation is occurring simultaneously.	
Solid Manre	Sample Prior to Loadout	Results can be used to plan for the upcoming crop.	Difficult to obatin representative sample because of heterogeneous nature of manure in storage and difficulty in accessing manure in the interior of the storage.
		May provide most economic option for N-based application rates.	May only be able to reach limited volume of manure, even with tools designed for sample selection.
		Can provide addition to historic manure analysis records.	Gas safety concern whem moving manure.
		Results can be used for export decisions and marketing. May be required by agencies in manure export situations.	
	Sample During Loadout	May provide most economic option for P-based application rates.	Not possible to use test results from sampling period for current year's timing.
		Most convenient and practical collection timing.	Gas safety concern whem moving manure.
		Can provide addition to historic manure analysis records.	
		Easy to get into deeper bulk areas of pile. Deeper manure is less influenced by volatilization and drying.	
		Subsample mixture is more homogenous.	
		In export scenarios, accurate analysis of delivered product.	

and can also be taken directly from the spreader. When irrigating or drag-lining, collection can occur in the field with the use of trays or containers placed in the field prior to swath application. In practice, however, this type of field collection rarely occurs, as it may be messy and requires more footwork than collection at the loading location.

If sampling during application, avoid sampling from the very first or very last manure being removed. If a small percentage of manure is noticeably different, such as thick manure at the bottom of a storage, do not place that manure into the composite sample. If a large volume of the manure is thicker (like that demonstrated in Table S-1.0.1), consider sampling that portion of manure separately. Such an approach is expected with stratified manure lagoons. This sampling approach may pay off agronomically, economically, and environmentally when different manures from the same storage are managed as separate manure groups for application planning.

When sampling during manure application, develop a sampling plan before the work begins. Sample collection can be planned out at uniform intervals. Ahead of sampling, estimate the number of loads that will be removed from the storage. Avoid a number of loads from the first and last loads from an unagitated storage. Even if agitation will occur, do not include the last loads that you expect to remove; agitation processes are not always perfect, and the last loads frequently contain higher solid content than the bulk of the removed manure. Often, personal experience with the manure handling system is the best way to determine which loads to disregard. Once the total number of loads to be removed is estimated, plan to sample at regular load intervals so that 10 or more samples are collected.

Solid Manure. As outlined for liquid manure, above, determine the load intervals from which samples will be taken. Again, take 10 or more samples of approximately equal volume from the pile, tractor bucket, manure spreader, or transportation truck. Sampling during removal can facilitate access to interior sample locations (Miller et al., 2019).

Solid manure is more commonly exported than liquid manure. It is often a legal requirement to provide a manure analysis when manure is exported. It is best to obtain a representative sample by collecting manure during the loading of transportation equipment.

Number of Samples Needed for Composite Sampling

The goal of manure analysis is to provide single values of manure nutrient concentrations taken from a single manure source. This single sample is best obtained by compositing a number of samples that are representative of variation in the manure storage, then taking a single subsample from the composited sample for delivery to the laboratory. While collecting the proper number of samples can lead to measurements that are within ±10% accuracy of average content for solid manures, attaining such accuracy may require significant investment of time and money (Miller et al., 2019). Attaining accuracy to this level may not be practical at the farm level.

A compromise should be sought that, with the time and effort available, allows collection of enough samples to accurately reflect the average nutrient content of the manure. More samples are always better, but not always practical. The effort of obtaining one more sample in pursuit of the accuracy goal of ±10% becomes inefficient at some point, and may not be worthwhile (Miller et al., 2019). Unfortunately, knowing when this breakpoint occurs is nearly impossible in the field. For liquid manures, Dou et al. (2001) noted that if thorough agitation occurs, five samples could be composited to accurately represent average manure storage content for N and P, but this increased to at least 40 samples without agitation. Davis et al. (2002) found that, for solid manure, 24, 27, and 21 samples were needed to achieve this goal for N, P, and K, respectively, but > 100 samples were needed for ammonium and nitrate. Because of variability in both liquid and solid storage scenarios, it is nearly impossible to specify the number of samples to collect. Indeed, many peer-reviewed and Extension publications avoid suggesting a specific number. Extension publications generally agree that 10 samples should be collected to achieve an acceptable balance of accuracy and practicality (e.g., Martin and Beegle, 2009; Murphy, 2006; Wortmann et al., 2014).

Gas Safety Concerns

It is always prudent to remember that all manures contain a large amount of organic matter that is under a continuous state of microbial degradation, and that potentially harmful and deadly gases are a by-product of microbial activity. Both liquid and solid manures should be treated with caution and respect. The deadliest gas produced from manure is hydrogen sulfide, which is commonly described to smell like rotten eggs at low concentrations. At higher concentrations, however, the gas can impair the sense of smell and be undetectable without safety monitoring equipment. Methane can also be produced from manure, and presents explosion hazards. Because any gas that displaces oxygen can result in oxygen-deficient breathing conditions, wearing a gas detection monitor is always recommended when handling manure. Workers should avoid confined space entry and low areas where heavy gases can accumulate, work in well-ventilated areas, keep the wind to their back, and avoid working alone. Movement of manure, such as that done when scooping solid manure or agitating liquid manure, greatly increases gas release (Fabian-Wheeler et al., 2017).

Composite Sample Attainment, Preparation, and Shipment

Sample Identification. Prior to filling, use a permanent marker to write identification information on the container or bag that will be sent to the laboratory. It is difficult to write on the container if manure spills onto its side. Do not assume that laboratory staff will be able to identify an unmarked container using the accompanying paperwork; confusion can occur if two unlabeled containers are simultaneously handled. If labeling guidance is not already provided with the container, include the sample location, operator's name, date of collection, animal species, production phase, and contact information. Identify the desired analysis results. Make sure the laboratory reports data in units that are useful for your planning requirements.

After the composite sample is collected, the subsample needs to be collected. The composite sample of liquid manure should be mixed thoroughly within the composite collection container, with the subsample for analysis taken while the manure is still swirling or moving. Solid manure should also be mixed thoroughly, and care should be taken to break up larger aggregates so that most of the material is similar in size. Because different sized particles may separate or settle differently within the container during mixing, the subsample should then be "grabbed" from several depths within the composite container. If solid manure is losing liquid in the composite container, mixing should be thorough enough to mix the liquid into the solids, or an equal proportion of liquid should be included in the subsample.

Once collected, place the composite subsample into the container that will be used for delivery to the laboratory. Most laboratories will provide containers that hold enough material for them to analyze. Utilize the shipping container and instructions provided by the laboratory.

Fill in all requested information. If a laboratory-provided container is not available, use a clean sealable plastic container. Do not use metal and glass containers, which may introduce contamination or break during transit. If a container is not supplied, approximately one pint (about 1 pound) of liquid manure or a one-gallon sealable bag filled ¾ full of solid manure are customarily adequate in volume. Place the first liquid sample container in a sealable plastic bag; double bagging of solid samples ensures less spillage. Do not fill any container or bag more than ¾ full.

Consider collection and shipping dates to avoid delays in transportation that can occur during weekends or holidays. Upon arrival at the laboratory, samples are often refrigerated at a temperature above freezing until analysis is performed. Laboratories may safely hold samples for several days in refrigeration.

If delivery or shipping to the laboratory will not occur immediately, place the sample on ice, in refrigeration, or in a freezer. If it will take more than one day to unload a manure storage facility, place the samples on ice overnight. Alternatively, it may be desirable to send a composite sample for each working day. If placed in a freezer, make sure there is expansion space in the container to avoid bursting due to expansion of freezing liquids. Cooling the sample will slow microbial processes in the manure to minimize nutrient transformations, such as conversion from organic-N to ammonium-N, providing greater confidence that the sample will accurately reflect on-site manure characteristics. Cooling will also produce less gas during transit. Gases can cause internal pressure and expansion of the container to the point that manure may leak from the container before it is delivered. For this reason, it is recommended that extra air space is squeezed from a container before it is sealed for transport.

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S-2.0
Laboratory Sample Processing & Storage

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Introduction

This chapter outlines recommended procedures for receiving, preserving, storing, homogenizing, and subsampling manure samples for routine analysis. An active microbial population and the volatility of many constituents can make animal manure an unstable and analytically challenging material. Sample preservation, storage, and homogenization procedures can have an important impact on microbial and chemical transformations, and consequently on the analytical results. Most manure samples are also highly heterogeneous, particularly those that include bedding material, and the solids content of manures can vary widely both among and within species, depending on handling and storage systems. A universal sample processing protocol is thus unlikely to perform well on all samples. The receiving technician must be adequately trained to carefully observe the physical characteristics of the material and use their best judgment to ensure that the secondary sample and test portion accurately represent the submitted primary sample. Variation also exists between labs in terms of analytes offered, equipment and labor resources, client sample volume, and regulatory requirements. Ultimately, the sample receiving procedures adopted in each lab will be a compromise, balancing the goals of obtaining the highest quality data and meeting regulatory obligations against analytical costs and labor resources.

Definitions

Air-dry. Sample material air-dried in a forced air oven at 36 °C for 48–72 h.

As-received. Sample material that is analyzed and/or reported at the moisture content in which it was initially received at the laboratory.

Holding time. The time between sample collection and analysis.

Homogenization. Mixing of manure material, either manually or by mechanical means, until physical, chemical, and /or biological homogeneity of the sample is achieved.

Oven-dry. Sample material dried in a forced air oven at 70±5 °C for 18–24 h, or until sample weight is constant and moisture losses diminish to nil (weight loss from second drying is less than 1% of initial weight loss).

Primary sample. The representative field sample submitted to the lab for analysis.

Secondary sample. The subsample collected from the primary sample for downstream analysis and archiving.

Storage time. The time between sample receipt in the laboratory and analysis.

Test portion. The volume or weight of the secondary sample used in each downstream analysis.

Biohazards and Laboratory Safety

During handling and processing of manure samples, laboratory workers risk exposure to numerous pathogenic organisms. These include viruses, bacteria, and protozoa (parasites), and can cause diarrhea, nausea, vomiting, fever, and even death. Pathogens are present not just in animal feces but in urine, blood, saliva, hair, bones, and feathers, all of which are common components of manure samples. In addition, laboratory workers are exposed to manure materials from numerous animal species—swine, cattle, poultry, horses, sheep, goats, bats, fish, and humans. These organisms host a wide range of known and emerging pathogens that vary in their deleterious effects on human health.

There are three primary routes of pathogen exposure for laboratory workers: respiratory, oral, and cutaneous. Respiratory transmission occurs when workers inhale airborne particulates that contain pathogens. This exposure can occur through routine laboratory practices such as sample drying, sample grinding, sweeping, and wiping lab benches. For processes that generate significant dust or other aerosols, a hood is recommended to contain airborne particulates. Cleaning of grinders or other contaminated equipment should be done using a HEPA vacuum, and drying ovens should be vented to the outside. Protective masks should

be worn when airborne particulates cannot be removed by other means.

Oral exposure to pathogens occurs when fecal pathogens are introduced to the mouth or nose, usually by touching of the face or by ingestion. While pathogen infection by the oral route is generally low in the U. S. due to the lack of routine exposure, workers handling manure are at much greater risk. Practices recommended to control oral exposure to pathogens include no eating, drinking, handling of contact lenses, or application of cosmetics in the lab. Personal protective equipment (PPE), such as disposable gloves, safety glasses, and lab coats, should be worn at all times in the lab and removed when exiting the lab. These PPE should be considered contaminated and not worn into common areas outside of the lab, such as break rooms and offices.

The cutaneous route introduces infectious agents through uncovered scratches, punctures, or cuts to the skin. Covering all skin below the neck is the simplest way to prevent cutaneous exposure to pathogens.

While cleaning with a routine surfactant is adequate for many lab settings, manure analysis labs also necessitate the use of disinfectants. A standard soap and water surfactant is generally sufficient for reducing bacterial and protozoal populations, but not viruses. Bench surfaces should be cleaned at least daily with a combination surfactant and disinfectant.

Sample Receiving, Examination, and Transfer

All samples submitted to the laboratory should be accompanied with an information sheet (e.g., chain of custody) that provides contact information for the client, a description of the sample (e.g., animal species, storage system), date sampled, and test requests. Information sheets should be reviewed and samples inspected immediately upon arrival at the laboratory. The date and initials of the person receiving the sample should be recorded, along with the condition of samples (e.g., temperature, shipped on ice) and sample container. Note any samples that are not in sealed containers, have insufficient volume, or show any evidence of leakage or sample loss. If a manure sample is not in a sealed container, it should immediately be transferred to an appropriate container. To minimize subsampling error, transfer as much of the original sample as possible, within reason. If the primary sample size exceeds the container, it should be homogenized before transfer.

If the sample received is much smaller than recommended, this should be noted in the sample report or results, and the client should be educated on proper sampling protocols. Inadequate sample volume may require modifications to the method, limit the ability of the lab to perform routine quality control (QC), and compromise the robustness of the analysis.

If the sample arrives frozen it should be thawed to room temperature before subsampling and transfer.

> ***Safety notes:*** 1) Glass containers should be avoided due to breakage hazards. Do not use glass to store samples, and educate clients who submit samples in glass to use plastic instead. 2) Use caution when opening samples in sealed containers, especially if inadequate head space is observed, as contents may be under pressure.

Sample Preservation

It is of the utmost importance to the integrity of the analysis to stabilize manure samples as soon as possible upon receipt. Stabilization preserves the analytes of interest as closely as possible to the same form and concentration as when the sample arrived at the laboratory and until the analysis is complete. This requires that microbial activity and volatilization/evaporation be minimized.

Acidification of sample material by lowering the pH to < 2.0 using H_2SO_4, HNO_3, or HCl is recommended for preservation of several analytes in aqueous environmental samples (e.g., drinking water); this extends the allowable holding time for most parameters (EPA, 2021; APHA, 2012). Acid preservation, for example, extends the allowable hold time for Kjeldahl nitrogen and ammonia (NH_3) analysis in regulatory samples from 7 days, refrigerated at ≤ 6 °C, to 28 days (APHA, 2012; US EPA, 2021). While the efficacy of the acidification procedure for stabilization of analytes in manure has not been evaluated, the US EPA does not require acid preservation prior to elemental analysis by ICP-OES of regulatory solid samples (EPA, 2021). Acidification of agricultural samples for preservation is also not generally practical due to its adverse effect on some analytes (e.g., pH, phosphorus, and calcium carbonate equivalent (APHA, 2012)) and to the high labor costs associated with regulatory and environmental sample handling protocols.

To retard microbial activity and volatilization losses, manure samples should be preserved by refrigeration at ≤ 6 °C upon receipt (EPA, 2021; USCC, 2003; APHA, 2012). In addition to refrigeration, drying of samples prior to analysis may be an acceptable approach to stabilizing the material for certain analytes (e.g., total elements measured by ICP-OES), but this is not recommended for the analysis of volatile compounds, such as NH_3, or measurements that will be otherwise impacted by drying (e.g., pH, conductivity, water extractable P). While the stability of elements such as P, K, Ca, Mg, Cu, and Zn in dried versus as-received manure samples has not been examined, it is assumed that the primary mechanisms affecting analytical consistency are 1) the potential for introducing error during drying and grinding, and 2) microbial decomposition of organic carbon, with subsequent loss of carbon dioxide (CO_2) and reduction in dry matter in as-received

materials. Drying of organic materials is a well-established mechanism for slowing or halting the microbial activity involved in decomposition of organic matter (Kalra, 1998). Storage procedures should thus focus on reducing microbial and enzymatic activity through reduction in moisture (drying at 65 °C or higher), reduction in temperature (refrigeration at < 6 °C), or both.

Freezing is not generally recommended for the analytes important in manure analysis (APHA, 2012; US EPA, 2021; US EPA, 2001). However, if a manure sample cannot be analyzed for one or more of the listed parameters within the specified time limit, it may be frozen at -18 °C to suspend microbial and chemical activity. Be aware that temperature affects pH and the ammonia-nitrite-nitrate balance within minutes of when microbial activity resumes (APHA, 2012), so frozen manure samples should be analyzed immediately after thawing to room temperature.

Table S-2.0.1. Maximum recommended storage times for liquid or solid manure stored at ≤ 6 °C before specified analyses.

Analyte	Recommended Storage Time
pH[1,2,3]	7 days
Total Kjeldahl nitrogen[3]	7 days
Ammonia nitrogen[3]	7 days
Nitrate nitrogen[1,2]	7 days
Near Infrared Reflectance	7 days
Electrical conductivity[2]	28 days
Minerals—Total P, K, Ca, Mg, Cu, Zn[1,2]	Sample: 14 days Digest: 6 months
Water-extractable phosphorus	21 days
Total solids	7 days
Chloride[1,2]	28 days

[1] Adapted from APHA, 2012.
[2] Adapted from US EPA, 2021, Table 2.
[3] Adapted from ASTM, 2015

Sample Storage Time

Once samples are preserved by refrigeration or other means, analysis should be performed as soon as possible. The shorter the time between sample collection and sample analysis, the more accurately the results will represent the original sample. Nevertheless, a storage period before analysis is to be expected, and the length of time that a sample can be held for retesting is an important component of the lab QC program. The term "holding time" is defined as the time between sample collection and analysis (EPA, 2021) rather than as the time between sample receipt in the lab and analysis. This is an important distinction to make when applying guidelines developed for regulatory water and wastewater samples preserved in the field to routine manure samples that infrequently arrive at the lab in a preserved state. Because a lab has little control over manure sample handling practices in the field, this guide offers recommended storage times for samples upon arrival in the lab. Suggested maximum storage times for routine analyses on manure are listed in Table S-2.0.1.

Sample constituents most sensitive to change during storage time are those prone to evaporative/volatilization loss (e.g., NH_3) or microbial/chemical transformations (e.g., pH, NO_3, total solids). While the recommended analytical timeframes suggested for regulatory wastewater samples may not be achievable for routine manure samples, parameters susceptible to change over short periods of time should be measured as soon as possible.

The recommended regulatory hold time for pH, regardless of preservation method, is 15 minutes in wastewaters (APHA, 2012, p. 145; US EPA, 2004) and two hours in sediment (EPA, 2021). For NO_3 in regulatory wastewater water samples, analysis within 48 hours of refrigerated samples (USCC, 2003; APHA, 2012) or 14 days for acidified samples is recommended to avoid potential denitrification losses (APHA, 2012). Unlike surface and ground waters, the stability of NO_3 levels in solid and liquid manures during storage has not been well established, but it is assumed to be a small fraction of total nitrogen losses compared to the dominant forms of organic N and NH_4. For these latter analytes, holding times for compost samples are 48 hours under refrigerated conditions (USCC, 2003) and 28 days for regulatory samples with acid preservation (EPA, 2021). ASTM (2015) suggests for sediments that, "in the absence of specific instructions, storage at a temperature of 4 °C or lower for a period of time not to exceed 1 week is recommended." For those parameters in manure that should be measured as soon as possible after collection (pH, NO_3, NH_3, total N) but where recommended regulatory timeframes or acid preservation are not feasible, this guide recommends a maximum storage time of 7 days.

Most minerals (i.e., P, K, Ca, Mg, Zn, Cu) that are analyzed by ICP-OES for total content in manure are assumed to be less sensitive to storage time before analysis relative to the analytes previously discussed. US EPA 40 CFR 136, Appendix C, which details the ICP-OES method for metals, states that solid samples require no preservation prior to analysis other than storage at ≤ 6 °C and that there are no established holding time limitations for solid samples (EPA, 2021). Additionally, the recommended holding time for compost samples is up to 6 months under refrigerated conditions (USCC, 2003). While the recommended manure storage time for elemental analysis in this guide is 6 months, this is an estimate of analyte stability, and future

research in this area or the judgment of the lab manager may supersede this recommendation.

Homogenization

As with field sampling, the ability to obtain a representative test portion is a critical component of accurate analytical reporting. While the purpose of field sampling protocols is to collect a primary sample that is representative of the larger manure stockpile, lab handling protocols are intended to produce an analysis that accurately reflects the submitted primary sample. While it is important to obtain a representative secondary sample, the analyst should be aware that excessive homogenization may alter the physical characteristics of the material by particle size reduction, and the chemical characteristics by mechanical cell lysis and oxidation of the analytes (Ditsworth et al., 1990; Stemmer et al., 1990a,b). Analytes subject to losses or change due to mechanical homogenization include pH, EC, NO_3^-, NH_3, and water extractable P. The primary sample should thus be manually mixed and subsampled for the subsequent homogenization of the secondary sample.

Homogenization methods are dependent on the characteristics of the manure material, most particularly the moisture content. In this manual, manure classification follows the definitions established by the MidWest Plan Service (MWPS), which are based on the estimated solids content (Lorimor et al., 2004) (Table S-2.0.2).

Liquids and slurries. While the recommended instrument for homogenization of liquid and slurry manures is a rotor-stator homogenizer, run for up to 60 seconds, mixing in a blender or manual shaking are acceptable alternatives if the desired uniformity is achieved. Liquid samples can be shaken within and poured from the original container, provided the solids stay uniformly suspended. Liquid and slurry samples without straw or other long-fiber material (e.g., hair) can be mixed in a blender or with a roto-stator homogenizer (Fig. S-2.0.1) to a uniform consistency. Long-fiber materials are challenging to homogenize using any device, and may require manual homogenization. Unlike solid and semi-solid manures, which are usually homogenized at the time of receipt, liquid samples should be homogenized immediately before subsampling for the secondary sample and/or for aliquoting the test portion. Analytical samples that have been stored after homogenization may require re-homogenization before subsampling for the test portion. Because of potential pressure build-up in the sample, caution should be used when opening a container after extensive shaking.

Solids and semi-solids. For samples containing greater than an estimated 10% total solids, a blade homogenizer should be utilized for up to 60 seconds. Solid manures or manures with coarse bedding or other materials can be chopped, divided, recombined, and/or mixed with a spatula to minimize heterogeneity prior to or during homogenization.

It is assumed that foreign materials such as glass, stones, metals, and plastics have been removed prior to submission. When this is not the case, it is acceptable to remove these unrepresentative materials prior to homogenization, and it is recommended to do so if the sample material will be dried and ground (USCC, 2003).

Table S-2.0.2 Dairy manure sample classifications by MWPS and recommended homogenization methods.

Designation	Estimated Total Solids Content (%) As-received[1]	Homogenization Method
Liquid	1–4%	Shaking, blender, roto-stator
Slurry	4–10%	Blender, roto-stator
Semi-Solid	10–20%	Food processor
Solid	> 20%	Food processor

[1] Adapted from Lorimor et al., 2004

Table S-2.0.3. Suggested test portion size for various manure analyses.

	Test portion size (solid)	Test portion size (liquid)	Chapter Reference
CCE	1.0 g (min. 0.2 g)	10 mL	M-7.1
C, Total	0.1–4.0 g	NA	M-2.1
Cl^-	1.0 g	10 mL	M-10.1–10.3
EC	20 cm^3(1:2; v/v) 40 g (1:5; w/v)	25 mL	M-9.1
ICP-OES	1.0 g (0.5–5.0 g)	10–15 g	M-5.2
N, Total	0.1–4.0 g	NA	M-3.1
NIR			M-3.3
NO_3-N	1.0 g	10 mL	M-4.3
NH_4-N	1.0 g	10 mL	M-4.2
NH_4-N (potentiometric)	2.0 g	50 mL	M-4.1
pH	20 cm^3(1:2; v/v) 40 g (1:5; w/v)	25 mL	M-8.1
PO_4-P	1.0 g	10 mL	M-5.3
Solids, Total	10–20 g	20–50 g	M-1.1
TKN	0.5–5.0 g	10–50 mL	M-3.2
WEP	2.0 g	2.0 g	M-6.1

Figure S-2.0.1 Homogenization devices used in manure analysis. Adapted from USFDA 2014.

Blender, countertop or immersion. A countertop blender is a stationary, upright electrical device consisting of a water-tight removal blade assembly built around a vessel in which the material is blended. An immersion blender (i.e., hand blender or wand blender) has no container of its own, but instead has a mixing head with rotating blades that can be immersed in a container. Immersion blenders are convenient for homogenizing samples in their original container and are easier to clean than countertop blenders, but they are less suitable for larger sample volumes (> 500 mL). Both types are suitable for liquids and slurries, but homogenization efficiency decreases as percent solids in the sample increases, making them unsuitable for solid samples.

Food Processor. A food processor is an electric-motor-driven appliance used for chopping solid or semi-solid foods and can be used to homogenize and grind manure samples with high percent solids content. Food processors differ from blenders in that they use swappable blades and disks instead of a fixed blade, making them easier to clean, and they have wider and shorter bowls, making them easier to use with the solid or semi-solid foods. They are not well suited for liquid samples.

Rotor-stator laboratory homogenizer. Also called high-shear mixers or colloid mills, rotor-stator homogenizers consist of a fast-spinning inner rotor with a stationary outer sheath (stator), which reduces particle size through mechanical shearing and cavitation (the rapid forming and collapsing of bubbles). This type of homogenizer is best suited for liquids and slurries. Efficiency and ease of cleaning is superior to that of the blender-type homogenizer, but the cost is higher.

Vortex mixer. A vortex mixer, or vortexer, consists of an electric motor with the drive shaft oriented vertically and attached to a flat or cupped rubber piece mounted slightly off-center. As the motor runs, the rubber piece oscillates rapidly in a circular motion. When a centrifuge tube or other appropriate container is pressed into the rubber cup or plate, the motion is transmitted to the liquid inside the container and a vortex is created. Not suitable for solid samples or large volumes. May be more suitable for re-homogenization of secondary samples.

Manures often contain organic materials such as bone, bedding, and feathers that may be difficult to homogenize using a blade homogenizer. The technician should endeavor to incorporate these materials into the homogenized sample by cutting them into smaller sections or increasing processing time.

Subsampling

Once the secondary sample is fully homogenized, the material can be subsampled for immediate analysis, storage prior to analysis, and/or archival. Containers should be made of plastic, should be a convenient size for storage, should seal sufficiently to prevent evaporative/volatilization loss or other leakage, and should be able to withstand freezing if needed. The volume of this secondary sample may vary across laboratories but should be at least 10 times the total volume of the analytical test portion. In general, the larger the test portion for any analysis, the more representative it will be and the higher the precision of the results. Likewise, the smaller the test portion, the more essential it is that the secondary sample be thoroughly homogenized.

Drying and grinding of samples. To reduce storage requirements and increase the homogeneity of the material, the secondary sample can be partially dried and ground for some analyses. Oven-dry samples should be dried in a forced air dryer at a minimum of 65 °C (to halt biological activity) and a maximum of 80 °C (to prevent thermal decomposition). Drying for 16 hours is generally sufficient for most materials, but samples with high moisture content may require longer drying times. Samples should be dried until the weight no longer changes (weight

loss from second drying is less than 1% of initial weight loss (EPA, 1973)). For parameters that are suitable for air drying, the sample should be dried in a forced air oven at 36 °C for 48–72 h (USCC, 2003). Partially dried samples should be stored in sealed containers to prevent changes in moisture content prior to analysis.

Because client results for manure samples are often reported on an as-received basis, analyses run on partially dried material require a correction for the moisture lost during drying. So that final results can be expressed on an as-received basis, the technician should record the weight of the subsampled material before and after drying using a moisture correction factor calculated as:

$$M = \left(\frac{D}{A}\right)$$

where:
M = moisture correction factor
D = dry weight at 36 °C or 70±5 °C
A = as-received weight

To express the concentration of a given analyte measured on a partially dried secondary sample on an as-received basis, multiply the result by the moisture correction factor, M.

The decision to dry a secondary sample should be based on the nature of the material and the analyte(s) of interest. Drying reduces the amount of space required for sample storage and allows for further homogenization and particle size reduction through grinding. Homogenization devices suitable for wet manure samples are less efficient at fine homogenization than high-powered laboratory grinders, so the additional step of grinding the dry manure can significantly improve analytical precision. Conversely, drying the material represents a change to the physical condition of the submitted material, which creates an opportunity for error when correcting for moisture, and may cause chemical changes to some analytes.

Note: Drying is not recommended for the analysis of total nitrogen, ammonium nitrogen, or other constituents that may be volatilized or transformed during the drying process. Subsampling for the analysis of these unstable constituents should be done directly from the as-received sample.

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Bedded beef pack manure being applied to a field.
Photo taken by Chryseis Modderman (University of Minnesota Extension).

Analytical Methods

M-1.1
Total & Volatile Solids

AUTHORS: John T. Spargo, Rachel Brimmer, Kristin Hicks, and Jason Lessl

1.0 Scope and Application

The method is suitable for determination of total, fixed, and volatile solids in manure and other organic residuals (e.g., biosolids, food waste, compost). It is adapted from methods developed for industrial wastewaters as well as for solid and semi-solid materials produced during water and wastewater treatment.

Determination of volatile solids is useful because it offers an approximation of organic matter present in the solid fraction of manures. Volatile solids can also be used to estimate organic C content by assuming a constant ratio between the two (Larney et al., 2005). It should be noted that the determination of fixed and volatile solids does not distinguish precisely between inorganic and organic matter; loss on ignition is not confined to organic matter, and includes decomposition or volatilization of some mineral salts.

2.0 Method Principle

2.1 This method was adapted from SM 2540 G. A well-mixed sample is weighed into an evaporating dish and dried to a constant weight at 103–105 °C. The total weight (sample plus dish) minus the weight of the empty dish represents the total solids. The total solids residue is heated to constant weight at 550 ± 50 °C. The remaining solids represent the fixed total solids, while the weight loss on ignition is the volatile solids.

2.2 Method Performance. A laboratory performing this method should be capable of achieving an MDL of 3 mg kg^{-1}, 7 mg kg^{-1}, and 7 mg kg^{-1} for total, fixed, and volatile solids, respectively (EPA, 2001). See Section 10.2 for method uncertainty.

2.3 Method interferences.

- **2.3.1** Wet samples lose weight by evaporation; collect initial sample weights as quickly as possible.
- **2.3.2** Post-drying residues are often hygroscopic and rapidly absorb moisture from the air; cool samples in a desiccator and collect weights quickly after drying.
- **2.3.3** Determination of total solids in manure is subject to negative error due to loss of free ammonia, ammonium carbonate, and volatile organic matter during drying.
- **2.3.4** For liquid manure (< 4% solids), excessive residue in the dish may form a water-trapping crust; limit sample size to ≤ 200 mg residue.
- **2.3.5** Results for residues high in oil or grease may be questionable because of the difficulty of drying to constant weight in a reasonable amount of time (volatile and semi-volatile oils).
- **2.3.6** Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error. In such cases, measure volatile components by another test (e.g., total organic carbon).

2.4 Method Reporting. Total solids are reported as mass percent (%) of the full sample mass. Fixed and volatile solids are reported as mass percent (%) of the oven-dry sample (or dry solids).

2.5 Method Advantages. The method is relatively simple. It requires a balance, a drying oven, and a muffle oven.

2.6 Method disadvantages. Oily samples, high-salt samples, or liquid samples that form a crust while drying may be difficult to dry to constant weight.

3.0 Definitions

3.1 Total Solids. The residue remaining after evaporation and subsequent oven-drying to constant weight at 103–105 °C.

3.2 Fixed Solids. The residue of total solids remaining after heating the same sample to 550 °C, expressed as a mass percent (%) on an as-received or dry-weight basis.

3.3 Volatile Solids. The mass of total solids lost on ignition at 550 °C, expressed as a mass percent (%) on an as-received or dry-weight basis.

3.4 Laboratory Control Sample (LCS). A laboratory-prepared solution with a known quantity of total and volatile solids. Its purpose is to assure that the results produced by the laboratory remain within the criteria specified in the method for precision and bias.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Samples should be stored at ≤ 6 °C for up to seven days in plastic containers.

4.2 Sample Processing. Subsamples should be collected from the primary representative sample for determination of total, fixed, and volatile solids. Do not grind or mill samples prior to subsampling. Require sufficient sample for laboratory duplicates, at least 100 g for liquid samples and 40 g for solid samples.

4.3 Sample Test Portion. Recommended test portion size for solids is 10–20 g and for liquids is 20–50 g. For particularly heterogeneous materials, larger samples will improve precision.

5.0 Apparatus

5.1 Analytical balance, capable of weighing to 0.0001 g.

5.2 Evaporating dishes, 50 and 100 mL. Evaporating dish should be made of a material that has been shown to be resistant to sample matrix and weight stable at the required drying and ignition temperatures.

5.3 Desiccator.

5.4 Drying oven, forced air. Capable of operation at 103–105 °C.

6.0 Reagents and Standards

6.1 Reagent grade water, ASTM Type I.

6.2 Laboratory Control Sample. NaCl-KHP solution:

- **6.2.1** Dissolve 1.0 g sodium chloride (NaCl) in approximately 500 mL reagent water in a 1 L volumetric flask. Swirl gently to dissolve.
- **6.2.2** Add 1.0 g potassium hydrogen phthalate (KHP) to the NaCl solution and swirl. Bring to volume. Transfer to a plastic bottle and store at 4 °C.
- **6.2.3** Assuming 100% volatility of the acid phthalate ion, this solution contains 2000 mg L^{-1} total solids (0.2% solids), comprised of 1190 mg L^{-1} (0.119%) fixed solids and 810.0 mg L^{-1} (0.081%) volatile solids.

7.0 Procedure

7.1 If volatile solids are to be measured, heat clean evaporating dishes at 550 ± 50 °C for 1–2 h in a muffle furnace. If only total solids are to be measured, place clean dishes in oven at 103–105 °C for 1 h. Store and cool dishes in a desiccator until cooled to room temperature. Weigh each dish to the nearest 0.1 mg and record weight for later use.

7.2 Bring samples to room temperature before weighing. Homogenize sample and weigh into a pre-weighed dish 20.0–50.0 g of liquid or 10.0–20.0 g of solid or semi-solid sample. Note: Standards Methods procedure for solids and slurries (2540 G) calls for 25.0–50.0 g sample size if using a balance with sensitivity ± 0.010 mg. If using a balance with higher sensitivity (± 0.0001 g) a smaller sample size is acceptable.

7.3 Dry sample in the oven at 103–105 °C overnight or, if shorter time is used, until constant weight is achieved.

> ***Note:*** Standards Methods procedure calls for an initial 1 h drying time, followed by repeated 1-h drying times until constant weight is achieved. The overnight drying period is utilized here to decrease the number of weighing steps required to achieve constant sample weight.

7.4 Cool to room temperature in desiccator and weigh. Record weight.

7.5 Heat total solids residue in muffle furnace at 550 ± 50 °C overnight or, if shorter time is used, until constant weight is achieved.

> ***Note:*** The Standards Methods procedure (2540 G) calls for igniting the sample for less than 1 h and repeating ashing and weighing cycles until constant weight is achieved. To eliminate the repeated ashing and weighing cycles while still ensuring sufficient time to volatilize organic components, one extended ashing cycle is utilized in the procedure.

7.6 Allow sample to cool in furnace to approximately 200 °C or transfer to an oven at 105 °C to cool. Transfer to desiccator for final cooling to room temperature. Weigh crucible. Be alert to change in weight due to air exposure. After removing the sample from dessicator, obtain the weight as soon as possible; otherwise keep the sample stored in the desiccator until the analyst is ready to obtain the final weight.

8.0 Calculations and Reporting

8.1 Calculate total, volatile, and fixed solids according to the following equations:

$$Total\ solids, \% = \frac{A - B}{C - B} \times 100$$

$$Volatile\ solids, \%\ (dry\ weight) = \frac{A - D}{A - B} \times 100$$

$$Fixed\ solids, \%\ (dry\ weight) = \frac{D - B}{A - B} \times 100$$

where:

A = mass of residue + dish after drying, g
B = mass of crucible, g
C = mass wet sample + dish, g
D = mass of residue + dish after ignition, g

8.2 Total organic C can be estimated using the following equation:

$$Total\ organic\ C, \% = VS \times \beta$$

where:

VS = volatile solids, %
β = is the ratio of organic C to volatile solids. Larney at al. (2005) reported an average ratio of 0.52 for 3000 manure and compost samples.

8.3 Client Reports. Total solids are reported as mass percent (%) of the full sample mass. Fixed (ash) and volatile solids are reported as mass percent (%) of the oven-dry sample (or dry solids).

If problems are noted during the procedure, such as difficulty in drying oily materials, a comment should be included on the final report. Where total organic carbon is calculated from volatile solids, it should be noted on the report as an estimated value.

9.0 Quality Control

9.1 Method blank. Prepare and analyze a laboratory blank with each analytical batch. The blank must be subjected to the same procedural steps as a sample and will consist of approximately 25 mL reagent water. *Acceptance criteria: If material is detected in the blank at a concentration greater than the MDL, analysis of samples must be halted until the source of contamination is eliminated and a new blank shows no contamination.*

9.2 Laboratory Control Sample (LCS). An aliquot of the NaCl-KHP LCS solution must be analyzed with each batch of samples. Calculate the Recovery (R) of total and volatile solids in the sample relative to the total and volatile solids measured in the LCS solution. *Acceptance criteria: Solids recovery†: 80–110%; Volatile solids recovery†: 70–110%.*

† Performance criteria to serve as initial quality objectives. Data quality objectives should be refined with laboratory observations.

If recovery is outside of the acceptable range, correct the problem, re-prepare the sample batch, and repeat the LCS test.

9.3 Duplicate. Perform duplicate analyses on a minimum of 10% of samples and calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference (RPD). *Acceptance criteria: An RPD of ≤ 10% should be routinely achievable for total and volatile solids when the analyte concentration is greater than the LOQ. The operator may select samples for duplicate analysis that are expected to exceed to the LOQ.*

9.4 Proficiency Testing. Participate in the Manure Analysis Proficiency (MAP) program. Sample size requirements for this method may limit the ability to perform on MAP samples with low solids content. *Perform bi-annually.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of quantification (LOQ). Method detection limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least

Table M-1.1.1. Precision data for manure total solids, as-received basis.

Sample ID	Mean Total Solids Content (%)	Intra-Lab Standard Deviation S_r Total Solids %	Intra-Lab Repeatability r Total Solids %	Inter-Lab Reproducibility standard deviation S_R Total Solids %	Inter-Lab Reproducibility R Total Solids %
M 2014-D	3.15	0.016	0.048	0.27	0.81
M 2015-D	11.2	0.019	0.057	0.31	0.93
M 2012-B	56.5	0.026	0.078	1.16	3.48
M 2016-F	68.4	0.023	0.070	1.12	3.36

MAP Program, 58–64 laboratories reporting.

nnually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method precision. Method repeatability and reproducibility for total solids were calculated in accordance with ISO 5725-2 from results of proficiency rounds conducted in 2015 and 2016 for 10 laboratories by the Manure Analysis Proficiency (MAP) Program. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained (Table M-1.1.1)

11.0 Safety and disposal

11.1 Laboratory safety. Wear gloves, lab coat, and safety glasses when weighing manure samples.

11.2 Caution. High temperatures in muffle furnaces can cause severe burns. Use precautions when removing labware from ovens and muffle furnaces. Cool furnace to below 200 °C prior to removing crucible trays. Use oven mitts and/or tongs to remove crucibles.

11.3 Reagents disposal. Not applicable.

11.4 Samples disposal. Not applicable.

12.0 Reference Documents and Additional Resources

U. S. Environmental Protection Agency. (1971). Method 160.3. Residue, total (gravimetric, dried at 103–105 °C). In: Methods for the chemical analysis of water and wastes (EPA/600/4-79/020).

U. S. Environmental Protection Agency. (2001). Method 1684. Total, fixed, and volatile solids in water, solids, and biosolids. Draft. (EPA-821-R-01-015).

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Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. (2017). Method 2540 G. Total, fixed, and volatile solids in solid and semisolid samples. In: Standard methods for the examination of water and wastewater. 23rd edition. Lipps WC, Baxter TE, Braun-Howland E, eds. APHA Press.

M-2.1
Carbon, Total by Combustion

AUTHORS: Kristin Hicks, Rachel Brimmer, and John T. Spargo

1.0 Scope and Application

This method quantitatively determines the concentration of total carbon (TC) in manure (slurry, semi-solid, or solid) and compost by combustion of an individual sample in an oxygen (O_2)-containing environment, with subsequent quantification by thermal conductivity detector. This method may not be suitable for measuring carbon in liquid (< 4% solids) manure.

At commonly used combustion temperatures, elemental analyzers measure TC, which includes both organic and inorganic C. In animal manures and compost, inorganic C in the form of carbonate is assumed low, and the C measured is assumed to be primarily organic C. In sample materials with high carbonate content, combustion at high temperature may overestimate organic C as reported in the carbon-to-nitrogen (C:N) ratio; this should be considered when interpreting the results.

An accurate measure of organic C is important for determining the C:N ratio of organic materials. The C:N ratio is an important predictor of N mineralization in manures and compost stability, and is used to determine optimum mixing rates of compost feed stocks. Because microbial decomposition of carbohydrates is limited by metabolic nitrogen requirements, high C:N ratios slow the composting and mineralization processes. When the C:N ratio is low (< 15:1), oxygen is rapidly depleted and the aerobic carbohydrate metabolism necessary for the production of quality compost shifts to anaerobic metabolic pathways.

2.0 Method Principle

2.1 Method Description. This method is adapted from AOAC 949.12 and 972.43. A sample is introduced into a high temperature (> 900 °C) furnace where organic and inorganic C is combusted in the presence of O_2 to form CO_2 and water. Helium carrier gas transports the combustion products through a column where water is absorbed, and the remaining gases are introduced into a programmed temperature column which separates and releases the gases separately. The gases flow along a thermal conductivity detector (TCD), which produces an electrical signal proportional to the concentration of C, N, and/or other elements. Combustion analyzers are available in a variety of configurations, such as CN, CHN, CHNS, or CNS, depending on manufacturer and user needs.

2.2 Method Performance. A laboratory using this method should be capable of achieving an MDL of 0.25% C. MDLs should be determined and reported individually by the lab upon completion of the MDL method outlined in Appendix B.

2.3 Method Interferences. If the sample is also being analyzed for total N when acetic acid is used to stabilize NH_3, the acetic acid will produce a C peak that will interfere with C quantitation. Duplicate analysis of the sample with and without acetic acid will be necessary for both total N and C. Note that the addition of acetic acid may affect the inorganic carbon quantitation by neutralizing carbonates in the sample.

2.4 Method Reporting. Carbon is reported in mg kg^{-1} or as a % on a dry-weight or as-received basis. The C:N ratio is reported as a mass:mass ratio, e.g., 25:1.

2.5 Method Advantages. Combustion analysis is a well-established method, and automated instruments are available that are capable of high-throughput analysis in multiple element configurations such as CN, CHN, CHNS, and CNS.

2.6 Method Disadvantages. At 900 °C or greater, both organic and inorganic C is recovered. For some organic waste materials such as poultry manure, paper waste, or ash by-products that may contain significant quantities of carbonate, results may be artificially inflated, resulting in higher C:N results.

3.0 Definitions

3.1 Carbon, Total (TC). The sum of organic and inorganic carbon as determined by oxygen combustion analysis.

3.2 Carbon, Total Organic (TOC). A measure of the total amount of organic C in an aqueous sample.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Microbial activity (decomposition) is the primary mechanism for organic C losses in manure. Carbon is stable for extended periods of time if maintained at ≤ 6°C or after drying at 65–80 °C for > 16 hours. Nitrogen is more susceptible to losses than C because of ammonia volatilization. For accurate determination of C:N, sample storage practices should be based on N stabilization. Not recommended for freezing.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) samples do not generally require subsampling, but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage. This method is not suitable for liquid (< 4%) manure samples; use a total organic carbon (TOC) method for analysis of liquid samples.

4.3 Sample Test Portion. Combustion analysis is performed on a 0.1–4.0 g test portion from the ≥ 200 cm^3 homogenized secondary sample. Sample weight is typically based on expected N concentration rather than C.

5.0 Apparatus

5.1 Analytical balance, capable of measuring to 0.0001 g.

5.2 Sample delivery vessels, specific to the instrument, such as stainless-steel crucibles, ceramic boats, or tin capsules.

5.3 An elemental combustion analyzer, such as a CN, CHN, CHNS, or CNS analyzer, capable of carbon measurement and with associated consumables.

6.0 Reagents and Standards

6.1 Helium (99.996% minimum purity). UHP Grade.

6.2 Oxygen (99.996% minimum purity). UHP Grade.

6.3 Certified Reference Materials (CRMs) for calibration and calibration verification, such as acetanilide (71.09% C), sucrose, glutamic acid, EDTA, and certified manure material. Plant CRMs, e.g., wheat flour (42.89% C) or spinach (40.36% C), can be used for independent calibration verification. Internally produced Reference Materials (RM) may be used for continuing calibration verification (CCV).

6.4 Consumable materials for packing columns. These materials will vary based on the instrument manufacturer, and may include:

- **6.4.1** Ascarite/Sicapent
- **6.4.2** Copper oxide
- **6.4.3** Copper wire
- **6.4.4** Corundum balls (aluminum oxide)
- **6.4.5** Platinum
- **6.4.6** Silver or quartz wool
- **6.4.7** Tungsten
- **6.4.8** Zinc

6.5 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the unknown samples.

7.0 Procedure

7.1 Follow instrument manufacturer's recommended procedure for packing combustion and reduction columns, performing leak check, conditioning, and calibration, and preparing the instrument for analysis.

7.2 Analyze quality control samples per Section 9.

7.3 Homogenize secondary sample again, if necessary. Homogenization should have already occurred during sample receiving, but samples with high water content can separate during the time between initial homogenization and analysis. If separation is noted, manually re-homogenize the sample.

7.4 Weigh sample to nearest 0.0001 g and place in sample crucible, boat, or capsule. Sample size should be between 0.1 to 4.0 g, based on anticipated N content of the sample (see Chapter 3.1, Nitrogen, Total by Combustion) rather than C content. Record weight and sample identification in instrument software.

7.5 Analyze samples and collect data automatically through instrument software.

8.0 Calculations and Reporting

8.1 Calculations. Carbon concentration (%) is calculated in the sample as follows:

$$C, \% = \frac{C, mg}{Sample\ mass, mg}\ x\ 100$$

8.2 Client Reports. The C concentration is reported in % or mg kg^{-1} on an as-received or dry-weight basis.

C:N ratio is usually reported as mass concentration of C / mass concentration of N.

9.0 Quality Control

9.1 Calibration. Combustion analyzers should not require daily calibration. When calibration is required, select a chemical calibration standard with total C content in the typical range of materials for analysis. Pure organic CRMs such as acetanilide, glutamic acid, and EDTA are suitable for instrument calibration and verification. Internal RMs should not be used for the initial calibration verification. A minimum five-point calibration curve should be used. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration.*

9.2 Method Blank (MB). Analyses of method blanks are required to demonstrate freedom from contamina-

tion in sample vessels and reagents. The type of MB will vary depending on instrument delivery method, but may include analysis of a blank tin capsule, crucible, or boat, with or without nitrogen-free sand (for solid matrix) or reagent water (for liquid matrix). Analyze a method blank at the beginning of each analytical batch. *Acceptance criteria: Detected carbon should not exceed the LOQ.*

9.3 Continuing Calibration Verification (CCV). To verify the continuing calibration of the instrument, two CCV samples should be analyzed at the start of each batch and at every 10–15 samples within each batch. The CRM used to calibrate the instrument or another RM may be used. One RM should have a C concentration within the lower 20% of the calibration range and one within the upper 80%. *Acceptance criteria: If using a CRM, measured %C values should fall within the certified uncertainty range for that CRM. If using an internal RM, the %C should be ± 10% of the mean %C for the RM, where the RM mean is determined by 30–40 analyses of the material and distributed across all instruments where the RM will be used.*

9.4 Independent Calibration Verification (ICV). The analyst should include one or more independent CRM or SRM with certified total C content at the start of each analysis batch. These CRMs must be a different source from the calibration standards. Examples of suitable CRMs are acetanilide, glutamic acid, EDTA, certified manure or sludge material, or certified plant material. *Acceptance criteria: Measured % C values of the ICVs should fall within the certified uncertainty range for that RM.*

9.5 Duplicate (Optional). The laboratory should analyze one sample in duplicate per batch. Calculate RPD according to Appendix A: Definitions: Laboratory Duplicate; Relative Percent Difference. *Acceptance criteria: RPD should be ≤ 10%.*

9.6 Matrix Spike Recovery. Not applicable.

9.7 Proficiency Requirement. Laboratory may participate in a proficiency testing program for carbon. Example, U. S. Composting Council's Compost Analysis Proficiency Program.

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least annually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Precision. Data not available.

11.0 Safety and disposal

11.1 Laboratory safety. Burn risk is a potential safety concern when changing columns or performing maintenance on heated internal parts. Materials containing explosives or materials that can create explosive gas mixtures are not suitable for combustion analysis, and can lead to injuries and/or equipment damage. All safety procedures recommended by the manufacturer of the combustion analyzer must be followed.

11.2 Caution. Not applicable.

11.3 Reagents disposal. Instruments that use reagents containing chromium or silver should be treated as hazardous waste and disposed of accordingly.

11.4 Samples disposal. Dispose of manure in accordance with local, state, and/or federal regulations.

12.0 Reference documents and additional resources

AOAC. (1990.) Method 972.43 — Microchemical determination of carbon, hydrogen, and nitrogen — Automated method. In Official methods of analysis (15th ed., p. 341). AOAC International.

U. S. Environmental Protection Agency. (2021). 40 CFR Part 136. *Guidelines establishing test procedures for the analysis of pollutants*. https://www.ecfr.gov/cgi-bin/text-idx?SID=57d5771415f8a36cb-8c0e2ca2a1583a2&mc=true&node=pt40.25.136&rgn=div5#ap40.25.136_17.c

M-3.1
Nitrogen, Total by Combustion

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1.0 Scope and Application

This method quantitatively determines the concentration of total nitrogen (organic and inorganic) in manure (slurry, semi-solid, or solid) and compost by combustion of an individual sample in an oxygen (O_2)-containing environment, with subsequent quantification by thermal conductivity detector. Unlike the Total Kjeldahl Nitrogen (Chapter M-3.2, Total Kjeldahl Nitrogen) method for nitrogen, the combustion method also measures NO_3^- and NO_2-N. Also, recovery of some heterocyclic N-compounds is more complete by the Dumas method than TKN. Since these heterocyclic N-compounds, as well as NO_3^- and NO_2-N concentrations, are typically low in manure, results from the two methods are generally comparable. Total N may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The Dumas method for determination of total N by combustion described here was adapted from AOAC 972.43 and 990.03. The sample is introduced into a high temperature (> 900 °C) furnace, where the material is combusted in the presence of O_2. Helium carrier gas transports the combustion products through a reduction column where the nitrous oxide gases are reduced to dinitrogen gas (N_2) and excess oxygen is absorbed. N_2 is separated from other interfering gases and transported to a thermal conductivity detector, where N_2 is measured quantitatively. Combustion analyzers are available in a variety of configurations, such as CN, CHN, CHNS, or CNS, depending on user needs, and have been used extensively for several decades for high-throughput analysis.

2.2 Method Performance. The Method Detection Limit (MDL; 40 CFR 136) should be achievable at 0.25 mg and the Limit of Quantitation (LOQ) at 1.0 mg, depending on laboratory instrumentation. MDL should be determined and reported individually by laboratory upon completion of the MDL procedure outlined in Appendix B.

2.3 Method Interferences. Volatilization losses of NH_3 may occur during sample preparation from exposure to air and during sample analysis due to exposure to heat from the combustion furnace. To reduce NH_3 volatilization loss, minimize exposure to air and maintain sample at < 6 °C upon receipt. During sample weighing for analysis, NH_3 loss may be minimized by acidifying the samples to less than pH 5.0 with glacial acetic acid. If the sample is also being analyzed for total carbon (C), note that acetic acid will produce a C peak that will interfere with C quantitation. Duplicate analysis of the sample with and without acetic acid will be necessary for both total N and C quantitation.

2.4 Method Reporting. Nitrogen is reported in mg kg^{-1} or as % on an as-received or dry-weight basis, as determined by state requirements.

2.5 Method Advantages. Combustion analysis is a well-established method, and automated instruments are available that are capable of high-throughput analysis in multiple element configurations such as N, CN, CHN, CHNS, and CNS. This method also avoids the hazardous chemicals and acid digestion associated with the TKN method.

2.6 Method Disadvantages. The MDL of combustion analysis is approximately 10-fold higher than that of TKN. While this is not a primary concern in most manure samples, which typically have N concentrations in excess of 0.2%, it makes the combustion method unsuitable for liquid (< 4% solids) manure samples with N concentrations < 0.05%.

3.0 Definitions

Nitrogen, Total (TN). The sum of organic and inorganic N as determined by oxygen combustion analysis.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Nitrogen is susceptible to losses due to denitrification of nitrate in stored samples or, more commonly in manures, volatilization of NH_3. For accurate deter-

mination of N, samples should be refrigerated promptly and analyzed without drying, to avoid increasing NH_3 volatilization. The NH_3 may be stabilized with the addition of glacial acetic acid prior to combustion analysis. Samples should be stored at ≤ 6° C for up to seven days. Not recommended for freezing.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Do not dry. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter 2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Combustion analysis is performed on a 0.1–4.0 g test portion from the ≥ 200 cm^3 homogenized, secondary sample.

5.0 Apparatus

5.1 Analytical balance, capable of measuring to 0.0001 g.

5.2 Sample delivery vessels, specific to the instrument, such as stainless-steel crucibles, ceramic boats, or tin capsules.

5.3 An elemental combustion analyzer, such as a N, CN, CHN, CHNS, or CNS analyzer, capable of performing the Dumas nitrogen method and with associated consumables.

6.0 Reagents and Standards

6.1 Sand, laboratory grade, such as BDH9274 (optional).

6.2 Helium (99.996% purity). UHP Grade.

6.3 Oxygen (99.996% purity). UHP Grade.

6.4 Glacial Acetic Acid (optional)

6.5 Certified Reference Materials (CRM) such as acetanilide (10.36% N), glutamic acid (9.52% N), and EDTA (9.59% N) and/or a Standard Reference Material (SRM) such as SRM 2781, Domestic Sludge, (4.78% N) for calibration and calibration verification. Plant CRMs, e.g., wheat flour (1.43% N) or spinach (5.9% N), can be used for independent calibration verification (ICV). Internally produced Reference Materials (RM) may be used for continuing calibration verification (CCV).

6.6 Consumable materials for packing columns. These materials will vary based on the instrument manufacturer, and may include:

- **6.6.1** Ascarite/Sicapent (drying agent)
- **6.6.2** Copper oxide
- **6.7.3** Copper wire
- **6.7.4** Platinum
- **6.7.5** Silver or quartz wool
- **6.7.6** Tungsten
- **6.7.7** Zinc
- **6.7.8** Corundum balls (aluminum oxide)

7.0 Procedure

7.1 Follow instrument manufacturer's recommended procedure for packing combustion and reduction columns, performing leak check, conditioning, and calibration, and preparing the instrument for analysis.

7.2 Analyze quality control samples per Section 9.

7.3 Homogenize sample again, if necessary. Homogenization should have already occurred during sample receiving, but samples with high water content can separate during the time between initial homogenization and analysis. If separation is noted, manually re-homogenize the sample.

7.4. Weigh sample to nearest 0.0001 g and place in sample crucible, boat, or capsule. Sample size should be 0.1–4.0 g, based on anticipated N content of the sample. For manure, taking a 0.2–0.5 g sample of solids and 0.8–2.0 g of liquids is generally adequate. Record weight and sample identification in instrument software.

7.5. (Optional) Add 200 µL of glacial acetic acid to each sample to lower pH to < 5. After mixing acetic acid with the sample, verify pH < 5 with pH test strip and record pH.

7.6 Analyze samples and collect data automatically through instrument software.

8.0 Calculations and Reporting

8.1 Calculations. Nitrogen concentration (%) is calculated in the sample as follows:

$$N, \% = \frac{N, mg}{Sample\ mass, mg} \times 100$$

8.2 Client Reports. The N concentration is reported in % or mg kg^{-1} on an as-received or dry weight basis.

9.0 Quality Control

9.1 Calibration. Combustion analyzers should not require daily calibration. When calibration is required, select a chemical calibration standard with total N content in the typical range of the materials for analysis. Pure, organic CRMs such as acetanilide (10.36% N), glutamic acid (9.52% N), and EDTA (9.59% N) are suitable for instrument calibration and verification. Internal RMs should not be used for the initial calibration verification. A minimum five-point calibration curve should be used. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration.*

9.2 Method Blank (MB). Analyses of method blanks are required to demonstrate freedom from contamination in sample vessels and reagents. The type of MB will vary depending on instrument delivery method, but may include analysis of a blank tin capsule, crucible, or boat, with or without nitrogen-free sand (for solid matrix) or reagent water (for liquid matrix). If acetic acid is used to stabilize samples, it should be included in the MB. Analyze a method

blank at the beginning of each analytical batch. *Acceptance criteria: Nitrogen concentration should not exceed the LOQ.*

9.3 Continuing Calibration Verification (CCV). To verify the continuing calibration of the instrument, two CCV samples should be analyzed at the start of each batch and at every 10–15 samples within each batch. The CRM used to calibrate the instrument or another RM may be used. One RM N concentration should lie within the lower 20% of the calibration range, and the other should lie within the upper 80% of the calibration range. *Acceptance criteria: If using a CRM, measured % N values should fall within the certified uncertainty range for that CRM. If using an internal RM, the % N should be ± 10% of the mean N concentration of the RM, based on the mean—measured across all instruments where the RM will be used—of 30–40 analyses of the material.*

9.4 Independent Calibration Verification (ICV). The analyst should include one or more independent CRM or SRM with certified total N content at the start of each analysis batch. These CRMs must be a different source from the calibration standards. Examples of suitable CRMs are acetanilide (10.36% N), glutamic acid (9.52% N), EDTA (9.59% N), certified manure or sludge material, or certified plant material. Internal RMs may not be used as ICV. *Acceptance criteria: Measured % N values should fall within the certified uncertainty range for that RM.*

9.5 Duplicate. Laboratories reporting N in waste manure samples for compliance purposes must include a duplicate in their QC program. The laboratory should analyze one sample in duplicate per every 20 samples. Calculate RPD according to Appendix A: Definitions: Laboratory Duplicate; Relative Percent Difference. *Acceptance criteria: RPD should be ≤ 10%. RPD as a statistical approach to evaluating precision is sensitive to analyte concentration and may exceed 10% for N found at low concentrations (< 0.5%) in certain manure types (e.g., liquids). For these sample types, RPD should be within the documented historical acceptance limits for each matrix or concentration range.*

9.6 Matrix Spike Recovery (MSR). Laboratories reporting N in waste samples for regulatory compliance purposes must include a matrix spike in their QC program. The laboratory should spike at least one sample in each analytical sample batch. The concentration of the spike should be approximately 3 to 5 times the LOQ. The addition of 1 mL of 10,000 mg N L^{-1} TRIS spike solution works well for most manure samples (spike addition of 10 mg N). Calculate the percent MSR of N according to Appendix A: Definitions; Matrix Spike Recovery. *Acceptance criteria: 100 ± 15% recovery of the standard is acceptable.*

9.7 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure nitrogen that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. For an example, see Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

Table M-3.1.1. Quality control guidelines for the total N method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	As needed	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Method Blank (MB)	Every batch	N < LOQ
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	CRM: Within supplier specified uncertainty range; RM: ± 10% of mean
Independent Calibration Verification (ICV)	After calibration	CRM: Within specified uncertainty range
Duplicate	Every batch	≤ 10% RPD
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%

10.0 Limit of quantification and method performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least annually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Precision. Total nitrogen method repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of proficiency rounds of 10 laboratories conducted in 2015 and 2016 by the Manure Analysis Proficiency (MAP) Program, coordinated by the Minnesota Department of Agriculture. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained (Table M-3.1.2).

11.0 Safety and Disposal

11.1 Laboratory Safety. Burn risk is a potential safety concern when changing columns or performing maintenance on heated internal parts. Materials containing explosives or materials that can create explosive gas mixtures are

Table M-3.1.2. Laboratory precision data for manure total nitrogen as-received basis, combustion method.

Sample ID	Total Solids Content (%)	Mean Content mg N kg^{-1}	Intra-lab Repeatability standard deviation S_r mg N kg^{-1}	Intra-lab Repeatability r mg N kg^{-1}	Inter-lab Reproducibility standard deviation S_R mg N kg^{-1}	Inter-Lab Reproducibility R mg N kg^{-1}
M 2014-A	3.2	840	38	118	166	481
M 2015-D	15.7	5710	127	370	616	1780
M 2012-B	56.5	26900	460	1290	1280	3710
M 2016-F	68.4	20210	270	810	1010	2930

MAP Program, 31–43 laboratories reporting.

not suitable for combustion analysis, and can lead to injuries and/or equipment damage. All safety procedures recommended by the manufacturer of the combustion analyzer must be followed.

11.2 Caution. Not applicable.

11.3 Reagents Disposal. Instruments that use reagents containing chromium or silver should be treated as hazardous waste and disposed accordingly.

11.4 Samples Disposal. Dispose of manure in accordance with local, state, and/or federal regulations.

12.0 Reference documents and additional resources

AOAC. (1990.) Method 972.43 — Microchemical determination of carbon, hydrogen, and nitrogen — Automated method. In Official methods of analysis (15th ed.) (p. 341). AOAC International.

AOAC. (2006). Official method 990.03: Protein (crude) in animal feed, combustion method. In Official methods of analysis of AOAC International (18th ed), Revision 1 (pp. 30–31). AOAC International.

U. S. Environmental Protection Agency. (2021). 40 CFR Part 136. *Guidelines establishing test procedures for the analysis of pollutants*. https://www.ecfr.gov/cgi-bin/text-idx?SID=57d5771415f8a36cb-8c0e2ca2a1583a2&mc=true&node=pt40.25.136&rgn=div5#ap40.25.136_17.c

Wolf, A.M. (2004). Total nitrogen in sewage sludge by combustion. Submitted to the US EPA and the PA Department of Environmental Protection as an alternative to the Kjeldahl procedure for evaluating total nitrogen in sewage sludges. Approved by DEP on 5/3/2004 and EPA on 5/28/2004 for use in total nitrogen in sewage sludges.

M-3.2
Total Kjeldahl Nitrogen (TKN) Method

AUTHORS: Kristin Hicks and Jason Lessl

1.0 Scope and Application

This method determines the amount of organically bound and inorganic ammonium (NH_4-N) nitrogen in manure (liquid, slurry, semi-solid, and solid) for crop nutrient management planning. The nitrogen in manure primarily exists in the forms of NH_4-N and organic nitrogen. The latter consists of urea, proteins, peptides, nucleic acids, and other cellular components of biological origin. Total Kjeldahl Nitrogen (TKN) is defined as the sum of organic nitrogen, which is converted to NH_4-N during digestion, and free NH_4-N, both of which are then quantified as NH_4-N by one of several methods. It should be noted that the TKN method does not measure nitrate or nitrite, which are assumed to be negligible in manure.

2.0 Method Principle

2.1 Method Description. This method is adapted from US EPA 351.2. Using Kjeldahl digestion with concentrated sulfuric acid, a metal catalyst, and salts, organically bound nitrogen is converted to NH_4^+ and quantified by one of several methods.

The first step in the TKN method is a hot sulfuric acid (H_2SO_4) digestion of the sample. Salts, commonly potassium sulfate (K_2SO_4) or sodium sulfate (Na_2SO_4), are used to raise the boiling point, preserving the digest acid for the duration of the digest and enhancing recovery of the analyte. The efficiency of organic matter oxidation is also increased by the addition of a metal catalyst. The earliest versions of the method used mercury (Hg) as a catalyst, but due to environmental concerns this is no longer used. Titanium (Ti), chromium (Cr), selenium (Se), and copper (Cu) are currently used as acceptable alternative catalysts, with Cu or a mix of Cu and other metals used most commonly. These digestions have historically been performed using an open digestion block with glass digestion tubes in a fume hood. However, due to safety concerns and labor intensity, multiple instruments capable of automated Kjeldahl digestion are now widely available.

After digestion, NH_4-N in the digest can be measured by diffusion-conductivity, continuous flow analysis, flow injection analysis, discrete analysis, or distillation and titration (Chapter M-4.2).

2.2 Method Performance. The TKN method has a detection limit in manures of approximately 1–10 mg L^{-1} for liquids or mg kg^{-1} for solids, but should be determined and reported individually by the laboratory upon completion of the MDL procedure outlined in Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. High nitrate concentrations (10 times or more than the TKN level) can result in inaccurately low TKN values. This rarely occurs in manures, and is a more common problem with industrial wastes. Where high NO_3-N concentrations are a factor, samples should be diluted and reanalyzed. If the acid to organic material ratio is too low, incomplete digestion, as evidenced by dark particles in digested samples, can occur. When digestion is incomplete, the original sample must be diluted and re-digested.

2.4 Method Reporting. Reporting of TKN is dependent on state permitting requirements, but is typically reported as TKN ppm, mg TKN L^{-1}, mg TKN kg^{-1}, or as a % N on an as-received basis.

2.5 Method Advantages. The advantages of TKN are: 1) it is a well-established method for accurate quantitation of nitrogen in manures, 2) low technical expertise is required for the digestion component of the method, 3) large sample sizes can be processed, and 4) it has a 10-fold lower MDL than the nitrogen combustion method (Dumas), which is the most common alternative method.

2.6 Method Disadvantages. The TKN method involves 1) relatively high volumes of concentrated sulfuric acid which poses significant lab safety concerns and requires hazardous waste disposal and 2) a metal catalyst that requires hazardous waste disposal 3) a labor-intensive procedure with a separate measurement for ammonium after digestion.

3.0 Definitions

3.1 Total Kjeldahl Nitrogen (TKN). The sum of N contained in organic substances plus the nitrogen contained in the inorganic compounds ammonia and ammonium ($NH_3 + NH_4^+$).

4.0 Sample Processing, Preservation, and Storage

4.1 Sample storage. Due to the effect of temperature on pH and NH_4^+, samples should be stored at ≤ 6 °C and no longer than 7 days. Not recommended for freezing.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Do not dry. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. For solid and semi-solid samples, digestion is performed on a 0.5–5.0 g test portion from the ≥ 200 cm^3 homogenized secondary sample. For liquid samples, the test portion for most manures (e.g., lagoons) should be 10 mL, but volume may be increased up to 50 mL for very dilute samples.

5.0 Apparatus

5.1 Analytical balance, capable of weighing to 0.001 g.

5.2 Boiling chips (optional). Acid-resistant, e.g., Alundum or Teflon.

5.3 Acid-resistant fume hood with exhaust system.

5.4 Block digestion system with heavy-walled, volumetric, glass digestion tubes capable of heating to 375 °C and analyzing up to 5.0 g solid samples or 50 mL liquid samples. Tube size for digesting manure is dependent on sample size, but should be appropriate for your digestion system.

5.5 Alternatively, 5.3 and 5.4 can be substituted with an automatic digestion system with a built-in exhaust manifold.

5.6 One of the following instruments commonly used for quantification of NH_4-N:

- **5.6.1** Distillation / Titration Analyzer. Manual or automated distillation apparatus unit with manual or automated titrimetric determination of NH_4-N. Manual distillation should include distillation rack fitted with reflux traps, water-jacket condensers, and in-flasks emitters.
- **5.6.2** Spectrophotometric continuous flow, flow injection, or discrete analyzer.
- **5.6.3** Diffusion-Conductivity Analyzer.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I or equivalent, ammonia-free.

6.2 Sulfuric acid (H_2SO_4), concentrated.

6.3 Salt, e.g., potassium sulfate (K_2SO_4).

6.4 Metal catalyst, e.g., copper sulfate ($CuSO_4 \cdot 5H_2O$).

6.5 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

> ***Note:*** Premixes of K_2SO_4/$CuSO_4$ powders and tablets (e.g., Kjeltabs) are readily available by multiple suppliers for convenience.

7.0 Procedure

7.1 Weigh 0.5–5.0 ± 0.05 g of semi-solid and solid sample or 10–50 mL of liquid sample into a digestion tube. Record the sample quantity and dilution factor.

7.2 Following protocols outlined in Section 9, prepare method blanks, duplicates, matrix spikes, and reference materials along with unknowns for digestion.

7.3 In the fume hood, add concentrated H_2SO_4 to each digestion tube. The amount of acid to add should be optimized by each lab based on the percent solids of the sample material. The greater the percent solids, the more acid that will be required to digest the material. The ratio of H_2SO_4 to sample should be approximately 5:1 mL g^{-1} for slurries and solids. The ratio of acid to sample for most liquids should be on a 1:1 mL mL^{-1} basis, but may decrease to 1:5 for very dilute liquids with < 1% solids.

7.4 Add boiling chips (optional), salt (e.g., K_2SO_4), and the metal catalyst (e.g., $CuSO_4 \cdot 5H_2O$) to each digestion tube. The ratio of salt:catalyst in the mixture is approximately 10:1. The amount of salt/catalyst mixture to add to each sample varies within the method, but is generally in a ratio of approximately 3:1 g g^{-1} of salts to solid samples and 0.03 g mL^{-1} of salts to liquid samples.

7.5 Place the digestion tubes in the digestion block and set the temperature of the block to 160 °C for 30 minutes. Raise the block temperature to 380 °C and heat for a minimum of one hour or until the digest clears. If using a Cu catalyst, the digest should be transparent with a bluish color.

7.6 Allow samples to cool (15–30 minutes) and then dilute with reagent grade, ammonia-free water. Low volume digests may crystallize if allowed to fully cool before adding water. If this occurs, samples can be gently warmed to re-dissolve the digest crystals. Record dilution factor if not using the distillation/titration method.

7.7 Quantify NH_4-N in the digest by one of the methods listed in Chapter M-4.2.

8.0 Calculations and Reporting

8.1 Calculations. Calculate the NH_4-N as described in Chapter M-4.2.

8.2 Client Reports. Reporting of TKN is dependent on state permitting requirements, but is typically reported as TKN ppm, mg TKN L^{-1}, mg TKN kg^{-1}, or as a % N on an as-received basis.

9.0 Quality Control

Note: For additional QC components associated with this method, see Chapter M-4.2.

9.1 Method Blank (MB). One MB should be included with each batch of digestions, including all reagents used in the digestion of manure samples, in order to test for contamination in the water, reagents, filters, or apparatus. *Acceptance criteria: Concentrations of detected analytes should not exceed the LOQ for NH_4-N by TKN.*

9.2 Duplicate. Digest and analyze one sample duplicate per batch. The duplicate should be separated in the batch from the first sample by at least 10 unknown samples when possible. Calculate RPD according to Appendix A: Definitions: Laboratory duplicate; Relative Percent Difference. *Acceptance criteria: Follow RPD% criteria for Chapter M-4.2.*

9.3 Matrix Spike Recovery (MSR). With each batch, digest a sample in duplicate, with the duplicate sample receiving a spike of known NH_4-N concentration. The spike concentration should be at least four times the LOQ and should be high enough to be detected above the NH_4-N concentration in the original sample. A mid-range calibration CCV or ICV solution is usually suitable. Calculate the recovery of NH_4-N according to Appendix A: Definitions; Matrix Spike Recovery. *Acceptance criteria: Follow MSR% criteria for Chapter M-4.2.*

9.4 Reference Material (RM). Include a minimum of one organic nitrogen reference standard (e.g., sludge reference material, L-Lysine Monohydrochloride) with each batch of digestions. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.5 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure TKN that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) for TKN shall be determined based on the method described in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to the practice of this method. Determine MDL and LOQ annually or anytime apparatus is repaired or replaced.

10.2 Method Precision. TKN method repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program, coordinated by the Minnesota Department of Agriculture, from proficiency rounds conducted in 2015 and 2016 of 10 laboratories. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained (Table M-3.2.1)

Table M-3.2.1. Laboratory precision data for manure Total Kjeldahl Nitrogen (TKN), as-received basis.

Sample ID	Total Solids Content (%)	Mean Content mg N kg^{-1}	Intra-lab Repeatability standard deviation S_r mg N kg^{-1}	Intra-lab Repeatability r mg N kg^{-1}	Inter-lab Reproducibility standard deviation S_R mg N kg^{-1}	Inter-lab Reproducibility R mg N kg^{-1}
M 2014-A	3.2	840	5	15	64	192
M 2015-D	11.2	2680	17	54	180	540
M 2012-B	56.5	25960	45	135	1200	3610
M 2016-F	68.4	18990	55	165	1580	4740

MAP Program, 32–42 laboratories reporting.

11.0 Safety and Disposal

11.1 Laboratory Safety. Personal protective equipment during digestion should include thick, chemical-resistant, elbow-length gloves; lab coat; apron; closed-toe, slip-resistant shoes; safety glasses; and acid-proof face shield. Safety eye wash stations and safety showers should be nearby and technicians should be trained in their use. In addition, a fully stocked spill kit with neutralizers and absorbent should be available in the work area.

The laboratory is responsible for being aware of current local, state, and federal regulations regarding the safe handling of the chemicals specified in this method. A reference file of Safety Data Sheets (SDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advised.

11.2 Caution. TKN digestion requires the use of concentrated sulfuric acid under high temperatures. Sulfuric acid is a Class 3 health hazard that can burn skin and damage eyes.

11.3 Reagents Disposal. Sulfuric acid is classified as a hazardous waste, regardless of the metal catalyst used, and must be disposed of accordingly. Reagents and spills should be neutralized with a suitable compound, e.g., sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), or limestone ($CaCO_3$).

11.4 Samples Disposal. Digests should be neutralized with a suitable compound, e.g., Na_2CO_3, $NaHCO_3$, or $CaCO_3$, before disposal. Digests should be disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

American Public Health Association (APHA). (2017). *Standard methods for the examination of water and waste water* (23rd Ed.). 4500-Norg C Semi-micro-Kjeldahl Method.

Kjeldahl, J., (1883). Neue methods zur bestimmung des stickstoffs in organischen korpern. *Zeitschrift für analytische Chemie*, 22:366-382.

U. S. Environmental Protection Agency. (1993). Method 351.2. Determination of total Kjeldahl nitrogen by semi-automated colorimetry. Revision 2.0.

M-3.3
Nitrogen, Total by Near-Infrared Spectroscopy (NIR) for Poultry Litter

AUTHORS: Jason Lessl and Jake Mowrer

1.0 Scope and Application

Near infrared reflectance (NIR) spectroscopy is a rapid, safe, proven, and reliable method for measuring total N in poultry litter samples as required for nutrient management planning. NIR is sensitive to the detection of molecules containing N-H bonds.

2.0 Method Principle

2.1 Method Description. NIR spectroscopy is an analytical technique that offers several advantages for routine laboratory operations: rapidity, the absence of requirements for high temperature or dangerous chemicals, simple sample preparation, multiple determinations in one operation, and non-destructive analysis. NIR spectroscopy is based on the absorption of radiation energy in the range of 700–2500 nm. Absorption is followed by a response at the molecular level as a change in the vibrational energy of molecular bonds, the frequency of which is often specific enough to identify the responsible bond type. A light source (lamp) and a detector are required. The instrument's detector records the spectral response as reflected light minus the losses due to absorbance, compared with a reference spectra. Other types of radiative losses are inherent in the process, including transmission, refractance, and scattering; as such, different sample matrices will require separate "calibrations."

The primary stretch overtones for N bonds in proteins, urea, ammonium, and nitrate all occur between 1100–1600 nm, though secondary and complex overtones are reported up to 2500 nm. This allows for the use of less expensive instruments capable of scanning only the lower range of NIR wavelengths.

In contrast to many other analytic methods, the calibration procedure for NIR spectroscopy, though crucial to performance, is secondary rather than direct. The reflectance or absorption spectra from test samples are compared with results from a "reference" analytic method through statistical analysis. Inclusion of many separate wavelength bands may be necessary to adequately describe the relationship. For total N in poultry litter, both total Kjeldahl and combustion have historically proven to be useful reference methods. Typical approaches used to handle large amounts of sample spectral data for comparison with reference values include principal components regression, modified least squares regression, and neural networking. While many manufacturers of NIR instruments provide calibration software packages that facilitate this process, third-party software is also frequently used.

Calibration sets should be large enough to contain as much expected variability as possible for the samples to be analyzed. Typical calibration sets are in the range of 500 or more samples. In-house calibrations may be started with fewer samples, but continually improved over time through the incremental inclusion of additional samples as resources allow. *It is crucial that the reference procedure be performed as carefully as possible to minimize avoidable "noise" from statistical analysis.*

> ***Note:*** Calibrations for water content and carbon content may also be easily obtained, provided the lab has capacity to perform the reference method(s). These complementary measurements can be simultaneously produced with N content.

2.2 Method Performance. Method detection limits are in the range of 200–500 mg N kg^{-1} dry matter. Reliable NIR spectroscopy values must be selected from carefully prepared reference samples to calculate the relationship of absorbance to N concentration. The accuracy of the reference method is fundamental to the performance of the NIR calibration in predicting the N content of a sample based on the spectral response. Samples must also be screened

for "closeness" of spectra, as large differences in spectral response within a group of samples complicate interpretation and comparison with the reference data. The Mahalanobis distance (H statistic) is commonly used for this evaluation. Successful calibrations may be evaluated through the comparison of derived values from predicted vs. reference methods, using simple linear regression and coefficient of determination (r^2). Values of $r^2 > 0.90$ are desirable.

A validation procedure is required in addition to the calibration procedure. Validation tests the ability of the calibration algorithm to correctly predict the composition of unknown samples. It is not uncommon to develop a "good" calibration model that fails to accurately predict unknowns due to small sample size or failure to include certain sources of variability. Briefly, a set of samples with values measured directly using the same reference method as the calibration set *and that have not yet been included in the calibration algorithm* are predicted and compared against the reference values using simple linear regression.

Validation testing should be continued on a routine basis as part of the NIR QC process. Calibrations may be strengthened by inclusion of validation sets. The targeted inclusion of difficult-to-predict or slightly distant spectra from the calibration set spectral mean (H statistic > 4) can often be most useful in accounting for new variabilities and improving calibrations over time.

2.3 Method Interferences. The method is sensitive to differences in manure material composition and particle size distribution. Substantial differences in amount or type of bedding material, contamination with soil or other foreign material, water content, and particle size homogenization can change the spectra pattern of absorption and reflection in a manner that compounds errors and spectral interpretation. Even small differences in sample preparation, such as the method of drying, may give rise to differences in spectral response. It is therefore important that all samples are handled and prepared exactly the same prior to analysis. Continued monitoring of the Mahalanobis distance (H statistic) value allows for rapid identification of samples that do not meet the criteria for use of a calibration built upon the spectral properties of a particular set of samples. Samples should all be allowed to reach room temperature prior to analysis, as temperature affects bond vibrational energy.

> ***Note:*** While drying samples has been found to moderately improve calibrations, it is not necessary for producing calibrations of high quality. To minimize ammonia volatilization and other N transformations that result, drying should be avoided in samples prepared for N analyses.

2.4 Method Reporting. When total solids content is known, report % N or mg kg^{-1} on an as-received or dry-matter basis.

2.5 Method Advantages. No exposure to dangerous chemicals or high heat. Easy sample preparation and quick overall turnaround time from receipt of sample to reporting results.

2.6 Method Disadvantages. Relies substantially on development of secondary NIR mathematical calibration model. Since commercial availability for calibrations is currently undeveloped, in-house calibration, though time consuming, is required. Sensitive to sample composition differences that give rise to substantial differences in spectral response.

3.0 Definitions

3.1 Near infrared reflectance (NIR). A spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from 700–2500 nm).

4.0 Sample Processing, Preservation, and Storage

4.1 Sample storage. Samples should be stored at ≤ 6 °C for up to seven days. Not recommended for freezing.

4.2 Sample processing. Samples may be sieved to improve accuracy, if also done with the calibration and verification samples. Sieving may bias sample composition.

4.3 Sample test portion. Sample size should be enough to fill the cuvette, typically 2–3 g.

5.0 Apparatus

5.1 NIR system capable of scanning from at least 1100–1600 nm. This is the primary range of response for N-containing bonds. Instruments using the full range of 700–2500 nm may also be used, but are generally more expensive.

5.2 NIR sample cell with quartz glass window.

6.0 Reagents and Standards

6.1 Reference material. May be developed in-house or purchased as certified RM. Store at ≤ 6 °C.

6.2 No reagents are required.

7.0 Development of NIR Prediction Model

7.1 Selecting representative samples for model development. A key component of the NIR prediction model is the size and variety of poultry litter samples scanned by the NIR spectroscopy instrument. The sample population should represent the full diversity of poultry litter materials to be scanned. For example, if only poultry litters from layer houses (which typically contain little or no bedding material) are used to develop the prediction model, it may not be appropriate to scan litters from a broiler or composted sources.

Numerous samples should be scanned by NIR and assayed by wet chemistry (i.e., nitrogen by total combustion) procedures to obtain calibration statistics. There is no specific rule for the minimum sample set to develop a calibration set. Generally, 100 samples demonstrate potential, and another 50 samples demonstrate validation (Burns, 2007). The more samples used, however, the more robust the prediction model will become.

Sample selection should represent the range of N values to be measured into consideration along with the diversity of different poultry litters in a population. Poultry litter, for example, can differ depending on the type of poultry production system (i.e., broilers, layers, breeders, pullets), how the litters are stored (i.e., fresh, stackhouse, composted, cleanout), the type of bedding material used (i.e., wood shavings, sawdust, straw, bark, peanut hulls), and the presence of any poultry litter amendments (i.e., Alum, KLASP, Poultry Guard).

Ideally, the prediction model must be derived from calibration samples that fully cover all of the expected diversity with minimal redundant information. Extrapolation outside the range of the reference samples is not recommended, since accuracy of prediction becomes suspect.

For developing reliable NIR spectroscopy prediction models and valid results, laboratories should:

1. Minimize sources of error during sampling, processing, and analysis.
2. Obtain values for reference samples using standard analytical methods with high precision and accuracy.
3. Standardize sample preparation and analytical procedures.
4. Standardize the NIR spectroscopy instrument.
5. Use regression methods like partial least square (PLS) to obtain accurate, predictive spectral information.
6. Perform routine instrument maintenance—e.g., maintaining a log of lamp use hours.
7. Analyze only samples representative of the original validation population.
8. Obtain routine diagnostics of all associated instruments and undergo yearly prediction model (calibration) updates.

7.2 Development of the NIR Prediction Model. The results of each sample scan are recorded as log(1/reflectance), typically at 2 nm intervals covering at least the 1100–2500 nm wavelengths. Statistical analysis of collected NIR spectra is performed by using a calibration software package, usually provided by the manufacturer of the NIR instrument (e.g., WinISI). The first step in the process is to compare all the spectral data and identify and remove outliers from the calibration dataset. Principal components regression analysis of the sample spectra can be used for sample scoring, which ranks spectra according to their Mahalanobis distances (H statistic). This indicates the difference between an individual sample spectrum and the average of the entire sample set, called the "global-H" or GH distance. Any sample with a GH > 3.0, for example, can be eliminated as an outlier. The algorithm also measures similarities between the sample spectrum and its closest neighboring spectrum using a second Mahalanobis distance, called the "neighboring-H" or NH distance, and allows for the elimination of samples (commonly with a NH < 0.6) from their nearest neighboring samples. As outliers are removed, the overall robustness of the prediction model is improved.

The calibration is further developed by using modified partial least squares regression—a stepwise protocol where each standardized wavelength is simultaneously cross-validated. Using the "leave-one-out cross-validation" procedure, the dataset is partitioned into several subgroups and used for cross-validation of the model. This is typically performed in five cross-validation steps, with the calibration and validation subgroups split into 80% and 20% of the entire dataset, respectively. The validation errors are combined into a single overall standard error of cross-validation (SECV). This procedure allows for every sample in the set to be used in the validation process, improving the calibration model. The results are averaged and used to calculate the SECV and the associated 1 – variance ratio statistics (1-VR), which are then used to compare the validation of the models. A lower SECV and higher 1 – VR indicates successful cross-validation of the model. Additional outliers are rejected based on their spectral differences (H statistic), and compositional outliers are removed from the model on the basis that the difference between predicted and laboratory-measured values exceeds three standard deviations of the SECV. Samples with poor laboratory-measured values that do not correlate well with the spectral features of the dataset are considered compositional outliers.

Once the model is complete, statistical analysis is performed to assess the overall robustness of the model and its accuracy in predicting sample values. The r^2 describes how well the data points fit the statistical model (the line of regression). The standard error of prediction (SEP) and the associated r^2 are used to evaluate the equations. A lower SEC and higher r^2 is considered optimal. The ratio of performance (SEP) to deviation (SD), called the ratio of performance deviation (RPDNIR), is used to evaluate the success of the independent/external validation of the model. The RPDNIR measures the coefficient of variation (CV) for the calibration and validation sets, respectively, and represents the factor by which the prediction accuracy increases compared to using the mean composition for the samples. This value also provides the average errors of pre-

diction during cross-validation and independent validation. Validation performance can be evaluated with the following metrics (see Table 3.3.1).

Table M-3.3.1. Assessment of NIR prediction model.

Category	r^2	RPD_{NIR}
Excellent	> 0.90	> 3.0
Good	0.81 – 0.90	2.5 – 3.0
Approximate	0.66 – 0.80	2.0 – 3.0
Poor	< 0.66	< 2.0

The quality (or robustness) of an NIR prediction model is dependent on the error evaluation, and typically involves statistics. A robust NIR prediction model should have an SEP that is equivalent to the standard deviation of the reference assay. This is determined by predicting values from an independent sample set (not used for the validation). A favorable prediction model will generally have a regression coefficient value > 0.90 (Table 3.3.1). Standard deviation of the reference assay should also be low. Method performance can be continually improved as the number of samples in the calibration set increases.

8.0 Sample Procedure

8.1 Homogenize a representative subsample of the as-received material unless it is too wet to hold shape (~30% or more water content).

8.2 Using the homogenized poultry litter sample, fill the cell with a quartz window, leaving sufficient room for the backing cover to secure the sample and ensure no movement during analysis. Pack all samples to similar depths and densities.

8.3 Place the cell cover on the back of the cell without excessively compacting the sample.

8.4 Place sample in NIR and obtain scan. If the spectra deviates from the predicted compositional value, the results are invalid and the sample should be analyzed by an alternative method.

8.5 Remove sample and repeat process until all sample spectra are obtained. Results transfer and storage will vary by instrument and software.

9.0 Calculations and Reporting

9.1 Calculations. Using the prediction model, the N content of the litter is calculated.

9.2 Client Reports. Report as total N in mg kg^{-1} or % on as-received basis. Water content, which can be analyzed concurrently with the N (assuming the calibration has been performed), may be used to calculate results on a dry matter basis. Otherwise, separate measurement of water content may be used.

10.0 Quality Control

10.1 Instrument Diagnostic Test. Run the manufacturer's built-in diagnostic test at least once a week to check the NIR response, wavelength accuracy, and repeatability. If any changes are noted, troubleshoot and repeat before proceeding with analysis.

10.2 Instrument Reference Standard (verifies spectral response and absence of spectral drift as per manufacturer's recommendations in most cases). Run a reference cell provided by the equipment manufacturer every day and record the result, which should indicate no change in results. *Acceptance criteria: The value of "T" should be less than 3.0. If it is not, troubleshoot the instrument before proceeding.*

10.3 Reference Material. Analyze one RM with every sample set. Any source of well-characterized, low moisture-containing poultry litter in house can be used to ensure that the NIR machine is performing optimally. *Acceptance criteria: Results should be within ± 10% of the mean value. Drift may indicate deterioration of the sample (e.g., ambient moisture absorption or breakdown of N), lamp performance decline, or detector malfunction. Procure a new aliquot of bulk sample from storage for recheck before troubleshooting instrument performance.*

10.4 Duplicate. Perform one duplicate per analytical batch. *Acceptance criteria: Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference. The RPD should be ≤ 10%. An RPD exceeding 10% indicates that a representative subsample was not obtained. Resample and repeat the duplicate analysis.*

11.0 Limit of Quantitation and Method Performance

11.1 Limit of quantification (LOQ). The LOQ is based on the sample set used in model development.

11.2 Method Performance. The accuracy is based on the robustness of the NIR prediction model and the capacity of the reference method. It is a frequently espoused position that NIR is more precise than the reference method, but often more biased. If a sample produces a spectra that falls outside the global H or neighboring H range, it will be flagged and should be run by a wet-chemistry method.

12.0 Safety and Disposal

12.1 Laboratory safety. Follow routine laboratory safety protocols for handling manure. Wear safety glasses, gloves, and appropriate lab clothing.

12.2 Caution. Not applicable.

12.3 Reagents disposal. Not applicable

12.4 Samples disposal. Dispose of manure in accordance with local/state and/or federal regulations.

13.0 Reference Documents and Additional Resources

Burns, D. A. & Ciurczak, E. W. (Eds.). (2007). *Handbook of near-infrared analysis*. CRC Press.

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Xing, L., Chen, L. J., & Han, L. J. (2008). Rapid analysis of layer manure using near-infrared reflectance spectroscopy. *Poultry Science,* 87:1281–1286. https://doi.org/10.3382/ps.2007-00464

M-4.1
Nitrogen, Ammonium (NH_4-N) by Ion-Selective Electrode (Potentiometric)

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1.0 Scope and Application

This method quantitatively determines the amount of ammonium nitrogen (NH_4-N) in liquid and solid animal manure and compost by potentiometric measurement using a NH_3 selective electrode in a reagent grade water. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Use standard solutions at the same temperature and containing approximately the same level of dissolved species as samples. NH_4-N concentrations may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The following method is adapted from SM 4500-NH_3 D. The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode with an internal solution of ammonium chloride. Dissolved ammonia (NH_3(aq) and NH_4^+) in an aqueous manure suspension is converted to NH_3(aq) by raising pH to above 11 with a sodium hydroxide. NH_3(aq) diffuses through the membrane and changes the internal solution pH, which is detected by a pH electrode. Potentiometric measurements are made with a pH meter that has an expanded millivolt scale or with a specific ion meter.

2.2 Method Performance. The user should be able to obtain an MDL of 1.0 mg NH_4-N kg^{-1} (as-received sample basis, dependent on instrument manufacturer). MDLs should be determined and reported individually by the laboratory upon completion of the MDL procedure outlined in Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. Amines are a positive interference. This may be enhanced by acidification. While mercury and silver interfere by complexing with ammonia, concentration of these metals in animal manure is expected to be negligible.

2.4 Method Reporting. NH_4-N content measured in mg NH_4-N L^{-1}, calculated as NH_4-N% or mg NH_4-N kg^{-1}, and reported on an as-received basis as lb NH_4-N ton^{-1} for solid samples and lb NH_4-N 1000 gal^{-1} or lb acre-$inch^{-1}$ for liquid manures.

2.5 Method Advantages. No sample digestion or extraction is necessary; analysis is direct and rapid.

2.6 Method Disadvantages. The ammonia-selective electrode responds slowly below 1.0 mg NH_3-N L^{-1} and requires a longer electrode immersion time (5–10 min) to obtain stable readings. The method is manual and multi-step, with lower precision relative to other methods.

3.0 Definitions

3.1 Ammonium nitrogen (NH_4-N). The nitrogen present in the form of ammonium — an inorganic, nontoxic salt of the ionized form of ammonia.

3.2 Potentiometric analysis. An electroanalytical technique whereby the potential between two electrodes is measured using a high-impedance voltmeter during a titration.

4.0 Sample Storage, Processing, and Test Portion

4.1 Sample Storage. NH_4-N is subject to volatilization losses as NH_3. Due to the effect of drying on NH_3 volatilization, samples should be analyzed on an as-received basis. NH_4-N concentrations in samples that are in closed containers, refrigerated upon receipt ≤ 6 °C, and analyzed within 48 hours are assumed to be stable. Storage time should not exceed seven days; do not freeze.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where

necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Recommended sample size for extraction is 5.0 g (solid and semi solid) and 50.0 mL (liquid) manures. Concentration of NH_4-N typically found in manure is sufficient to quantify.

5.0 Apparatus

5.1 Analytical balance capable of weighing to 0.01 g.

5.2 Extraction vessel with sealable caps, 200 mL. Polypropylene- or nitrogen-free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm).

5.4 Pipette dispenser capable of dispensing 5–50.0 mL.

5.5 Ammonia ion-selective electrode and specific ion meter.

5.6 Stir plate and magnetic stir bar.

5.7 Acid-washed beads.

6.0 Reagents

6.1 Reagent grade water, ASTM Type I.

6.2 10 M NaOH. Dissolve 400 g NaOH (100 g at a time) in 800 mL of reagent grade water. Cool to room temperature, transfer to 1 L plastic volumetric flask, and dilute to volume with reagent grade water.

6.3 Ammonium chloride stock solution (1000 mg NH_4-N L^{-1}): Dissolve 3.819 g of anhydrous NH_4Cl *(Note: to maximize surface area, dry 4–5 grams in a 250 mL beaker at 104 °C for 8 hours)*. Dilute in reagent grade water to 1.0 L. This solution contains 1000 mg N L^{-1} as NH_4. Store in the refrigerator. Alternatively, a high-purity calibration standard of 1000 mg NH_4-N L^{-1} with guaranteed analysis may be commercially purchased from an outside vendor for use as the stock ammonium chloride solution.

6.4 Ammonium calibration working standards: Bring the 1000 mg L^{-1} stock ammonium chloride solution to room temperature and make five standards with concentrations of 1.0, 10, 50, 100, and 200 mg NH_4-N L^{-1}. Solution is stable for 6 months at ≤ 6 °C.

6.5 Independent Calibration Verification (ICV) stock solution standard: The purpose of the ICV standard is to verify the low and high calibration points of the calibration curve by utilizing a standard solution made up from a different source of chemicals than that used for the standard curve.

6.5.1 Stock ICV solution (1000 mg NH_4-N L^{-1}): Dissolve 3.819 g of anhydrous NH_4Cl—purchased from source independent from the one used to make up the standard stock solution—in reagent grade water and dilute to 1.0 L. This solution contains 1000 mg N L^{-1} as NH_4. *(Note: prior to weighing, dry anhydrous NH_4Cl in a drying oven at 105 °C for eight hours and cool in desiccator until ready for use)*. Store the stock solution in the refrigerator at 4 °C. Alternatively, a high-purity calibration solution standard of 1000 mg NH_4-N L^{-1} (purchased from a different source than the stock ammonium chloride solution) with guaranteed analysis may be purchased from an outside vendor. Solution is stable for 6 months at ≤ 6 °C.

6.5.2 Working high ICV standard (200 mg NH_4-N L^{-1}): Pipette 200 mL of the 1000 mg NH_4-N L^{-1} ICV stock into a 1.0 L volumetric flask and bring to volume with reagent grade water. Store at ≤ 6 °C for up to 6 months. This solution can also be used as a CCV.

6.5.3 Working low ICV standard (1.0 mg NH_4-N L^{-1}): Pipette 10 mL of the 100 mg NH_4-N ICV stock into a 1 L volumetric flask and bring to volume with reagent grade water. Store at ≤ 6 °C for up to 6 months. This solution can also be used as a CCV.

6.6 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to that of the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Manure samples should be brought to room temperature (20–24 °C) and thoroughly mixed or, if necessary, blended before analysis. Sample preparation guidelines listed below are general. Samples high in ammonium may need to be diluted further to ensure that measured concentration is within the standard calibration curve.

7.1.1 If the sample is a pipettable slurry (less than 5% solids), weigh 50.0 ± 0.1 g sample into a 200 mL extraction vessel. Record sample weight.

7.1.2 If the sample is a concentrated slurry (5–15% solids), weigh approximately 5.0 ± 0.05 g of sample into the extraction vessel. Record sample weight. Dilute to 50 g with reagent grade water and mix thoroughly.

7.1.3 If the sample is moist or a dry solid, weigh approximately 2.0–5.0 ± 0.05 g of sample into an extraction vessel and record sample weight. Dilute to 50 mL with reagent grade water and mix thoroughly.

7.2 Place extraction vessel on reciprocating horizontal mechanical shaker set to 180 epm for 20 minutes.

7.3 Ammonia Meter Calibration. Calibrate the meter with the NH_4-N standards following the manufacturer's instructions.

7.3.1 Immerse the ammonia electrode in the first NH_4-N standard and mix with a magnetic stirrer. If necessary, place a piece of insulating material (e.g., a plastic washer) between the stirrer and beaker to minimize heat transfer to sample. Do not stir so rapidly that air bubbles are created, as they may become trapped on the electrode membrane and interfere with measurement. Maintain constant stirring rate (approximately 320 rpm) and a temperature of about 24 ± 2 °C throughout calibration and testing procedures. Immerse a combination pH electrode in the same solution (see Chapter M-8.1 for pH meter calibration).

7.3.2 Add a sufficient volume of 10 M NaOH solution (0.5 mL is usually sufficient) to raise pH above 11. If additional NaOH is needed, record the additional volume of NaOH that was added to each sample. *(Note: Do not add NaOH solution before immersing electrode, as NH_3 may be lost from a basic solution.)* Keep electrode in solution until a stable reading is obtained. If in calibration mode, set concentration reading on the meter to the concentration of the standard (1.0 mg L^{-1}). If in millivolt mode, record the millivolt reading of the standard.

7.3.3 If in calibration mode, record the slope of the curve. It should be 60 ± 5. If it is not, repeat the calibration procedure. Record the calibration information from the meter and determine the coefficient of determination (r^2), which must be 0.99 or greater. If it is not, determine the source of the problem and repeat the calibration procedure until an $r^2 \geq 0.99$ is obtained.

7.3.4 If in millivolt mode, enter the millivolt readings associated with each standard in the appropriate Excel spreadsheet. Determine the statistics associated with the linear curve (slope, intercept, and r^2). The r^2 must be 0.99 or greater. If it is not, determine the source of the problem and repeat the calibration procedure until an $r^2 \geq 0.99$ is obtained.

7.3.5 Once an appropriate curve is obtained, place the ammonia electrode in reagent grade water until concentration drops to below 1.0 mg L^{-1}. Check concentration of a blank (reagent grade water), of the 1.0 mg NH_4-N L^{-1} ICV standard, and of the 200 mg NH_4-N L^{-1} ICV standard. These must read less than 1.0 mg NH_4-N L^{-1}, between 0.9–1.1 mg NH_4-N L^{-1}, and between 180–220 mg NH_4-N L^{-1}, respectively. If the correct concentrations are achieved, proceed with sample analysis. If not, determine the source of the problem and re-measure the solutions until the correct concentrations are achieved.

7.4 Analyze unknown samples

7.4.1 Immerse the ammonia electrode in the sample and mix with a magnetic stirrer. If necessary, place a piece of insulating material (e.g., plastic washer) between the stirrer and beaker to minimize heat transfer to sample. Do not stir so rapidly that air bubbles are created, as they will become trapped on the electrode membrane and interfere with measurement. Maintain the same stirring rate (320 rpm) and temperature as those used for calibration. Immerse a combination pH electrode in the same solution (See Chapter M-8.1 for pH meter calibration).

7.4.2 Add a sufficient volume of 10 M NaOH solution (0.5 mL is usually sufficient) to raise the pH above 11. If additional NaOH is needed, record the additional NaOH that was added to each sample. Place a check mark in the box provided on the sample worklist to verify that a pH greater than 11 was achieved. *(Note: Do not add NaOH solution before immersing electrode, as ammonia may be lost from a basic solution.)* Keep electrode in the solution until a stable reading is obtained. Record analysis result.

7.4.3 Rinse the pH and ammonia electrodes and stir bar with reagent grade water in between each sample.

8.0 Calculations and Reporting

8.1 Calculate the NH_4-N content in mg kg^{-1} on as-received basis of the manure sample according to the following equation:

$$NH_4\text{-}N, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:

C = extract result, mg NH_4-N L^{-1}
V = extract volume, mL
M = sample mass, g
DF = additional dilution factor, when applicable

Note: Final volume should be adjusted for solids in manure (i.e., 50 minus mass of solids in sample) if significant. If more than 0.5 mL NaOH is added to sample, amount in excess of 0.5 mL should be added to 50 mL final volume.

8.2 Client reports. Note: final results may be reported as either NH_3-N or NH_4-N. Report all values to 1 mg NH_4-N L^{-1} on an as-received basis to three significant digits. Alternative reporting units: solid manure as mg kg^{-1} and/or lb ton^{-1}; liquid manure as mg L^{-1}, lb 1000 gal^{-1}, or lb $acre\text{-}inch^{-1}$. Any NH_4-N concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. As needed. *Acceptance criteria: The coefficient of determination, r^2, should be ≥ 0.99. Otherwise perform maintenance and recalibrate.*

9.2 Method blank (MB). One MB per batch should be analyzed with every batch of 20 samples or fewer. *Acceptance criteria: Background NH_4-N should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until source of contamination is eliminated.*

9.3 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured NH_4-N concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × LOQ, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the batch. The frequency of CCV analysis is every 10 samples. Calculate the percent error (PE) between the CCV known and the as-measured NH_4-N concentrations according to Appendix A: Definitions; Percent Error. The ICV standards may be used for CCV. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed CCV.*

9.5 Duplicate. Analyze a minimum of one laboratory duplicate sample per analytical batch and calculate the RPD. When possible, the duplicate should be separated in the batch from the original sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Laboratory duplicate; Relative Percent Difference. *Acceptance criteria: An RPD of ≤ 20% should be routinely achievable for NH_4-N when the analyte concentration is greater than the LOQ.*

9.6 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch of 20 samples or fewer. Used to evaluate precision, matrix interferences and extraction efficiency, a spike of known NH_4-N concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background NH_4-N but not exceeding the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the LOQ is usually suitable. Once added to the sample aliquot, the NH_4-N spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate percent MSR according to Appendix A: Definitions: Matrix Spike Recovery. *Acceptance criteria: If recovery of NH_4-N falls outside the control limits of 100 ± 15%, NH_4-N is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.7 Reference Material (RM). A RM with a matrix similar to that of materials tested should be analyzed with every batch. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.8 Initial demonstration of capability. Prior to using this method, each analyst should demonstrate capability to

Table M-4.1.1. Quality control guidelines for NH_4-N potentiometric method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	As-needed	$r^2 \geq 0.99$
Method Blank (MB)	Every batch	NH_4-N < MDL
Continuing Calibration Verification (CCV)	After calibration, every 20 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 20% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%

Table M-4.1.2. Precision data for manure NH_4-N as-received basis, potentiometric method.

Sample ID	Total Solids (%)	Mean Content mg NH_4-N kg^{-1}	Intra-lab Standard Deviation S_r mg NH_4-N kg^{-1}	Intra-lab Repeatability r mg NH_4-N kg^{-1}	Inter-lab standard deviation S_R mg NH_4-N kg^{-1}	Inter-lab Reproducibility R mg NH_4-N kg^{-1}
M 2014-D	3.15	263	9	28	37	111
M 2015-D	11.2	484	36	108	54	162
M 2012-B	68.7	7032	140	420	1840	5520
M 2016-F	90.5	1257	78	234	177	531

MAP Program, 4–6 laboratories reporting.

generate acceptable precision and accuracy by performing the following:

9.8.1 Calibrate the instrument following the procedure detailed in the method.

9.8.2 Analyze a calibration blank, 1.0 mg NH_4-N L^{-1} ICV standard, and 200 mg NH_4-N L^{-1} ICV standard and ensure that limits specified in the method are met.

9.8.3 Analyze four samples with known ammonia content (proficiency or QC samples from Environmental Resource Associates or other vendor may be used for this purpose) and achieve the following:

9.8.3.1 Average of four results must be within the designated limits or, if limits are not designated, within 20% of the true value.

9.8.3.2 Coefficient of variation (std dev/mean × 100) must be less than 15%.

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure NH_4-N that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). The Method Detection Limit (MDL) shall be determined based on the method described by the US EPA in Part 136, described in Appendix B. The LOQ shall be calculated by the following equation.

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. NH_4-N potentiometric repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program, coordinated by the Minnesota Department of Agriculture, from proficiency rounds conducted in 2012–2016. Laboratories utilized NH_4-N selective electrode from different manufacturers, and the following precision data were obtained (Table M-4.1.2).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear lab coat, gloves, and eye protection when handling manure and reagents.

11.2 Caution. NaOH is reactive. Use caution when adding and dissolving NaOH in water, as a large amount of heat and corrosive vapors are generated. NaOH causes severe skin burns and eye damage. Prepare under hood, use gloves, and avoid contact with skin.

11.3 Reagents Disposal. Dispose of reagents in accordance with local/state and/or federal regulations.

11.4 Sample Disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

American Public Health Association. (2005). Standard methods for the examination of water and waste water. Method 4500- NH3 D. Ammonia-Selective Electrode Method (22nd Ed.).

U. S. Environmental Protection. (1974). Method 350.3. Nitrogen, Ammonia (Potentiometric, Ion Selective Electrode). https://www.nemi.gov/methods/method_summary/4871/

M-4.2
Nitrogen, Ammonium (NH_4-N) by Distillation, Spectrophotometry, and Diffusion-Conductivity

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1.0 Scope and Application

This method determines the amount of ammonium nitrogen (NH_4-N) in liquid and solid animal manure and compost on an extract with acidified 1.0 M KCl or on a digest by total Kjeldahl nitrogen (TKN), with subsequent analysis by one of three analysis methods: distillation/titration, spectrophotometry, or diffusion-conductivity analysis. NH_4-N concentrations may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. Ammonium nitrogen (NH_4-N) is extracted from manure using acidified 1.0 M KCl, and concentration is determined by one of the following methods: distillation/titration, adapted from US EPA 350.2; spectrophotometry, adapted from US EPA 350.1 or modified by Krom (1980); or diffusion-conductivity, as adapted from Carlson et al. (1990) analysis and SM 4500-N D. This method is also applicable for the determination of NH_4-N in the TKN digest (Chapter M-3.2).

2.1.1 The spectrophotometric method is based on either the alkaline phenol Berthelot reaction (Shozaburo and Nakano, 1959) or the sodium salicylate modified Berthelot reaction (Krom, 1980). In the Berthelot reaction, alkaline phenol reacts with sodium hypochlorite in the presence of a catalyst (sodium nitroprusside) to form indophenol blue. The modified Berthelot reaction replaces phenol with sodium salicylate, which is far less toxic, and replaces hypochlorite with sodium dichloroisocyanurate, a more stable chlorine donor. Spectrophotometric systems capable of measuring either reaction chemistry include continuous flow, flow injection, and discrete analysis.

2.2 Method Performance. The user should be able to attain an MDL < 5.0 mg NH_4-N L^{-1} (extract basis, instrument and/or method dependent). An MDL should be determined by each laboratory following the procedure outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. Ammonium contamination from filter paper, reagents, equipment, and lab environment may occur readily, and care must be taken.

2.3.1 Distillation/Titration. There is no known interference with determination of NH_4-N using the distillation/titration or diffusion-conductivity methods of analysis.

2.3.2 Spectrophotometry. Samples that are colored in the absorption range may interfere, and may be clarified through the use of a dialyzer membrane or by filtering the sample using a smaller membrane filter. Alternately, some instruments include a color blanking feature that measures the sample color with and without reagent and corrects for the absorbance effect of the sample color. Metal hydroxides can precipitate at high pH of the reaction. A complexing agent such as EDTA and sodium potassium tartrate may be added to prevent precipitation.

2.3.3 Diffusion-Conductivity. Volatile amines can diffuse through the hydrophobic membrane to produce a conductivity response. Interference from low-molecular weight amines is generally not a significant problem in animal manures. Calcium and Mg precipitate formation can clog instrument lines. A chelate like DTPA is added to the alkaline feed to prevent precipitation.

2.4 Method Reporting. NH_4-N concentration is measured as mg NH_4-N L^{-1} of extract or digest, corrected for sample dilution, and reported on an as-received basis as NH_4-N%, mg NH_4-N L^{-1} , or mg NH_4-N kg^{-1}. Solid and semi-solid samples may be reported as lb NH_4-N ton^{-1}, while liquid or slurry samples may be reported as lb NH_4-N 1000 gal^{-1} or lb $acre\text{-}inch^{-1}$.

2.5 Method Advantages. Diffusion-conductivity and distillation methods have lower MDLs compared to spectrophotometric methods, and are not affected by color or turbidity. Diffusion-conductivity and spectrophotometric methods have rapid throughput.

2.6 Method Disadvantages. NH_3 volatilizes readily at ambient temperatures and at pH > 7.5; care must be taken to minimize loss. Distillation method has low throughput and is laborious.

3.0 Definitions

3.1 Ammonium nitrogen (NH_4-N). The nitrogen present in the form of ammonium—an inorganic nontoxic salt of the ionized form of ammonia.

3.2 Distillation/titration analysis for ammonium. Distillation converts NH_4-N to NH_3 (gas) when treated with an alkali solution and recovered as NH_4-N in a boric acid trap, with subsequent determination by titration with a standardized NaOH solution.

3.3 Spectrophotometric analysis. Determination of an analyte in a liquid matrix by the reaction with a chromophore and measurement of the amount of light absorbed at a specific wavelength, where the change in absorption is proportional to the analyte concentration.

3.4 Diffusion-conductivity analysis. Determination of an inorganic analyte via gas diffusion across an in-line hydrophobic membrane followed by electrical conductivity measurement, where the change in conductivity is proportional to the inorganic analyte concentration.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. NH_4-N is subject to volatilization losses as NH_3. Due to the effect of drying on NH_3 volatilization, samples should be analyzed on an as-received basis. NH_4-N concentrations in samples that are in closed containers, refrigerated upon receipt ≤ 6 °C, and analyzed within 48 hours are assumed to be stable. Storage time should not exceed 7 days without acidification. Samples stabilized in a 1:10 KCl extraction solution (Section 6.2) may be stored at ≤ 6 °C for up to 28 days; do not freeze.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Recommended sample size for extraction is 1.0 g (s) and 10.0 mL (l). Concentrations of NH_4-N typically found in manure are sufficient to quantify content. Lower test portion sizes are acceptable.

5.0 Apparatus

5.1 Analytical balance capable of weighing to 0.01 g.

5.2 Extraction vessel with sealable cap, 100–200 mL. Polypropylene- or nitrogen-free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm), with stroke length of 0.75 or 1.0 inch.

5.4 Pipette dispenser capable of dispensing 5.0–50.0 mL.

5.5 Filter papers, highly retentive, NH_4-N free. *Note: specific filter suppliers may have significant NH_4 contamination, and content should be verified.*

5.6 Centrifuge, high-speed, capable of RCF 2400 × g for 50 mL centrifuge tubes.

5.7 Centrifuge tubes and caps, 50 mL capacity, compatible with centrifuge.

5.8 Ammonium nitrogen analysis instrument (select one):

- **5.8.1** Distillation / titration analyzer for ammonium. Manual or automated distillation apparatus unit based on distillation of NH_3 into boric acid trap with titrimetric determination of NH_4-N. Instrument detection limit of ≤ 1.0 mg L^{-1}.
- **5.8.2** Spectrophotometric continuous flow, flow injection, or discrete analyzer; or instrument of similar design capable of performing NH_4-N analysis by alkaline phenol / sodium hypochlorite or modified Bertholet reaction chemistry. Instrument detection limit ≤ 1.0 mg NH_4-N L^{-1}.
- **5.8.3** Diffusion-Conductivity Analyzer. Instrument based on the method described by Carlson (1990). Instrument detection limit ≤ 0.05 mg NH_4-N L^{-1}.

6.0 Reagents

6.1 Reagent grade water, ASTM Type I.

6.2 *For extraction of NH_4-N only. Not applicable to analysis of TKN digests.* Potassium chloride (KCl) acidified extraction solution:

- **6.2.1** 1.0 M KCl extraction solution: Dissolve 74.551 g of ACS grade KCl in 500 mL reagent grade water, add 20 mL ACS grade glacial acetic acid CH_3COOH (99%), and dilute to a 1 L final volume.

6.2.2 2.0 M KCl matrix matching solution: Dissolve 149.102 g of ACS grade KCl in 500 mL reagent grade water, add 40 mL ACS grade glacial acetic acid CH_3COOH (99%), and dilute to a 1 L final volume.

6.3 NH_4-N stock solution for calibration standards, 1000 mg NH_4-N L^{-1}. Weigh 3.819 g of dried ACS grade ammonium chloride NH_4Cl. *(Note: to maximize surface area, dry 4–5 grams in a 250 mL beaker at 104 °C for 8 hours).* Allow to cool in a desiccator to room temperature before use. Dissolve in 500 mL of reagent grade water and dilute to 1 L. Solution is stable for 6 months at ≤ 6 °C. Prepare working standards from 1.0 to 100 mg L^{-1} matrix matched in 1.0 M KCl acidified solution. Ammonium chloride 1000 mg L^{-1} standard may be commercially purchased. Alternate ammonium compounds may be used for calibration (e.g., ammonium sulfate).

6.4 NH_4-N for Independent Calibration Verification (ICV) solution.

6.4.1 NH_4-N solution, 1000 mg NH_4-N L^{-1}. Dissolve 3.819 g of ACS grade dried ammonium chloride NH_4Cl from a source other than that of the stock solution. To maximize surface area, dry 4–5 grams in a 250 mL beaker at 105 °C for 8 hours. Dissolve in 500 mL of reagent grade water and dilute to 1 L. Solution is stable for 6 months at ≤ 6 °C. Prepare two ICV solutions at the low and high end of the calibration range, e.g., to 1.0 and 10.0 mg L^{-1}, in 1 M KCl (second source) solution. Alternatively, purchase externally.

6.4.2 1000 mg NH_4-N L^{-1} certified solution. Source: Sigma Aldrich, Fisher Scientific, ASI Sensors, or any supplier who can certify content.

6.5 Distillation analysis:

6.5.1 Magnesium oxide (MgO).

6.5.2 Indicator solution. Dissolve 0.400 g of bromocresol green and 0.800 methyl red in 480 mL ethanol.

6.5.3 Boric acid (H_3BO_3). Add 280 g of reagent grade boric acid to 3000 mL reagent grade water in 4000 mL beaker. Stir and heat until dissolved. Add 164 mL of indicator solution. Mix and dilute to 14 L with reagent grade water.

6.6 Spectrophotometric analysis *(Note: Reagents and reagent concentrations may vary by instrument method and manufacturer):*

6.6.1 Phenolic solution (Choose one of two chemistry options):

- Alkaline phenol. Dissolve 83 g phenol in a 1 L Erlenmeyer flask containing 500 mL reagent grade water. While stirring, slowly add 32 g NaOH. Cool, dilute to 1 L with reagent grade water, and filter through a glass fiber filter if necessary. Store in a dark glass bottle. Use considerable caution to minimize exposure.
- Salicylic acid. Dissolve 25 g NaOH in 50 mL reagent grade water. Add 800 mL reagent grade water followed by 80 g sodium salicylate and dilute to 1 L. Store in a dark glass bottle.

6.6.2 Chlorine donor solution (Choose one of two chemistry options):

- Sodium hypochlorite solution. Dilute 500 mL NaClO containing 5.25% available chlorine to 1 L with reagent grade water, and filter through a 0.45 µm membrane filter, if necessary. Store at ≤ 6 °C.
- Sodium dichloroisocyanurate [$Na(C_3N_3O_3Cl_2)$; DIC]. Dissolve 2.0 g DIC in 800 mL reagent grade water and dilute to 1 L. DIC solution does not require refrigeration. Both NaClO and DIC offer the same sensitivity as a chlorine donor. The advantage of DIC is that it is available as a stable solid, the solution is more stable than a corresponding NaClO solution, and it does not require frequent standardization, which is particularly beneficial in an automated system (Krom et al., 1980)

6.6.3 Buffer/complexing agent. Dissolve 50.0 g disodium ethylenediamine-tetraacetate (Na_2 EDTA) and 12.5 g NaOH in 900 mL and dilute to 1 L with reagent grade water. Alternate agents may be recommended (e.g., potassium sodium tartrate, tri-sodium citrate) by instrument manufacturer.

6.6.4 Sodium nitroprusside. Dissolve 7.0 g of $Na_2Fe(CN)_5NO \cdot 2H_2O$ (alternate name: sodium nitroferricyanide) in 900 mL and dilute to 1 L with reagent grade water. Reagent is light sensitive; store in a dark glass container.

6.6.5 Follow manufacturer's instructions for preparation of reagents.

6.7 Diffusion-Conductivity analysis:

6.7.1 Boric acid (H_3BO_3), 1.0% solution. Dissolve 5.0 g of H_3BO_3 in 100 mL reagent grade water and dilute to 500 mL.

6.7.2 Ammonium hydroxide (NH_4OH), 0.01 M solution. Dissolve 10 mL of 0.0987 M NH_4OH in 80 mL reagent grade water and dilute to 100 mL.

6.7.3 Buffer solution. Dissolve 10.0 mL of 1% boric acid solution in 990 mL of reagent grade water. Using a pH meter adjust pH to between 6.9–7.0 drop wise with 0.01 M NH_4OH solution. Dilute to 1 L with reagent grade water and stir to mix.

6.7.4 Sodium hydroxide (NaOH), 10.0% solution. Dissolve 200 mL of a 50% NaOH solution in 800 mL reagent grade water, stir, and dilute to 1.0 L final volume.

6.8 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to that of the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

Note: For measurement of NH_4-N in TKN digest, proceed directly to step 7.2.

7.1 Extract NH_4-N from the manure matrix.

7.1.1 Semi-solid and solid waste. Weigh 1.0–5.0 ± 0.05 g of sample into a bottle and dilute with 10–50 mL of KCl extraction solution. The ratio of KCl to sample size should be 10:1. Shake the capped bottle at the mid-oscillation or excursions range for a minimum of 30 minutes. After shaking, clarify extract to remove turbidity using filtration and/or by centrifugation.

7.1.2 Filter 20–50 mL of liquid waste material through a highly retentive filter to remove particulate matter that may clog the instrument probes or tubing. Thicker samples should first be centrifuged at 4000 rpm for 10 minutes to settle organic particulates, and the supernatant filtered through a filter. In order matrix match, dilute filtered extract 1:1 (v/v) with 2 M KCl to achieve final extract KCL concentration of 1.0 M KCl.

7.2 Initialize and calibrate the instrument according to manufacturer instructions. Follow protocols outlined in manufacturer instructions and Section 9.0 for measurement of NH_4-N in extracts and digests.

7.3 Samples with NH_4-N concentrations exceeding the highest calibration standard should be diluted using the same mixed ratio of 1.0 M KCl extraction solution.

8.0 Calculations and Reporting

8.1 Calculations. Instrument data is converted by the operating system to extract/digest concentration in mg L^{-1}.

8.1.1 Solid and semi-solid manure. Results are converted to mg kg^{-1} on an as-received basis, as follows:

$$NH_4\text{-}N, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:

C = extract/digest result, mg NH_4-N L^{-1}

V = extract/digest volume, mL

M = sample mass, g

DF = additional dilution factor, when applicable

8.1.2 Liquid manure. Results are converted to mg L^{-1} on an as-received basis as follows:

$$NH_4\text{-}N, mg\ L^{-1} = C \times DF$$

where:

C = extract/digest result, mg NH_4-N L^{-1}

DF = additional dilution factor, when applicable

8.2 Client reports. Report all values to 0.01% or 1 mg NH_4-N L^{-1} on an as-received basis to three significant digits. Alternative reporting units: solid manure as mg NH_4-N kg^{-1} and/or lb ton^{-1}; liquid manure as mg NH_4-N L^{-1} and/or lb 1000 gal^{-1} or lb $acre\text{-}inch^{-1}$. Any NH_4-N concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of six calibration standards for every batch. The calibration range is specific to the analysis method, but a calibration range of 0.5–50.0 mg NH_4-N L^{-1} will include most manure samples. A higher calibration in the range of 50.0–250.0 mg NH_4-N L^{-1} may be necessary for some samples. Any extract concentration above the highest calibration standard should be diluted with 1 M KCl acetic acid extractant and rerun. The lowest standard should ≤LOQ. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration.*

9.2 Calibration Blank (CB). CB should be analyzed after calibration and every 10 samples. *Acceptance criteria: The matrix-matched NH_4-N concentration must be lower than the MDL. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination is eliminated.*

9.3 Method Blank (MB). After the CB, run one MB with each batch of 20 or fewer samples. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, fil-

ters, or apparatus. *Acceptance criteria: Background NH_4-N should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until the source of contamination is eliminated.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured NH_4-N concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed CCV.*

9.5 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured NH_4-N concentration according to Appendix A: Definitions. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.6 Duplicate. Analyze a minimum of one duplicate sample one per analytical batch of 20 or fewer samples and calculate the RPD. The duplicate should be separated in the batch from the first sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference (RPD). *Acceptance criteria: An RPD of ≤ 20% should be routinely achievable for NH_4-N when the analyte concentration is greater than the LOQ. The operator may select samples for duplicate analysis that are expected to exceed to the LOQ, or may consider the use of a statistical test such as Relative Standard Deviation (RSD), also known as the Coefficient of Variation (CV).*

9.7 Matrix Spike Recovery (MSR). Run one MSR with each batch of 20 or fewer samples. The MSR evaluates precision, matrix interferences, and extraction efficiency. A spike of known NH_4-N concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background NH_4-N, but should not exceed the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the MDL is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the NH_4-N spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate percent MSR according to Appendix A: Definitions: MSR. *Acceptance criteria: If recovery of NH_4-N falls outside the control limits of 100 ± 15%, NH_4-N is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.8 Reference Material (RM). An RM with a matrix similar to that of materials tested should be analyzed with every batch of 20 or fewer samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure NH_4-N that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two

Table M-4.2.1. Quality control guidelines for the NH_4-N distillation, spectrophotometric, and diffusion-conductivity methods.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Calibration Blank (CB)	After calibration, every 10 samples	NH_4-N < MDL
Method Blank (MB)	Every batch	NH_4-N < LOQ
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 10% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%
Reference Material	Every batch	Within uncertainty limits

Table M-4.2.2. Precision data for manure NH_4-N as-received basis, distillation- titration method.

Sample ID	Total Solid Content (%)	Mean Content mg NH_4-N kg^{-1}	Intra-lab Repeatability Standard Deviation S_r mg NH_4-N kg^{-1}	Intra-lab Repeatability r mg NH_4-N kg^{-1}	Inter-lab Reproducibility standard deviation S_R mg NH_4-N kg^{-1}	Inter-lab Reproducibility R mg NH_4-N kg^{-1}
M 2014-D	3.16	278	3.3	9.8	35	105
M 2015-D	11.2	407	7.2	21.6	118	354
M 2012-B	56.5	8474	26.7	80.1	465	1398
M 2016-F	68.4	1218	21.5	64.5	366	1098

MAP Program, 19–23 laboratories reporting.

Table M-4.2.3. Precision data for manure NH_4-N as-received, spectrophotometric method.

Sample ID	Total Solid Content (%)	Mean Content mg NH_4-N kg^{-1}	Intra-lab Repeatability Standard Deviation S_r mg NH_4-N kg^{-1}	Intra-lab Repeatability r mg NH_4-N kg^{-1}	Inter-lab Reproducibility standard deviation S_R mg NH_4-N kg^{-1}	Inter-lab Reproducibility R mg NH_4-N kg^{-1}
M 2014-D	3.16	247	2.4	7.1	80	241
M 2015-D	11.2	444	4.0	11.9	65	194
M 2012-B	56.5	6750	82.0	246	1001	3003
M 2016-F	68.4	1089	5.6	16.9	114	342

MAP Program, 21–24 laboratories reporting.

Table M-4.2.4. Precision data for manure NH_4-N as-received, diffusion-conductivity method.

Sample ID	Total Solid Content (%)	Mean Content mg NH_4-N kg^{-1}	Intra-lab Repeatability Standard Deviation S_r mg NH_4-N kg^{-1}	Intra-lab Repeatability r mg NH_4-N kg^{-1}	Inter-lab Reproducibility standard deviation S_R mg NH_4-N kg^{-1}	Inter-lab Reproducibility R mg NH_4-N kg^{-1}
M 2014-D	3.16	269	8.5	25.5	46	138
M 2015-D	11.2	428	7.0	22.0	46	139
M 2012-B	56.5	7428	73.2	220	1035	3105
M 2016-F	68.4	1281	29.3	87.8	144	431

MAP Program, 9 laboratories reporting.

rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

9.10 Analyte carry-over. Carryover should be determined for NH_4-N for the spectrophotometric and diffusion-conductivity methods based on the procedure outlined in Appendix A. *Perform as needed or during preventive maintenance.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). The Method Detection Limit (MDL) shall be determined based on the method described by the US EPA in Part 136, described in Appendix B. The LOQ shall be calculated by the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. NH_4-N method repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program, coordinated by the Minnesota Department of Agriculture, from proficiency rounds conducted in 2012–2016. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained for the spectrophotometric method (Table M-4.2.2), the distillation/titration method (Table M-4.2.3), and the diffusion-conductivity method (Table M-4.2.4).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear safety glasses with side shields, chemical resistant gloves, and lab coat or apron.

11.2 Caution. Phenol is mutagenic and acutely toxic and corrosive to skin, lungs, and eyes. Use extreme caution to limit exposure.

11.3 Reagents Disposal. Phenol is damaging to the environment and should be treated as hazardous waste. Dispose of other reagents in accordance with local/state and/or federal regulations.

11.4 Samples Disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

American Public Health Association (APHA). (2012). *Standard methods for the examination of water and waste water* (22nd Ed.).

Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. 4500-n nitrogen In: Lipps, W.C., Baxter, T.E., Braun-& Howland, E., Eds. Standard methods for the examination of water and wastewater. APHA Press. DOI: 10.2105/SMWW.2882.086

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U. S. Environmental Protection Agency. (2001). Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses. Chapter 2. Document # EPA-823-B-01-002.

U. S. Environmental Protection Agency. (2001). Method 1689. Ammonia-N in water and biosolids by ion-selective electrode potentiometry with preliminary distillation.

U. S. Environmental Protection Agency. (1974). Method 350.2. Nitrogen, ammonia (colorimetric, titrimetric, potentiometric distillation procedure).

U. S. Environmental Protection Agency. (1993). Method 350.1. Methods for the chemical analysis of water and wastes. Ammonia nitrogen by semi-automated colorimetry.

U. S. Environmental Protection Agency. (1993). Method 351.2. Determination of total Kjeldahl nitrogen by semi-automated colorimetry. Revision 2.0.

M-4.3
Nitrogen, Nitrate by Cadmium or Hydrazine Reduction; Automated Spectrophotometry

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1.0 Scope and Application

This method quantitatively determines the concentration of combined nitrate (NO_3^-) and nitrite (NO_2^-) in liquid and solid animal manure and compost, using extraction with a KCl solution followed by spectrophotometric analysis with a cadmium or hydrazine reduction method. It is assumed that these samples contain negligible amounts of NO_2^-, and that combined NO_3^- and NO_2^- are therefore reported as nitrate-nitrogen (NO_3-N). NO_3-N concentrations may be requested for nutrient management planning and regulatory compliance.

2.0 Method Principle

2.1 Method Description. Nitrate and nitrite are extracted from manure using 1.0 M KCl, and concentration is determined by automated spectrophotometric colorimetry, based on either the cadmium reduction method, where nitrate is reduced to nitrite by a passage through a copperized cadmium column (US EPA 353.2; Keeney & Nelson, 1982), or the hydrazine reduction method, where nitrate is reduced to nitrite with hydrazinium sulfate catalyzed by Cu^2+, under alkaline conditions and at elevated temperature (US EPA 353.1; Kempers & Luft, 1988). The NO_2-N concentration (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with α-naphthyl-ethylenediamine dihydrochloride to form a highly-colored azo dye which is measured at 540 nm (modified Griess reaction). Instruments capable of automated analysis include flow and discrete analyzers.

2.2 Method Performance. The user should be able to attain an MDL in the range of 0.2–0.5 mg NO_3-N L^{-1} for an applicable calibration range of 0.2–50 mg NO_3-N L^{-1}. An MDL should be determined and reported individually by the laboratory upon completion of the MDL procedure outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. Filtered samples that are colored in the absorption range (540 nm) of the method can interfere. While most interferences can be removed by using in-line dialysis, dialysis reduces method sensitivity. Filtering the sample with a membrane filter can reduce some interference. Soluble organic components smaller than the membrane filter only interfere at very high concentrations (> 200 mg N L^{-1}). Alternatively, some instruments include a color blanking feature that measures the sample color with and without reagent and corrects for the absorbance effect of the sample color.

High concentrations of Ca or Mg can interfere with nitrate determinations during analysis. In the cadmium reduction method, iron, copper, and other metals can interfere and cause low results. EDTA can be used to eliminate these interferences. Samples that contain large concentrations of dissolved carbon, oil, and grease can interfere by coating the cadmium. Filtering and dilution can mitigate dissolved carbon interference, and oil and grease interference can be eliminated by extracting with an organic solvent.

2.4 Method Reporting. Nitrate content is measured as mg NO_3-N L^{-1}, calculated as mg NO_3-N kg^{-1} or mg NO_3-N L^{-1} and reported on an as-received basis as lb NO_3-N ton^{-1} for solid samples and as lb NO_3-N 1000 gal^{-1} or lb NO_3-N acre-$inch^{-1}$ for liquid manures.

2.5 Method Advantages. The method is less sensitive to high ionic strength and is faster and more precise than ion-selective electrode (ISE). Compared to the hydrazine reduction method, cadmium reduction is more suitable for saline samples. It is more widely used than the hydrazine method and, like the hydrazine method, is less costly than other methods such as vanadium (III) and enzymatic reduction. Compared to the cadmium reduction

method, hydrazine is less toxic, and less costly. The hydrazine method can measure smaller sample volumes and has higher throughput because no cadmium column is required.

2.6 Method Disadvantages. Cadmium reduction chemistry requires the preparation and monitoring of cadmium columns/coils. Cadmium is a toxic, carcinogenic, and mutagenic heavy metal, and the method requires hazardous waste disposal. While hydrazine is less toxic than cadmium, the method nevertheless utilizes hazardous chemicals that are also toxic and carcinogenic and require hazardous waste disposal. Hydrazine reduction efficiency requires careful monitoring of the heating temperature and of the quality and quantity of the hydrazine. The method is prone to contamination from filter paper, reagent water, reagents, glassware, and other sample processing apparatus. Care must be taken to avoid introduction of NO_3^- and NO_2^- contamination.

3.0 Definitions

3.1. Nitrate nitrogen (NO_3-N). The nitrogen present in the form of the nitrate ion (NO_3^-) — a salt or ester of nitric acid.

3.2 Reduction Efficiency Verification (REV). A mid-calibration range nitrite standard used to test the reaction efficiency by comparison to a nitrate standard of the same concentration.

3.3 Spectrophotometric analysis. Determination of an analyte in a liquid matrix by reaction with a chromophore and measurement of the amount of light absorbed at a specific wavelength, where the change in absorption is proportional to the analyte concentration.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Nitrate is subject to losses in the environment, primarily by biological denitrification under anaerobic conditions and by leaching. Nitrate concentrations in samples that are in closed containers, refrigerated upon receipt ≤ 6 °C, and analyzed within 48 hours (APHA, 2012) are assumed to be stable. Storage time should not exceed 7 days. Solid materials dried at > 65 °C for 24–48 hours can be stored for up to six months. *Note: If analysis includes ammonium nitrogen (NH_4-N), samples should be analyzed as-received.*

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Minimum recommended sample size is 1.0 g (solid and semi-solid) and 10.0 mL (liquid and slurry). Due to the low concentration of NO_3-N typically found in manure, sample sizes below these levels are not recommended and larger sizes may be required to accurately quantify NO_3-N concentrations.

5.0 Apparatus

5.1 Analytical balance, capable of weighing to 0.01 g.

5.2 Extraction vessels with sealable caps, 100 mL. Polypropylene or nitrogen-free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm), stroke length of 0.75 or 1.0 inch.

5.4 Pipette dispenser capable of dispensing 5.0–50.0 mL.

5.5 Filter papers, highly retentive, nitrate- and nitrite-free. *Note, specific filter suppliers may have significant NO_3 contamination, and content should be verified.*

5.6 Centrifuge tubes, 50 mL.

5.7 Centrifuge, high-speed, capable of RCF 2400 × g for 50 mL centrifuge tubes.

5.8 Spectrophotometric flow injection or discrete autoanalyzer capable of performing nitrate-nitrogen analysis based on cadmium or hydrazine reduction of NO_3-N to NO_2-N and subsequent quantification of azochromophore measured spectrophotometrically at 540 nm.

6.0 Reagents and Standards

Note: To prevent contamination, use only reagents that are labeled for nitrogen analysis.

6.1 Reagent grade water, ASTM Type I.

6.2 Potassium chloride (KCl) extraction solutions. *If also extracting for NH_4^+, may also include 2% glacial acetic acid in the extracting solution. See Method M-4.2, Nitrogen, Ammonium by Distillation, Spectrophotometry, and Diffusion-Conductivity.*

- **6.2.1** 1.0 M KCl extraction solution: Dissolve 74.551 g of ACS grade KCl in 500 mL reagent grade water, and dilute to a 1 L final volume.
- **6.2.2** 2.0 M KCl matrix matching solution: Dissolve 149.102 g of ACS grade KCl in 500 mL reagent grade water and dilute to a 1 L final volume.

6.3 NO_3-N stock solution for calibration standards and Continuing Calibration Verification (CCV) solution, 1000 mg L^{-1}. Dissolve 7.218 g KNO_3 or 6.068 g $NaNO_3$ in 500 mL and dilute to 1.0 L in reagent grade water. Preserve with 1 mL chloroform. Stable for 6 months. If no preservative is used, the solution is stable for 4 weeks. Store at ≤ 6 °C. Prepare working standards ranging from 0.5 to 50.0

mg L^{-1} matrix matched in 1 M KCl. Standard(s) may be commercially purchased.

6.4. NO_3-N stock solution for Independent Calibration Verification (ICV), 1000 mg L^{-1}. Dissolve 7.218 g of KNO_3 or 6.068 g $NaNO_3$ from a source other than that of the stock solution in 500 mL of reagent grade water and dilute to 1000 mL. Solution is stable for 6 months at ≤ 6 °C. Prepare two ICV solutions at the low and high end of the calibration range, e.g., to 1.0 and 10.0 mg L^{-1} in 1 M KCl (second source) solution.

6.5 NO_2-N stock solution for Reduction Efficiency Verification (REV), 1000 mg L^{-1}. Dissolve 6.072 g KNO_2 or 4.924 g $NaNO_2$ in 500 mL reagent grade water, dilute to 1000 mL. Solution is stable for 6 months at ≤ 6 °C. Prepare one REV solution at the mid-point of the calibration range, e.g., 10.0 mg L^{-1} in 1 M KCl solution. Because NO_2^- oxidizes readily in the presence of moisture, keep stock solution tightly stoppered and prepare working solution daily.

6.6 Copper (II) sulfate stock solution: Dissolve copper sulfate ($CuSO_4 \cdot 5H_2O$) in 1 L reagent grade water to concentration recommended by instrument manufacturer. Stable for 7 days or 12 weeks depending on concentration. Refer to instrument manufacturer recommendation.

6.7 Cadmium reduction reagents are based on US EPA 353.2. Reagents and concentrations may be instrument manufacturer specific:

6.7.1: Buffer solution: Dissolve 86 g reagent grade ammonium chloride and 1.0 g disodium ethylenediamine tetracetate (EDTA) in 800 mL reagent grade water. Adjust the pH to 8.5 with sodium hydroxide pellets as needed and dilute to 1 L. Add 0.5 mL Brij solution (30%). Store in a glass bottle. Stable for 12 weeks.

6.7.2 Color developing reagent: To approximately 800 mL reagent grade water, add, while stirring, 100 mL concentrated phosphoric acid (H_3PO_4 ; 85%), 40 g sulfanilamide ($C_6H_8N_2O_2S$), and 1.0 g N-(1-naphthyl)-ethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$). Stir until dissolved and dilute to 1 L. Store in brown glass bottle and keep in the dark when not in use. Stable for 4 weeks.

6.7.3 Copper-cadmium column: cadmium initially cleaned with dilute HCI, then flushed with 2% copper(II) sulfate solution (20 g $CuSO_4 \cdot 5H_{20}$ in 1 L) for five minutes for until the blue color fades and followed with 10 reagent grade water rinses or until the cadmium turns black.

6.8 Hydrazine reduction reagents are based on US EPA 353.1. Reagent and concentrations may be specific to the instrument manufacturer.

6.8.1: Buffer solution: Dissolve 33 g potassium sodium tartrate ($C_4H_4O_6KNa \cdot 4H_2O$) and 24 g tri-sodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$) in 900 mL reagent grade water and dilute to 1 L. Add 3 mL Brij 35 (30%) solution. Solution is stable for 1 week. Store at ≤ 6 °C.

6.8.2 Color developing reagent: To approximately 500 mL reagent grade water add 150 mL concentrated phosphoric acid (H_3PO_4 85%) and 10 g sulfanilamide ($C_6H_8N_2O_2S$), followed by 0.5 g N-(1-Naphthyl) ethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$). Dilute the solution to 1 L with reagent grade water. Store in brown glass bottle and keep in the dark when not in use. Solution is stable for 2 weeks.

6.8.3 Hydrazinium sulfate solution: Dissolve 2.5 g hydrazinium sulfate ($N_2H_6SO_4$) in 900 mL reagent grade water. Add 1.5 mL 1.2% copper (II) sulfate stock solution and dilute to 1 L. Solution is stable for 1 week.

6.8.4 Sodium hydroxide (NaOH) solution: Dissolve 6 g NaOH in I L reagent grade water. Add 3 mL Brij 35 (30%). Stable for one week. Store at ≤ 6 °C.

6.9 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Extract NO_3-N from sample matrix.

7.1.1 Semi-solid and solid waste. Weigh 1.0–5.0 ± 0.05 g of sample into a bottle and dilute with 10–50 mL of KCl extraction solution. The ratio of KCl to sample size should be 10:1. The capped bottle is shaken at the mid-oscillation or excursions range for a minimum of 30 minutes. After shaking, clarify extract to remove turbidity using filtration or by centrifugation.

7.1.2 Filter 10–50 mL of liquid waste material through a highly retentive filter to remove particulate matter that may clog the instrument probes or tubing. Thicker samples should first be centrifuged at 4000 rpm for 10 minutes to settle organic particulates and filter the supernatant. In order matrix match, dilute filtrated extract 1:1 in 2 M KCl.

7.3 Initialize and calibrate instrument by flow or discrete analysis instrument according to manufacturer instructions. Follow protocols outlined in Section 9.0. Transfer the filtrate to sample tubes and place in an autosampler tray in preparation for analysis.

7.4 Samples with concentrations exceeding the highest calibration should be diluted in reagent grade water matrix matched to 1 M KCl acidified extraction solution.

8.0 Calculations and Reporting

8.1 Calculations. Absorbance data are converted by the operating system to extract concentration in mg NO_3-N L^{-1}.

8.1.1 Solid and semi-solid manure. Extract solution data are converted to mg kg^{-1} on an as-received basis as follows:

$$NO_3\text{–}N, mg\ kg^{-1} = \frac{C \times V}{M} \times DF$$

where:
C = extract result of NO_3-N, mg L^{-1}
M = sample mass (g) used in the extraction
V = extract volume, mL
DF = dilution factor, when applicable

8.1.2 Liquid manure. Extract solution data are converted to mg L^{-1} as follows:

$$NO_3\text{–}N, mg\ L^{-1} = C \times DF$$

where:
C = extract result of NO_3-N, mg L^{-1}
DF = dilution factor, when applicable

8.2 Client reports. Report all values to 0.1 mg kg^{-1} or mg L^{-1} as NO_3-N on an as-received basis to three significant digits. Report solid manure as mg kg^{-1} and/or lb ton^{-1}. Report liquid manure as mg L^{-1} and/or lb 1000 gal^{-1} or lb acre-$inch^{-1}$. Any NO_3-N concentration below the LOQ is reported as < LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of six calibration standards. The calibration range is specific to the autoanalysis method, but a calibration range of 0.5–50.0 mg NO_3-N L^{-1} will include most manure samples. A higher calibration in the range of 50.0–250.0 mg NO_3-N L^{-1} may be necessary for some samples. Any extract concentration above the highest calibration standard should be diluted with 1M KCl and rerun. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration, otherwise perform maintenance and recalibrate.*

9.2 Calibration Blank (CB). After calibration and every 10 samples a zero standard CB should be analyzed. *Acceptance criteria: The matrix-matched NO_3-N concentration must be lower than the MDL. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until the source of contamination is eliminated.*

9.3 Method Blank (MB). After the CB, one MB consisting of 1 mL reagent grade water and 25 mL 1M KCl per batch should be analyzed. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, filters, or apparatus. *Acceptance criteria: Background NO_3-N should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If the second MB passes, calibration is verified. If the second MB fails, halt analysis until the source of contamination is eliminated.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured NO_3-N concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed.*

9.5 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and as-measured NO_3-N concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10% PE. If criteria is exceeded, re-analyze ICV. If a second ICV passes, calibration is verified. Otherwise, recalibrate with new standards and re-analyze all samples since the last valid ICV.*

9.6 Reduction Efficiency Verification (REV). To detect problems with reduction efficiency, a mid-calibration range nitrite should be included daily. The conversion of nitrate to nitrite is dependent on the quality and quantity of the cadmium or hydrazine and, in the case of hydrazine, on the heat supplied to the reaction. Inefficiencies in the temperature or concentrations during the reaction will cause reduced conversion. The mid-range nitrite REV follows a mid-range nitrate CCV of the same concentration, and the reduction efficiency is calculated as:

$$Efficiency, \% = \frac{NO_3\text{–}N\ response}{NO\text{–}N\ response} \times 100$$

Acceptance criteria: The percent efficiency between the NO_2-N and NO_3-N standards must be > 80%. If not, correct the problem with one or more of the following approaches: a) replace the cadmium reduction column with a new column or reactivate the cadmium granules by following the procedure in Section 6.7.3, b) make new solutions of the copper sulfate catalyst or the hydrazine sulfate reactant, and c) test the heating element in the hydrazine reaction system to verify that it is reaching the required temperature.

9.7 Duplicate. Due to the typically low NO_3-N concentrations in manure, duplicate analysis may be of limited value for this analyte. An RPD of ≤ 10% is difficult to obtain as concentrations approach the LOQ and may offer little benefit for quality control. For this reason, comparison of a spiked to an unspiked duplicate (MSR) is a more useful measure of precision in NO_3-N analysis than comparison of two unspiked duplicates.

9.8 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch of 20 or fewer samples. Used to evaluate precision, matrix interferences, and extraction efficiency, a spike of known NO_3-N concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background NO_3-N but should not exceed the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the MDL is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the NO_3-N spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate MSR according to Appendix A: Definitions; Matrix Spike Recovery. *Acceptance criteria: If recovery of NO_3-N falls outside the control limits of 100 ± 15%, NO_3-N is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure NO_3-N that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

9.10 Analyte Carryover. Manure NO_3-N concentrations are typically low enough that carryover effects are negligible. Where this is not the case, refer to Chapter M-4.2, Nitrogen, Ammonium (NH_4-N) by Distillation, Spectrophotometry, and Diffusion-Conductivity.

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). The Method Detection Limit (MDL) shall be determined based on the method described by the US EPA in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. NO_3-N spectrophotometric repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program coordinated by the Minnesota Department of Agriculture, in proficiency rounds conducted on 10 laboratories in 2012 and 2016. Laboratories utilized instruments from different manufactur-

Table M-4.3.1. Quality control guidelines for the NO_3-N method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Calibration Blank (CB)	After calibration, every 10 samples	NO_3-$N < MDL$
Method Blank (MB)	Every batch	NO_3-$N < LOQ$
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Reduction Efficiency Verification (REV)	Every batch	Recovery, 100 ± 10%
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%
Reference Material	Every batch	Within uncertainty limits

Table M-4.3.2. Laboratory precision data for manure as-received basis, NO_3-N method.

Sample ID	Total Solids Content (%)	Mean Content mg NO_3-N kg^{-1}	Intra-Lab Standard Deviation S_r mg NO_3-N kg^{-1}	Intra-Lab Repeatability r mg NO_3-N kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg NO_3-N kg^{-1}	Inter-Lab Reproducibility R mg NO_3-N kg^{-1}
M 2014-D	3.2	1.3	0.07	0.5	1.0	3.1
M 2015-D	11.2	2.6	0.15	0.8	2.1	6.3
M 2012-B	56.5	165	1.5	234	44	132
M 2016-F	68.4	507	9.4	74	143	430

MAP Program, 19–22 laboratories reporting.

ers, and the following precision data were obtained (Table M-4.3.2).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear safety glasses with side shields, chemical resistant gloves, and lab coat or apron. Hydrazinium sulfate and phosphoric acid are corrosive. Sodium nitrate is an oxidizer. Ammonium chloride is an irritant.

11.2 Caution. Cadmium and hydrazinium sulfate are carcinogenic and, in the case of cadmium, mutagenic. Minimize human exposure.

11.3 Reagent Disposal. Reagents should be treated as hazardous waste and disposed of according to state requirements.

11.4 Sample Disposal. Sample extracts should be treated as hazardous waste and disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

APHA. (2017). *Standard methods for examination of water and wastewater*, (23nd ed.). Method 4500-NO3- E-F. American Public Health Association.

Bremmer, J. M., & Keeney, D. R.. (1965). Determination and isotopic ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation for and determination of ammonium. *Proceedings – Soil Science Society of America,* 29:504–507.

Dorich, R. A., & Nelson, D. W. (1984). Evaluation of manual cadmium reduction methods for determination of nitrate in potassium chloride extracts of soils. *Proceedings – Soil Science Society of America,* 48:72–75.

U. S. Environmental Protection Agency (1978). Method 353.1, Methods for chemical analysis of water and wastes: Nitrogen, nitrate-nitrite (colorimetric, automated hydrazine reduction). https://www.nemi.gov/methods/method_summary/5250/

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FiaLab. Method for nitrate determination by cadmium reduction, Version 2.3.

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Mulvaney, R.L. (1996). Extraction of exchangeable ammonium and nitrate and nitrite. In D. L. Sparks, Ed.), Methods of Soil Analysis Part 3 - Chemical Methods. Number 5. Soil Science Society of America.

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M-5.1
Digestion & Dissolution for Elemental Analysis

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Introduction

The objective of these methods is to remove the organic matrix and solubilize elements of interest in preparation of analysis. There are numerous procedures that can be used (Bock, 1979), but for the purpose of this manual, methods deemed accurate, reproducible, and safe are recommended for routine use. Briefly, these methods include: i) the addition of concentrated acid and hydrogen peroxide to the sample followed by heating (US EPA, 1996); ii) the addition of concentrated acid to the sample in a sealed vessel and heating with microwave energy (US EPA, 2007); or iii) heating the sample in a high-temperature muffle furnace under normal atmospheric oxygen conditions and the addition of acid to the resulting ash (Isaac, 1998). Digestion can take place in open or closed vessels. Closed vessels are preferred if volatile elements or potential cross contamination is a concern.

While each of these methods will approximate a total digestion of most elements in most manures and organic waste residuals, none will effectively solubilize silicate minerals. To determine the "total" element constituents in manures with significant silicate minerals, further treatment is necessary to dissolve silica complexes which prevent complete dissolution of some elements (US EPA, 1996). Those methods are beyond the scope of this manual.

Selection of the appropriate method depends on several factors, including available laboratory equipment, sample type, element(s) of interest, gaseous fume removal, contamination considerations, and necessary safety precautions. These methods have been used routinely for the analysis of plant tissue, compost, and other organic matrices (Jones and Case, 1990; Thompson, et al., 2001; Bock, 1979).

Reference Documents

Bock, R. (1979). *A handbook of decomposition methods in analytical chemistry*. International Textbook Publishers.

Isaac, R. A. (1998). AOAC Method 985.01. Metals and other elements in plants. In Cunniff (Ed.), Official methods of analysis of AOAC International (16th Ed.) AOAC International.

Jones, J. B., Jr., & Case, V. W. (1990). Sampling, handling, and analyzing plant tissue samples. p. 389-427. In Westerman, R. L. (Ed.), *Soil testing and plant analysis*. No. 3. American Book Series.

Thompson, W. H., Leege, P. B., Millner, P. D., & Watson, M. E. (Eds.) (2001). Test methods for the examination of composting and compost. U. S. Composting Council.

U. S. Environmental Protection Agency. (1996). Method 3050B: Acid digestion of sediments, sludges, and soils, Revision 2.

U. S. Environmental Protection Agency. (2007). Method 3051A: Microwave assisted acid digestion of sediments, sludges, and soils. Revision 1.

M-5.1a Microwave-Assisted Digestion, Closed Vessel

1.0 Scope and Application

This method is based on destruction of the organic matrix of manure by nitric acid digestion in a closed vessel via microwave heating for the subsequent quantitative determination of P, K, Ca, Mg, S, Zn, Mn, Fe, B, Cu, and Mo (Stripp and Bogen, 1989). The method is also suitable for the preparation of samples for determination of additional analytes including US EPA 503 metals As, Cd, Hg, Pb, Ni, and Se. This method does not address the dissolution of silicate minerals that may prevent the complete dissolution of some elements.

2.0 Method Principle

2.1 Method Description. This method is adapted from US EPA method 3051A. A representative manure sample is digested in concentrated nitric acid using microwave heating with a suitable laboratory microwave unit. The sample and acid are placed in a fluorocarbon (PFA or TFM) microwave vessel. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle, and then diluted

to volume and analyzed by methods described in Chapter M-5.2 or M-5.3. This method is ideally suited to elemental constituents that may be lost through volatilization in open vessel digestion.

2.2 Method Performance. Not applicable.

2.3 Method Interferences. Not applicable.

2.4 Method Reporting. Following analysis of digested sample, the concentration of analytes is reported as a mass fraction (% or mg kg^{-1}) of the as-received sample. Results may also be reported as a mass fraction on a dry-weight basis if the total solids content is determined on a parallel subsample.

2.5 Method Advantages. Volatile elements are retained as digestion is performed in closed vessels; minimal potential for cross-contamination of samples; rapid digestion time; minimal handling of sample is required; relatively safe procedure.

2.6 Method Disadvantages. Costly equipment (laboratory microwave and vessels) required; relatively small number of samples can be handled at one time; the process for preparing digestion vessels and then transferring samples from vessels to final containers is tedious and labor intensive; concentrated acids are required; potential sample loss if venting of vessel occurs during digestion.

3.0 Definitions

Not applicable.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample storage. Store samples in pre-washed plastic or glass containers at ≤ 6 °C. Samples should be digested as soon as possible, but no longer than 14 days after sample receipt. Sample digests may be held for up to 6 months.

4.2 Sample Processing. Subsample at least 200 cm^3 of solid (> 20% solids) and semi-solid (10–20% solids) primary samples and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Solid, semi-solid, and slurry samples may be analyzed as-received or after drying and grinding. Slurry (4–10% solids) and liquid samples do not generally require additional homogenization but, where necessary, refer to Chapter S-2.0. Solid, semi-solid, and slurry samples may be analyzed as-received or after drying and grinding. Liquid samples should be run as-received, without drying.

4.3 Sample Test Portion. Digestion is performed on a 0.50–5.0 g of solid and semi-solid sample. For liquid samples, the test portion for most manures (e.g., lagoons) should be 10–15 g.

4.4 Total Solids Content. If sample was analyzed as-received and dry weight concentrations are needed for reporting, determine total solids content according to Chapter M-1.1.

5.0 Apparatus

5.1 Microwave digestion system — commercially designed for laboratory use with minimum power delivery of 600 watts and with digestion vessel pressure and temperature monitoring. Microwave unit cavity must be corrosion resistant and well-ventilated, with electronics protected against corrosion for safe operation. Unit should have a rotating turntable or other design features to ensure homogeneous distribution of microwave radiation within the unit.

> ***Caution:*** Kitchen-type microwave ovens must not be used for this method due to safety concerns with acid corrosion and pressure buildup.

5.1.1 Microwave calibration: If the microwave digestion system uses temperature feedback control to meet the performance specifications of the method, performing a calibration procedure is not necessary. If calibration is needed, follow instrument manufacturer's recommendations or the procedure described in US EPA method 3051 (US EPA, 2007).

5.2 Digestion vessel with liner and safety membranes Fluorocarbon (PFA or TFM) digestion vessels, 100–120 mL capacity, capable of withstanding pressures up to 7.5 ± 0.7 atm (110 ± 10 psi) and capable of controlled pressure relief at pressures exceeding 7.5 ± 0.7 atm (110 ± 10 psi). Inclusive of microwave temperature and/or pressure monitoring system with limit control.

5.2.1 All digestion vessels, as well as glass and plasticware, must be carefully acid washed and rinsed with reagent grade water. If performing trace element or metal analysis, a more extensive washing procedure should be performed.

5.3 Volumetric dispenser, acid resistant, capable of delivering 10 mL.

5.4 Volumetric flasks, 100 mL, or graduated disposable polypropylene resin digestion cups with certified volume accuracy within ASTM 1272-02 Class A tolerance.

5.5 Plastic storage bottles.

5.6 Filter funnels of glass, plastic, or disposable polypropylene.

5.7 Whatman No. 40 or 41 filter paper, or equivalent retentive filter.

5.8 Acid digestion fume hood, minimum draft 150 cfm.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM type 1.

6.2 Boiling chips. PTFE chips used for method blank and lab fortified blank (e.g., Chemware PTFE Ultra-Pure Boiling Stones Part # 26397-103).

6.3 Concentrated nitric acid (HNO_3), 16 M. Trace metal grade if trace elements are of interest.

7.0 Procedure

7.1 Calibrate microwave digestion unit. If the microwave unit uses temperature feedback control capable of replicating the performance specifications of the method, the calibration procedure may be omitted. Otherwise, follow the calibration procedure recommended by US EPA (2007).

7.2 Weigh a well-mixed sample to the nearest 0.001 g into a fluorocarbon sample vessel equipped with a single ported cap and pressure relief valve. Adjust sample size to obtain a sample of 0.10–0.50 g of solids.

7.2.1 For solid or semi-solid samples, weigh 0.100–5.000 g of sample into the vessel to achieve a final sample size of 0.1–0.5 g of solids. Record sample weight. Bring all samples up to approximately 5 mL to ensure equal sample mass of vessels during the microwave digestion.

7.2.2 For liquid samples, weigh 10–15 g of the sample into the vessel. Record sample weight.

7.3 In a fume hood, add 10 mL of concentrated nitric acid to the sample. Cap the vessel and torque the cap according to the unit manufacturer's directions. Weigh the vessels, with sample plus acid, to the nearest 0.001 g and place in the microwave carousel.

Caution: Toxic nitrogen oxide fumes are usually produced during digestion; all work must be performed in a properly operating fume hood. The analyst should also be aware of the potential for a vigorous reaction between the sample and the digestion acid. If a vigorous reaction occurs, allow to cool before capping the vessel.

Caution: When digesting samples containing volatile or easily oxidized organic compounds, initially weigh a sample of no more than 0.1 g dry matter and observe the reaction before capping the vessel. If a vigorous reaction occurs, allow the reaction to cease before capping the vessel. If no appreciable reaction occurs, a sample weight containing up to 0.5 g of dry matter can be used.

7.4 It is important that all vessels in the microwave contain the same volume of liquid. Consequently, samples of liquid manure containing a final vessel volume of 30 mL (20 mL of sample plus water and 10 mL of nitric acid) should be digested with other liquid manures. Samples of semi-solid or solid manures with a final vessel volume of 15 mL (5 mL of sample plus water and 10 mL of nitric acid) should be digested with other semi-solid and solid manures.

7.5 When fewer than the full complement of samples are to be digested, the remaining vessels should be filled with 20 mL of water and 10 mL of nitric acid (when digesting liquid manures) or with 5 mL of water and 10 mL of nitric acid (when digesting semi-solid or solid manures) to achieve the full complement of vessels. This provides an energy balance, since the microwave energy absorbed is proportional to the total mass in the cavity.

7.6 Irradiate each group of samples for 12–17 minutes. The temperature of each sample should rise to 175 °C in less than 6–7 minutes and remain between 170–180 °C for the balance of the 12- to 17-minute period. While the original US EPA 3051A procedure called for a 10-minute digestion time (up to 175 °C in less than 5.5 minutes and between 170–180 °C for the balance of the 10 minutes), this procedure was developed for samples containing a total final volume of 10 mL. Because of the larger sample volume when digesting manures, a longer digestion period may be required in some microwaves to reach the temperature specified and to ensure complete digestion of all samples.

Note: The pressure should peak at less than 6 atm for most samples. The pressure will exceed these limits in the case of high concentrations of carbonate or organic compounds. In these cases, the pressure will be limited by the relief pressure of the vessel to 7.5 ± 0.7 atm (110 ± 10 psi). All vessels should be sealed according to the manufacturer's recommended specifications.

7.7 At the end of the microwave program, allow the vessels to cool for a minimum of 5 minutes before removing them from the microwave unit. When the vessels have cooled to room temperature, weigh and record the weight of each vessel assembly. If the weight (recorded in 7.3) has decreased by more than 10% from the original weight, determine the reason for the weight loss and repeat the digestion process following steps 7.2–7.7.

7.8 Complete the preparation of the sample by carefully uncapping and venting each vessel in a fume hood. Transfer the sample to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with the injection of the sample into the instrument, the sample may be centrifuged, allowed to settle, or filtered.

7.8.1 Settling: Allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sam-

ple. Dilute to final volume with reagent grade water.

7.8.2 Centrifugation: Centrifugation at 1,500–2,000 g for 10 minutes is usually sufficient to clear the supernatant. Dilute to a final volume with reagent grade water.

7.8.3 Filtering: Filter the sample through Whatman 40, 41, or equivalent filter paper into 100 ml volumetric flasks. Dilute to a final volume with reagent grade water.

7.9 The digest is now ready for analysis for elements of interest by methods described in Chapter M-5.2 or M-5.3. Ensure that the samples and standards are matrix-matched, 10% v/v HNO_3.

8.0 Calculations and Reporting

8.1 Calculations. Following analysis of the digestated sample, the concentration of analytes are reported on the basis of the actual weight of the original sample. Dilutions must be included when calculating final results. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

8.2 Client Reports. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

9.0 Quality Control

Note: For additional quality control criteria associated with this method, see Chapters M-5.2 and M-5.3.

9.1 Method Blank (MB). Carry a MB consisting of 0.5 g PTFE boiling chips through the entire digestion procedure. Include one MB with each batch of 20 or fewer samples. *Acceptance criteria: The concentration of analytes of interest should be less than the LOQ.*

9.2 Laboratory fortified blank (LFB). Prepare an LFB by adding a known quantity of analyte(s) of interest to a MB. Include one LFB with each batch of 20 or fewer samples. *Acceptance criteria: Recovery of analyte(s) of interest should be 100 ± 15%.*

9.3 Duplicate. Digest and analyze one sample duplicate per batch of 20 or few samples. Calculate the RPD according to Appendix A. *Acceptance criteria: The RPD for analytes of interest should be ≤ 10 to 20%, depending on analyte.*

9.4 Matrix Spike Recovery (MSR). Prepare a matrix spike by adding a known quantity of analyte(s) of interest to an unknown duplicate sample. Include one MSR with each batch of 20 or fewer samples. *Acceptance criteria: Recovery should be 100 ± 15% for macro elements and 100 ± 25% for micro elements or as dictated by regulatory agencies.*

9.5 Reference Material (RM). An RM with a matrix similar to that of materials tested should be obtained to assess the accuracy and overall reliability of the analytical process and analyzed with each batch of 20 or fewer samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.6 Proficiency Requirement. Laboratories performing this method shall participate in a single blind proficiency testing program at least once per year for a matrix type similar to manure and in which the analytes of interest are included as test parameters. A recommended program for this purpose is the Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) program. If the laboratory fails to successfully analyze an analyte of interest on the proficiency test sample, it shall determine the cause for the failure and take corrective action. The laboratory will document the corrective action taken for future reference.

10.0 Precision and Accuracy

10.1 See determinative methods for additional information (Chapters M-5.2 and M-5.3).

11.0 Safety and Disposal

11.1 Laboratory Safety. Use nitric acid in a fume hood during digestion. During nitric acid digestion, wear safety glasses, lab coat, chemical-resistant gloves, apron, and face shield. Review safety procedures related to microwave digestion system operation before using.

11.2. Caution. Concentrated nitric acid is extremely irritating to skin and mucous membranes and can cause severe burns to skin. If skin contact occurs, flush with large volumes of water. Concentrated nitric acid is a Category 3 oxidizer and can cause fire when in contact with combustible material.

11.3 Reagents Disposal. Reagents should be treated as hazardous waste and disposed of according to state requirements. Reagents and spills should be neutralized with a suitable compound, e.g., sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), or limestone ($CaCO_3$).

11.4 Samples Disposal. Digests should be neutralized with a suitable compound, e.g., Na_2CO_3, $NaHCO_3$, or $CaCO_3$ before disposal. Digests should be disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

Kingston, H. M., & Jassie, L. B. (1986). Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Annals of Chemistry,* 58:2534–2541.

Sah, R.N., & Miller, R. O. (1992). Spontaneous reaction for acid dissolution of biological tissue in closed vessels. *Annals of Chemistry,* 64:230–233.

Stripp, R. A., & Bogen, D. 1989. The rapid decomposition of biological materials by using microwave acid digestion bomb. *Journal of Analytical Toxicology,* 13:57–59.

U. S. Environmental Protection Agency. (2007). Method 3051A: Microwave-assisted acid digestion of sediments, sludges, and soils. Revision 1.

M-5.1b Nitric and Hydrochloric Acid Digestion with Peroxide, Open Vessel

1.0 Scope and Application

This method is based on the acid dissolution of the organic matrix of manure by digestion via external heating with the subsequent quantitative determination of P, K, Ca, Mg, Na, S, Zn, Mn, Fe, B, Cu, and Mo. The method is also suitable for preparation of samples for determination of additional analytes, including US EPA 503 metals As, Cd, Pb, Ni, and Se. This method does not address the dissolution of silicate minerals that may be in the sample and may prevent the complete dissolution of some elements.

2.0 Method Principle

2.1 Method Description. This method was adapted from US EPA Method 3050B. A representative subsample containing up to 0.5 g dry matter is digested in nitric acid and hydrogen peroxide solution with heat. The digest is then refluxed with nitric and hydrochloric acid. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle, diluted to volume, and analyzed by methods described in Chapter M-5.2 or M-5.3.

2.2 Method Performance. Not applicable.

2.3 Method Interferences. Not applicable.

2.4 Method Reporting. Following analysis of digested sample, the concentration of analytes is reported as a mass fraction (% or mg kg^{-1}) of the as-received sample. Results may also be reported as a mass fraction on a dry-weight basis if the total solids content is determined on a parallel subsample.

2.5 Method Advantages. Equipment required is relatively inexpensive and easy to use; process can be automated and a large number of samples can be digested simultaneously.

2.6 Method Disadvantages. Requires a fume hood, lengthy digestion time, and concentrated acids; potential for cross-contamination of samples during refluxing; volatile elements may be lost in the digestion process.

3.0 Definitions

Not applicable.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample storage. Store samples in pre-washed plastic or glass containers at ≤ 6 °C. Samples should be digested as soon as possible, but no longer than 14 days after sample receipt. Sample digests may be held for up to 6 months.

4.2 Sample Processing. Subsample at least 200 cm^3 of solid (> 20% solids) and semi-solid (10–20% solids) primary samples and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid samples do not generally require additional homogenization but, where necessary, refer to Chapter S-2.0. Solid, semi-solid, and slurry samples may be analyzed as-received or after drying and grinding. Liquid samples should be run as-received, without drying.

4.3 Sample Test Portion. Digestion is performed on a 0.50–5.0 g of solid and semi-solid sample. For liquid samples, the test portion for most manures (e.g., lagoons) should be 10–15 g.

4.4 Total Solids Content. Subsample and determine as-received total solids content according to Chapter M-1.1.

5.0 Apparatus

5.1 Heating source. Adjustable and capable of heating a reference digestion vessel with 50 mL of water to 95 ± 5°C (hot plate, digestion block, or automated digestion block system).

5.2 Digestion vessels. 50-mL beakers or 50-mL graduated disposable polypropylene resin digestion cups with certified volume accuracy within ASTM 1272-02 Class A tolerance. Larger vessels may be used to allow for a larger subsample to improve precision of analysis of heterogeneous materials.

5.3 Disposable polypropylene resin reflux caps.

5.4 Glass thermometer or acid-resistant thermocouple covering the range of 0–200 °C.

5.5 Volumetric dispensers, 10 and 15 mL.

5.6 Volumetric flasks (50 mL) or graduated disposable polypropylene resin digestion cups with certified volume accuracy within ASTM 1272-02 Class A tolerance.

5.7 Polypropylene or HDP plastic storage bottles, 50 mL.

5.8 Filter funnels of glass, plastic, or disposable polypropylene.

5.9 Whatman No. 41 filter paper or equivalent retentive filter.

5.10 Analytical top-loading balance, 100 g capacity and resolution of 0.1 mg.

5.11 Acid digestion fume hood, minimum draft 150 cfm.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM type 1.

6.2 Boiling chips. PTFE chips used for method blank and lab fortified blank (e.g., Chemware PTFE Ultra-Pure Boiling Stones Part # 26397-103).

6.3 Concentrated nitric acid (HNO_3), 16 M. Trace metal grade if trace elements are of interest.

6.4 Concentrated hydrochloric acid (HCl), 12 M. Trace metal grade if trace elements are of interest.

6.5 Hydrogen peroxide (H_2O_2), 30%. ACS Grade.

7.0 Procedure

7.1 Weigh a well-mixed sample (as-received) to the nearest 0.001 g into a digestion vessel. Adjust sample size to obtain a sample of approximately 0.5 g of total solids.

7.1.1 For solid or semi-solid samples, weigh a subsample into the digestion vessel to achieve a final sample size that yields approximately 0.5 g of solids. Record sample weight.

7.1.2 For liquid or slurry samples, weigh 5–15 g of the sample into the digestion vessel. Record sample weight.

Caution: Toxic nitrogen oxide and chlorine fumes are produced during digestion; all work must be performed in a properly operating fume hood.

7.2 Add 5.0 mL of 1:1 HNO_3, mix the slurry, and cover the vessel with a watch glass. Heat the sample to 95 ± 5° C. Reflux for 10–15 minutes without boiling. Allow samples to cool, add 5.0 mL of concentrated nitric acid, and reflux at 95 ± 5° C for 30 minutes. US EPA method 3050B recommends repeating this last step until no brown fumes are given off, indicating complete oxidation of organic matter. Experience has shown that repeating the nitric acid addition and reflux step two times is sufficient for most manures. This should be verified and adjusted by the laboratory before use of the method, and re-evaluated anytime a new manure type is digested. Following the last addition of nitric acid, reflux the sample at 95 ± 5 °C for an additional 60 minutes.

7.3 After step 7.2 is complete, remove the digestion vessel from the heat, allow the sample to cool, and add 2–5 mL of reagent grade water and 0.5 ml of 30% H_2O_2. Wait 5–10 minutes, then return the digestion vessel to the hot plate or digestion block and reflux at 95 ± 5 °C for 30 minutes. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence caused by the exothermic reaction occurring with the addition of H_2O_2. Reducing the hot plate or block temperature by 10 °C has been shown to reduce the effervescence while still maintaining temperature.

7.4 Continue to add H_2O_2 in 0.5-mL aliquots with heating and reflux until the effervescence is minimal or until the general sample appearance is unchanged. Experience has shown that repeating the 0.5-mL H_2O_2 addition and reflux step three times is sufficient for most manures. This should be verified and adjusted by the laboratory before using the method, and re-evaluated anytime a new manure type is digested. Note: do not add more than a total of 5 mL of 30% H_2O_2.

7.5 Remove the vessels from the heat source, allow to cool for 5 minutes, and add 5.0 mL of concentrated HCl. Return the vessels to the heat source and reflux at 95 ± 5 °C an additional 20 minutes.

7.6 Dilute the sample to 50 mL and transfer to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with the injection of the sample into the instrument, the sample may be centrifuged, allowed to settle, or filtered.

7.6.1 Settling: Allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample. Dilute to volume with reagent grade water.

7.6.2 Centrifugation: Centrifugation at 1,500–2,000 g for 10 minutes is usually sufficient to clear the supernatant. Dilute to volume with reagent grade water.

7.6.3 Filtering: Filter the sample through Whatman 41 or equivalent filter paper into a volumetric flask or graduated disposable polypropylene resin digestion cups with volume accuracy within Class A tolerance, and dilute to volume with reagent grade water.

7.7 The digest is now ready for analysis for elements of interest by methods described in Chapter M-5.2 or M-5.3. Ensure that the samples and standards are matrix-matched. The diluted sample has an approximate acid concentration of 10% (v/v) HCl and 12.5% (v/v) HNO_3.

8.0 Calculations and Reporting

8.1 Calculations. Following analysis of the digested sample, the concentrations of analytes are reported on the basis of the actual weight of the original sample. Dilutions must be included when calculating final results. See determinative methods for additional information (Chapters M-5.2 and M-5.3)

8.2 Client Reports. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

9.0 Quality Control

Note: For additional quality control criteria associated with this method, see Chapters M-5.2 and M-5.3.

9.1 Method Blank (MB). Carry a blank sample consisting of 0.5-g PTFE boiling chips through the entire digestion procedure. Include one method blank with each batch of 20 or fewer samples. *Acceptance criteria: The concentration of analytes of interest should be less than the LOQ.*

9.2 Laboratory Fortified Blank (LFB). Prepare an LFB by adding a known quantity of analyte(s) of interest to a MB with each batch of 20 or fewer samples. *Acceptance criteria: Recovery of analyte(s) of interest should be 100 ± 15%.*

9.3 Duplicate. Digest and analyze one sample duplicate per batch of 20 or fewer samples. Calculate the RPD according to Appendix A. *Acceptance criteria: The RPD for analytes of interest should be ≤ 10 to 20%.*

9.4 Matrix Spike Recovery (MSR). Prepare a matrix spike by adding a known quantity of analyte(s) of inter-

est to an unknown duplicate sample. Include one MSR with each batch of 20 or fewer samples. *Acceptance criteria: Recovery should be 100 ± 15% for macro elements and 100 ± 25% for micro elements or as dictated by regulatory agencies.*

9.5 Reference Material (RM). An RM with a matrix similar to that of materials tested should be obtained to assess the accuracy and overall reliability of the analytical process and analyzed with each batch of 20 or fewer samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.6 Proficiency Requirement. Laboratories performing this method shall participate in a single blind proficiency testing program at least once per year for a matrix type similar to manure and in which the analytes of interest are included as test parameters. A recommended program for this purpose is the Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) program. If the laboratory fails to successfully analyze an analyte of interest on the proficiency test sample, it shall determine the cause for the failure and take corrective action. The laboratory will document the corrective action taken for future reference.

10.0 Precision and Accuracy

10.1. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear protective clothing and eye protection when preparing reagents. especially concentrated acids. Use nitric and hydrochloric acid in a fume hood with lab coat, chemical-resistant gloves, chemical-resistant apron, and face shield.

11.2 Caution. Concentrated hydrochloric acid and nitric acid cause severe skin burns and eye damage and are respiratory irritants. Concentrated nitric acid is a Category 3 oxidizer and can cause fire when in contact with combustible material. Concentrated hydrochloric acid is corrosive to metals. Review safety procedures related to block digestion system operation before using.

11.3 Reagent Disposal. Reagents should be treated as hazardous waste and disposed of according to state requirements. Reagents and spills should be neutralized with a suitable compound, e.g., sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), or limestone ($CaCO_3$).

11.4 Sample Disposal. Sample digests should be neutralized with a suitable compound, e.g., Na_2CO_3, $NaHCO_3$, or $CaCO_3$, and disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

Chapman, H.D., & Pratt, P. F. (1961). Methods of analysis for soils, plants, and waters. University of California Berkeley, Division of Agriculture Sciences. Priced Publication 4034.

Haung, C.L., & Schulte, E. E. (1985). Digestion of plant tissue for analysis by ICP emission spectroscopy. *Communications in Soil Science and Plant Analysis,* 16: 945–958.

U. S. Environmental Protection Agency. (1996). Method 3050B: Acid digestion of sediments, sludges, and soils, Revision 2.

M-5.1c Muffle Furnace Dry Ashing

1.0 Scope and Application

This method is based on the destruction of the organic matrix of manure via high temperature dry oxidation in a muffle furnace under normal atmospheric oxygen conditions and subsequent dissolution of the ash using nitric and hydrochloric acid (Isaac, 1998). The procedure is not quantitative for S or B which are volatilized from the sample during ashing. Ashing temperatures exceeding 500 °C will result in poor recoveries of Al, Fe, K and Mn (Issac and Jones, 1972). If the temperature is sufficiently high, other elements will also be volatilized. This method is applicable to P, K, Ca, Mg, Cu, Mn, and Zn.

2.0 Method Principle

2.1 Method Description. This procedure was adapted from AOAC 985.01. A representative manure sample is heated under normal atmospheric conditions at high temperature, resulting in the oxidation of the carbon compounds. The residual ash is acidified to ensure dissolution of the remaining elements, diluted to volume and analyzed by methods described in Chapter M-5.2 or M-5.3.

2.2 Method Performance. Not applicable.

2.3 Method Interferences. Not applicable.

2.4 Method Reporting. Following analysis of digested sample, the concentration of analytes is reported as a mass fraction (% or mg kg^{-1}) of the as-received sample. Results may also be reported as a mass fraction on a dry-weight basis if the total solids content is determined on a parallel subsample.

2.5 Method Reporting. Following analysis of the digestated sample, the concentration of analytes is reported as a mass fraction (% or mg kg^{-1}) of the as-received sample. Results may also be reported as a mass fraction on a dry-weight basis if the total solids content is determined on a parallel subsample.

2.6 Method Advantages. Relatively simple equipment can be used; minimum sample handling; relatively safe procedure; samples can be ashed overnight; large numbers of samples can be easily handled at one time.

2.7 Method Disadvantages. It is critical to maintain appropriate ashing temperature; optimum ashing temperatures may vary by sample matrix; volatile elements can be lost depending on the ashing temperature; losses due to spray and dust; losses due to incomplete working up of the ash; possible sample reaction with the crucible; potential

contamination from the muffle furnace lining if the furnace is not kept clean.

3.0 Definitions

Not applicable.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample storage. Store samples in pre-washed plastic or glass containers at ≤ 6 °C. Samples should be digested as soon as possible, but no longer than 14 days after sample receipt. Sample digests may be held for up to 6 months.

4.2 Sample Processing. Subsample at least 200 cm^3 of solid (> 20% solids) and semi-solid (10–20% solids) primary samples and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Solid, semi-solid, and slurry samples may be analyzed as-received or after drying and grinding. Slurry (4–10% solids) and liquid samples do not generally require additional homogenization but, where necessary, refer to Chapter S-2.0. Solid, semi-solid, and slurry samples may be analyzed as-received or after drying and grinding. Liquid samples should be run as-received, without drying.

4.3 Sample Test Portion. Digestion is performed on a 0.50–5.0 g of solid and semi-solid sample. For liquid samples, the test portion for most manures (e.g., lagoons) should be 10–15 g.

4.4 Total Solids Content. Subsample and determine as-received total solids content according to Chapter M-1.1.

5.0 Apparatus

5.1 Muffle furnace capable of heating to 500 °C with an accuracy of ± 50 °C.

5.2 Analytical balance. 100 g capacity and resolution of 0.1 mg.

5.3 Crucible with cover. High-form glazed porcelain or silica-glass, acid-washed.

5.4 Hotplate capable of heating to 120 °C.

5.5 Desiccation chamber and desiccant.

5.6 Volumetric dispensers. Acid-resistant, capable of delivering 1–10 mL.

5.7 Volumetric containers. 100 mL volumetric flask or graduated disposable polypropylene resin digestion cups with certified volume accuracy within ASTM 1271-02 Class A tolerance.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM type 1.

6.2 Concentrated nitric acid, (HNO_3) 16 M. Reagent grade acid should be analyzed to determine level of impurities. If the method blank is less than the MDL, the acid can be used.

6.3 Nitric acid, (HNO_3), 50% (v/v). Dilute nitric acid 1:1 (v/v) with reagent water.

6.4 Concentrated hydrochloric acid, (HCl) 12 M. Reagent grade acid should be analyzed to determine level of impurities. If method blank is less than MDL, the acid can be used.

6.5 Hydrochloric acid, (HCl), 50% (v/v). Dilute hydrochloric acid 1:1 (v/v) with reagent water.

7.0 Procedure

7.1 Dry sample. Samples must be dry before ashing to prevent a violent reaction inside of the furnace during the ashing procedure. Weigh a well-mixed sample into a porcelain crucible to provide a sample with an estimated 0.50–1.0 g of dry matter. Record sample weight. For liquid or slurry manure samples, a high-form porcelain crucible may be required to accommodate the sample size needed to provide 0.50 g of dry material. Dry sample according to the procedures specified by Chapter M-1.1. Record sample weights to the nearest 0.1 mg before and after drying as specified in this method if a percent solids determination of the sample is desired.

7.2 Ash sample.

7.2.1 Place crucibles with dried sample into the muffle furnace, ramp temperature to 500 °C, and ash for 2 h at 500 °C. Remove from furnace and allow sample to cool for approximately 1 h.

7.2.2 Wet ash with approximately 10 drops of reagent water and carefully add 4.0 mL of dilute HNO_3 (1:1). Evaporate excess HNO_3 on a hotplate set at 100–120 °C. Return crucible to muffle furnace and ash 1 h at 500 °C. Remove from furnace and allow sample to cool in desiccator for approximately 1 h.

7.2.3 Dissolve ash in 10 mL HCl (1:1) and transfer quantitatively to 100 mL volumetric flask or graduated disposable polypropylene resin digestion cups with volume accuracy within Class A tolerance. Dilute to volume with reagent water.

7.3 The samples are now ready for analysis for elements of interest by methods described in Chapter M-5.2 or M-5.3. Ensure that the samples and standards are matrix-matched. The diluted sample has an approximate acid concentration of 5% (v/v) HCl and 2% (v/v) HNO_3.

8.0 Calculations and Reporting

8.1 Calculations. Following analysis of the digestated sample, the concentration of analytes is reported on the basis of the actual weight of the original sample. Dilutions must be included when calculating final results. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

8.2 Client Reports. See determinative methods for additional information (Chapters M-5.2 and M-5.3).

9.0 Quality Control

Note: For additional quality control criteria associated with this method, see Chapters M-5.2 and M-5.3.

9.1 Method Blank. Carried through the entire digestion procedure. Include one method blank with each batch of 20 or fewer samples. The concentration of analytes of interest should be less than the LOQ.

9.2 Laboratory Fortified Blank (LFB). Blank Spike Recovery. Prepare an LFB spike blank by adding a known quantity of analyte(s) of interest to an MB. Include one spike blank with each batch of 20 or fewer samples. *Acceptance criteria: Recovery of analyte(s) of interest should be 100 ± 15%.*

9.3 Duplicate. Digest and analyze one sample duplicate per batch of 20 or fewer samples. Calculate the RPD according to Appendix A. *Acceptance criteria: The RPD for analytes of interest should be ≤ 10 to 20%.*

9.4 Matrix Spike Recovery (MSR). Prepare a matrix spike by adding a known quantity of analyte(s) of interest to an unknown duplicate sample. Include one MSR with each batch of 20 or fewer samples. *Acceptance criteria: Recovery should be 100 ± 15% for macro elements and 100 ± 25% for micro elements or as dictated by regulatory agencies.*

9.5 Reference Material (RM). An RM with a matrix similar to that of materials tested should be obtained to assess the accuracy and overall reliability of the analytical process and analyzed with each batch of 20 or fewer samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.6 Proficiency Requirement. Laboratories performing this method shall participate in a single blind proficiency testing program at least once per year for a matrix type similar to manure and in which the analytes of interest are included as test parameters. A recommended program for this purpose is the Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) program. If the laboratory fails to successfully analyze an analyte of interest on the proficiency test sample, it shall determine the cause for the failure and take corrective action. The laboratory will document the corrective action taken for future reference.

10.0 Precision and Accuracy

10.1 See determinative methods for additional information (Chapters M-5.2 and M-5.3).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear protective clothing and eye protection when preparing reagents, especially concentrated acids. Dispense concentrated acids in fume hood.

11.2 Caution. Review safety procedures related to muffle furnace operation before using.

11.3 Reagent and Sample Disposal. Use sodium bicarbonate and water to neutralize and dilute spilled acids. Dispose of sample digest as hazardous waste in accordance with all local and federal regulations.

12.0 Reference Documents and Additional Resources

Baker, D.E., Gorsline, G. W., Smith, C. G., Thomas, W. I., Grube, W. E., & Ragland, J. L. (1964). Techniques for rapid analysis of corn leaves for eleven elements. *Agronomy Journal,* 56:133–136.

Chapman, H. D., & Pratt, P. F. (1961). *Methods of analysis for soils, plants, and waters*. University of California Berkeley, Division of Agriculture Sciences. Priced Publication 4034.

Isaac, R. A. (1998). AOAC Method 985.01. Metals and other elements in plants. In Cunniff, P.(Ed.), *Official methods of analysis of AOAC International* (16th ed.). AOAC International.

M-5.2
Phosphorus, Potassium, & Metals by Inductively Coupled Plasma — Optical Emission Spectroscopy

AUTHORS: Kristin Hicks, Robert O. Miller, and John T. Spargo

1.0 Scope and Application

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) is used for the determination of elemental (e.g., P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, B, Mo, As, Cd, Cr, Ni, Pb, Se) concentrations in aqueous solutions. For solid or liquid manure samples, digestion is first required to dissolve the sample into an aqueous solution. ICP-OES is generally regarded as the method of choice for the elemental analysis of manure digests. The technique is capable of simultaneous multi-element analysis, has excellent sensitivity, and is linear across a concentration range of 4–5 orders of magnitude for most elements of interest, including macronutrients and micronutrients needed for nutrient management planning, and trace-element pollutants needed for contaminant monitoring.

2.0 Method Principle

2.1 Method Description. This method was adapted from US EPA methods 6010B and 200.7 and is based on the use of a high-energy plasma to ionize and excite elements in an aqueous matrix, with subsequent determination of element concentration using established defined wavelengths. To produce the plasma, ionized argon gas is passed through a quartz torch located inside an induction coil that is connected to a radio frequency generator. An alternating electric current flowing through the induction coil generates a changing magnetic field. Electrons and ions flowing through the magnetic field are heated by magnetic induction within the torch. Adding energy to the ionized argon by use of the induction coil is known as inductively coupling. The ionized argon gas reacts to the changing magnetic field, causing electron collisions and sufficient heat to establish and maintain an argon plasma.

An aqueous sample is first introduced to a nebulizer, where it is aerosolized and then carried via argon gas into the plasma torch. Aerosol droplets containing the analyte are desolvated, leaving microscopic salt particles. These analyte salts/oxides in the high-temperature plasma dissociate into their elemental ion constituents, and excitation and ionization shift ion electrons from lower to higher energy levels. As the atoms transit through the plasma to cooler regions, the ions' electrons decay from their excited state to ground state and energy is emitted in the form of photons at characteristic wavelengths unique to each element. Wavelengths are separated by a polychromator and photon intensity detected by a photosensitive detector. Elemental concentration is determined through calibration of peak intensity against known standard element concentrations.

Radial vs. axial viewing. The plasma zone for measuring photon intensity can be viewed from either of two directions: *radial* (side-on viewing) and *axial* (end-on viewing). It is important to note that radial and axial viewing refer to the angle at which the detector views the plasma analytical zone, not to the orientation of the torch itself. Earlier ICP-OES were constructed in the vertical direction for radial viewing. Modern instruments (after the mid-1990s) may have a torch orientation in vertical or horizontal directions, and can be used for radial or axial viewing or dual synchronous view of the analytical zone.

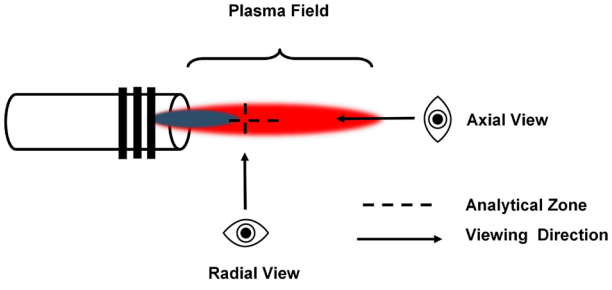

Figure M-5.2.1. Plasma viewing orientation in an ICP-OES torch in axial and radial view.

In radial view, the older and original orientation design, the spectrometer views a vertical slit in the plasma from the perpendicular direction (Figure M-5.2.1). For axial view, the spectrometer views the central channel of the plasma and collects analyte emissions over the entire length of the plasma. The net effect is that the viewing of the emission path length is increased relative to a radial view, which increases the spectrometer sensitivity and lowers the detection limit by an order of magnitude. It should be noted that axial view does not extend the linear calibration range further than in radial view. Accurate determination of analytes present in low concentrations such as molybdenum and some heavy metals is more difficult with ICP-OES operated in radial view. Conversely, the increased sensitivity of axial viewing makes this orientation more prone to interferences, and more dilutions may be needed than with radial view. For samples with high analyte concentration, the operator may need to use dual wavelengths. In addition, an ionization buffer is usually needed for axial viewing to quench easily ionized elements, such as K and Na. An ionization buffer is not typically needed for an ICP operated in radial view. Ultimately, the choice of radial or axial orientation depends on the requirements of the lab and the other sample matrices analyzed by the instrument. For an ICP-OES instrument dedicated to manure and soil, radial viewing may be preferable. For an ICP-OES instrument dedicated to manure and water or environmental compliance samples, an axial orientation may be preferable.

2.2 Method Performance. Detection limits and linear ranges for the elements will vary depending on wavelength selected, spectrometer, and sample matrices. Method detection limits (See Appendix B) should be determined for all analytes with a validation study. Estimated instrument detection limits (IDL) at specific wavelengths for the primary elements of interest are given in Table M-5.2.1 and can be used to estimate the MDL. Note that the exact wavelength will vary slightly depending on whether the lines were measured in a vacuum, in air, or in a purged system such as nitrogen. An MDL should be determined and reported individually by the laboratory upon completion of the MDL outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. Three main types of interferences are encountered during ICP-OES analysis: physical, chemical, and spectral.

Physical interferences occur due to differences between the aqueous sample digest and the pure calibration standards. These differences can affect nebulization or sample transport to the torch plasma, and may include viscosity, density, or matrix differences, with the latter having the largest relevance to manure analysis. Solutions that overcome these issues include sample dilution, matrix matching, increasing the nebulizer internal diameter, and internal standardization. Matrix spiking and recovery can help detect matrix effects, and a matrix spike duplicate should be a required component of the quality control program. Internal standardization can help when the matrix is not entirely known. Yttrium (Y) is commonly used as an internal standard, but other elements such as scandium (Sc), platinum (Pt), lanthanum (La), and lutetium (Lu) may also be used.

Chemical interferences occur when there is a difference in the way the sample and the calibration standard react in the plasma, resulting in unintended ionization, molecular formation, or plasma loading. Some elements (e.g., alkali metals) ionize easily and can produce second-order response curves due to ionization and self-absorption effects. These effects are particularly pronounced in instruments operated in axial view, but can be minimized by using an ionization buffer such as cesium or lithium.

Spectral interferences are the most common ICP-OES interference issue and are caused by overlap of a spectral line from other elements, unresolved overlap of molecular band spectra, background contribution from continuous or recombination phenomena that originate from the matrix, and stray light from the line emission of high-concentration elements. Spectral overlap can be avoided by selecting alternate wavelengths, computer-correcting the spectral data by determining and applying Interfering Element Correction (IEC) factors, or using a spectral mathematical modeling software. Unresolved overlap requires selection of alternate wavelengths. An element may have multiple energy levels and therefore multiple emission wavelengths. To correct for spectral overlap, multiple wavelengths can be chosen for each element. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

2.4 Method Reporting. Elemental concentrations are reported in mg kg^{-1} or as a % on an as-received or dry-weight basis, as determined by state requirements. Phosphorus and potassium may be reported as P_2O_5 and K_2O; see Appendix A for conversions.

2.5 Method Advantages. Chemical interferences are minimal; 4–6 orders of magnitude in linearity of intensity versus concentration are attainable; rapid, multi-element analyses are possible; analyses are more accurate and precise compared with other emission methods.

2.6 Method Disadvantages. Spectral interferences frequently occur, depending on the element and background; matrix interferences may be caused by differences between the sample and calibration standards; the most useful spectral lines may fall outside the range of the spectrometer; the initial purchase, operation, and maintenance of the equipment are expensive relative to other methods; the instrument requires a strict temperature and humidity-controlled

Table M-5.2.1. Wavelengths for ICP detection, known interferences, and approximate instrument detection limits (IDL) in radial or axial view for each analyte at the specified wavelength. IDL estimates are for dual-view ICP-OES instruments and may be lower for other instruments.

Analyte	Wavelength (nm)	Radial IDL µg/ml	Axial IDL µg/ml	Interferences
Aluminum (Al)	394.401	0.05	0.006	U
	396.152	0.03	0.006	Mo, Zr
	167.078	0.1	0.009	Fe
Arsenic (As)	189.042	0.05	0.005	Cr
	193.696	0.1	0.01	V, Ge
	228.812	0.1	0.01	Cd, Pt, Ir, Co
Boron (B)	249.678	0.004	0.003	Co, Cr, Hf
	249.773	0.003	0.001	Co, Th, Mn, Mo, Fe
	208.959	0.007	0.0005	Mo
Cadmium (Cd)	214.438	0.003	0.0003	Pt, Ir
	228.802	0.003	0.0003	As, Pt, Co, Ir
	226.502	0.003	0.0003	Ir
Calcium (Ca)	315.887	0.015	0.004	
	183.801	0.014	0.003	
	318.128	0.03	0.008	
	393.366	0.0002	0.00004	U, Ce
	396.847	0.0005	0.00006	Th
	422.673	0.01	0.001	
Chromium (Cr)	276.654	0.01	0.001	Cu, Ta, V
	267.716	0.005	0.0008	
	205.552	0.006	0.0008	
	284.325	0.008	0.0007	
Copper (Cu)	324.754	0.06	0.001	
	219.958	0.01	0.002	
	224.700	0.01	0.001	
Iron (Fe)	259.940	0.006	0.001	Hf, Nb
	238.204	0.005	0.001	Co, Ru
	239.562	0.005	0.001	Co, Cr
Lead (Pb)	168.215	0.03	0.003	Co
	220.353	0.04	0.006	Bi
	217.000	0.09	0.03	Ir, Th
Magnesium (Mg)	279.079	0.03	0.01	
	279.553	0.0002	0.00003	Th
	280.270	0.0003	0.00005	U,V
	285.213	0.002	0.00003	Cr, U, Hf, Zr
Manganese (Mn)	257.610	0.0014	00002	Ce
	259.373	0.0016	00002	Mo, Fe, U
	260.569	0.0021	0.00002	Co
Molybdenum (Mo)	202.030	0.008	0.0002	
	203.844	0.012	0.002	
	204.598	0.012	0.001	
Nickel (Ni)	231.604	0.02	0.002	Si
	221.647	0.01	0.0009	Fe, Cr, Ag, Pt
	232.003	0.02	0.006	
Phosphorus (P)	178.287	0.03	0.002	I
	177.495	0.01	0.005	Cu, Hf
	213.618	0.08	0.03	Cu, Mo
Potassium (K)	404.721	1.1	0.05	U, Ce
	771.531	1.0	0.03	
	766.490	0.4	0.001	
Selenium (Se)	196.026	0.08	0.006	Fe
	203.985	0.2	0.05	Cr, Ir
	206.279	0.3	0.16	Cr, Pt
	196.090	0.032	0.005	
Sodium (Na)	588.995	0.03	0.006	
	330.237	2.0	0.09	Zn, Pd
	589.595	0.07	0.00009	
Sulfur (S)	182.034	0.3	0.024	
	166.669	0.2	0.19	Si, B
	143.328	0.4	0.035	
Zinc (Zn)	213.856	0.002	0.0004	Ni, Cu, V
	206.200	0.006	0.0006	Bi
	202.548	0.004	0.0002	Cu, Co

Sources: US EPA 200.7, Inorganic Ventures, https://www.inorganicventures.com/periodic-table, and Spectro Analytical.

environment and may perform erratically outside of optimum conditions; the level of technical expertise required to operate the instrument and troubleshoot analytical problems is higher than for many other manure analysis methods.

3.0 Definitions

3.1 Inductively coupled plasma-optical emission spectroscopy (ICP-OES). An analytical technique used for the spectrophotometric detection and quantification of chemical elements in an aqueous solution.

3.2 Internal Standard (IS). Pure analyte added to all samples, standards, and quality controls in a known amount in order to correct for matrix interferences in the ICP-OES

method. The responses of other method analytes are referenced to the internal element. The internal standard must be an analyte that is not present in the unknown sample. Also known as an internal reference (IUPAC).

3.3 Inter-Element Correction (IEC). A technique used in ICP-OES to determine the relationship between an interfering element concentration and response at the wavelength of the analyte of interest. IEC factors are determined on the individual instruments and may be used within the tested concentration ranges to compensate for the effects of interfering elements. See US EPA 200.7.

3.4 Interference Check Solution (ICS). A solution containing known concentrations of the primary interfering elements in ICP-OES analysis that is used to verify the accuracy of the correction factors for inter-element interferences. Also known as a Spectral Interference Check Solution. See US EPA 200.7.

3.5 Ionization Buffer. A solution such as cesium or lithium introduced in-line in order to suppress ionization interference in ICP-OES.

4.0 Sample Processing, Preservation, and Storage

4.1 See Chapter M-5.1, Digestion and Dissolution for Elemental Analysis.

5.0 Apparatus

5.1 Apparatus for digestion and dissolution according to Chapter M-5.1, Digestion and Dissolution for Elemental Analysis. ***Note:*** *borosilicate glass should be avoided where boron is an analyte of interest. For accurate determination of boron, use quartz or PTFE beakers during digestion followed by transfer to plastic centrifuge tubes for analysis.*

5.2 Argon gas, 99.996%.

5.3 Auto-sampler. Use the auto-sampler recommended by the instrument manufacturer.

5.4 Inductively coupled plasma-optical emission spectrophotometer, equipped with background correction.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I grade.

6.2 Nitric acid (HNO_3), concentrated, trace metal grade.

6.3 Stock solutions. May be purchased from commercial sources or prepared in the lab from ultra-high purity grade chemicals (99.99–99.999% pure), reagent grade water, and 2–5% HNO_3. Purchased stock solutions are prepared at typical concentrations of 1,000 mg L^{-1} for macronutrients and 100 mg L^{-1} for micronutrients. If preparing in the lab, refer to US EPA 200.7 for detailed instructions.

6.4 Calibration standards. Working calibration standards are prepared by diluting the stock solutions in reagent grade water and 2–5% HNO_3. The calibration standards should be prepared at the same acid concentration as the sample digests to minimize matrix inferences. Prepare a blank (zero standard) and at least five calibration standards that bracket the typical concentration range of each analyte in the sample material. Store calibration standards in FEP fluorocarbon bottles or unused polyethylene bottles.

6.5 Ionization buffer (for axial view). Cesium (Cs) is often used as an ionization buffer and is introduced in-line using a working standard of 0.5% Cs prepared from a concentrated Cs (5.0–10.0%) solution.

6.6 Internal Standard. Used for both radial and axial view to correct for matrix interferences. From a 0.1–1.0% Y stock standard, make a 2–5 mg L^{-1} Y working standard for in-line introduction.

6.7 Calibration Blank (CB). Reagent water prepared in 2–5% HNO_3. Prepare a sufficient quantity to flush the system between standards and samples. Store in an FEP bottle.

6.8 Independent Calibration Verification (ICV) solution. Prepare the ICV solution with concentrations of all elements in the range of the mid-point of the calibration curve, from a different source than that used to make the calibration standards and at the same HNO_3 concentration. Store in an FEP bottle.

6.9 Interference Check Solution (ICS). Optional. Prepare the ICS solution with concentrations of the primary interfering elements (Al, Ca, Mg, K, Na, Fe, and Mg) and elements of interest at approximately 10 times their detection limits. To make, add 50 mL of conc. HNO_3 to about 300 mL of reagent water in 500 mL volumetric flask. Add 50 mL of interferent stock solution and 0.05 mL (50 µl) of trace stock solution and dilute to volume.

6.10 Matrix Spike Recovery (MSR) solution. Prepare the MSR solution with concentrations of all elements from a different source than that used to make the calibration standards and at the same HNO_3 concentration. The element concentrations of the matrix spike solution should be approximately 1–5 times the background concentration of the analytes of interest in the sample or approximately 100 times the MDL for each analyte. In general, concentrations of 200 mg kg^{-1} for macronutrients, 20 mg kg^{-1} for micronutrients, and 10 mg kg^{-1} for heavy metals is appropriate for most samples.

6.11 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

Note: Differences among the various makes and models of ICP spectrometers make it impractical to outline detailed instructions that apply to every instrument.

Follow the manufacturer's operating instructions for a particular instrument.

7.1 Digest samples according to the specified procedures (Chapter M-5.1).

7.2 Transfer an aliquot of the diluted digest into tubes and transfer to auto-sampler trays.

7.3 Set up the ICP-OES instrument with proper operating parameters as specified by the manufacturer.

7.4 Calibrate the instrument according to the instrument manufacturer's recommended procedures. Instrument should be set to rinse with reagent grade water between each sample analysis.

7.5 Analyze QC samples according to Section 9.0.

7.6 Elemental concentration (mg L^{-1}) determined in the digests are used to calculate the concentration in the manure, according to the calculation procedure outlined for each of the dissolution and digestion methods.

8.0 Calculations and Reporting

8.1 Calculations.

8.1.1 For samples analyzed and reported as-received or analyzed and reported on a dry-weight basis, calculate results as:

$$Analyte, mg\ kg^{-1} = \frac{C \times V}{S}$$

where:
C = analyte concentration from ICP, mg L^{-1}
V = extraction volume, mL
S = sample size, g

8.1.2 For samples analyzed as-received and reported on a dry-weight basis, a separate determination of percent solids must be performed on a homogeneous aliquot of the sample (Chapter M-5.1, Digestion and Dissolution for Elemental Analysis). Divide results (mg kg^{-1}) on as-received basis by (% solids/100) to determine results on dry-weight basis.

8.1.3 For samples analyzed on a dry-weight basis and reported on an as-received basis, a separate determination of the % dry matter must be performed. Record the weight of the secondary sample before and after drying and determine a moisture correction factor (M) as follows:

$$M = \left(\frac{D}{A}\right)$$

where:
M = moisture correction factor
D = dry weight at 36 °C or 70±5 °C, mg
A = as-received weight, mg

Multiply results (mg kg^{-1}) on a dry-weight basis by the moisture correction factor to determine results on an as-received basis (mg kg^{-1}). See Chapter S-2.0, Laboratory Sample Processing and Storage, for additional details.

8.2 Client Reports. Report results on a dry-weight or as-received basis in mg kg^{-1}. The client report should also provide the elemental concentrations in lb ton^{-1} or lb 1000 gal^{-1} on an as-received basis. Phosphorus and potassium may be reported as P_2O_5 and K_2O; see Appendix A for conversions.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of five calibration standards using calibration standard solutions and the calibration blank. If the concentration for any of the elements exceeds the highest calibration standard, the sample must be diluted and rerun for that element. The lowest standard should be ≤LOQ. *Acceptance criteria: The coefficient of determination, r^2, should be ≥ 0.998. Otherwise, perform maintenance and recalibrate.*

9.2 Calibration Blank (CB). The CB is a volume of reagent water acidified at the same acid concentration as the calibration standards and is used for initial and continuing calibration of the ICP instrument. *Acceptance criteria: Concentrations of detected analytes must be below the MDL for each analyte. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination eliminated.*

9.3 Method Blank (MB). The method blank is used to test for contamination during the sample preparation process. An MB should be carried through the entire sample analysis procedure, including digestion. The MB must contain all of the reagents in the same volumes used in the processing of the samples. *Acceptance criteria: Concentrations of detected analytes must be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until source of contamination is eliminated.*

9.4 Laboratory fortified blank (LFB). Include one prepared LFB with each batch of 20 or fewer samples. *Acceptance criteria: Recovery of analyte(s) of interest should be 100 ± 15%.*

9.5 Continuing Calibration Verification (CCV). Two CCV solutions should be used for initial and continuing calibration of the ICP instrument. One CCV should be within the lower 20% of the calibration range and not more than 5 times the LOQ, and one should be within the upper 20% of the calibration range. It is acceptable to alternate between the low and high CCV. The CCV solutions are prepared from the same stock solutions as the standards. Calculate the percent error (PE) between the CCV

Table M-5.2.2. Quality control guidelines for the ICP-OES method.

QC Parameter	Minimum Frequency	Acceptance Criteria
Calibration	Daily	$r^2 \geq 0.99$
Calibration Blank (CB)	Start and end of run and every 10 samples	< MDL
Method Blank (MB)	Start of each batch	< MDL
Laboratory fortified blank (LFB)	Every batch	100 ± 15%
Continuing Calibration Verification (CCV)	Start and end of run and every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	Every batch	Within ± 10 PE
Reference material	Every batch	Within specified uncertainty range
Duplicate	Every batch	RPD ≤ 10% or within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery 100 ± 15% for macro elements and 100 ± 25% for trace elements

known and the as-measured concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Ensure that results are within ± 10 PE of calibration. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed.*

9.6 Independent Calibration Verification (ICV). One ICV should be analyzed with each batch. The ICV should be prepared from a source other than the one from which the calibration stock standards are prepared. Calculate the PE between the ICV known concentration and as-measured concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Ensure that results are within ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.7 Interference Check Solution (ICS). Optional or as required. The ICS solution contains known concentrations of the primary interfering elements and is used to test for the correction factors for interelement interferences between the primary interfering elements (Al, Ca, Mg, K, Na, Fe, and Mg) and elements of interest. Spectral overlaps may be avoided by using an alternate wavelength. Where this is not feasible or where regulatory compliance requires strict adherence to the US EPA method, ICS solutions should be included in the QC program and analyzed at same time as the element of interest. If not included in each batch, an ICS should be analyzed bi-annually. *Acceptance criteria: Ensure that results for analytes of interest are ± 20% PE. If not, recalibrate, re-evaluate element concentrations, or use an alternate, less sensitive line for which quality control data is already established.*

9.8 Duplicate. A duplicate sample should be processed with each batch and should be separated in the analytical batch from the original sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference. *Acceptance criteria: An RPD of ≤ 10% should be routinely achievable for macronutrients. The RPD should not exceed 20% when the analyte is greater than the LOQ or should be within the documented historical acceptance limits for that element. For micronutrients, it should be understood that RPD may exceed 20% for analytes typically found at low concentrations in manures or as the measured analyte approaches LOQ. The laboratory operator may establish alternate acceptance criteria in these circumstances based on the historically documented limits of these analytes.*

9.9 Matrix Spike Recovery (MSR). Laboratories reporting ICP-OES elements in waste samples for regulatory compliance purposes must include a matrix spike in their QC program to evaluate the recovery efficiency of elements of interest to the regulatory body. Known quantities of the analytes of interest are added in the laboratory to one of two aliquots of the same manure sample. The element concentrations of the matrix spike solution should be approximately 10 times the LOQ of the analytes of interest in the sample or approximately 100 times the MDL for each analyte. The spiked and unspiked duplicates are digested and analyzed exactly like an unknown sample, and the MSR is calculated according to Appendix A: Definitions; Matrix Spike Recovery. A*cceptance criteria: Recov-*

Table M-5.2.3. Laboratory precision data for manure total phosphorus as-received basis, by ICP-OES.

Sample ID	Total Solids Content (%)	Mean Content mg P kg^{-1}	Intra-Lab Standard Deviation S_r mg P kg^{-1}	Intra-Lab Repeatability r mg P kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg P kg^{-1}	Inter-Lab Reproducibility R mg P kg^{-1}
M 2014-D	3.15	256	5	15	27	80
M 2015-D	11.2	4788	8	23	49	1475
M 2012-B	56.5	9330	17	51	896	2688
M 2016-F	68.4	3490	12	37	576	1728

MAP Program, 66–71 laboratories reporting.

Table M-5.2.4. Laboratory precision data for manure potassium as-received basis, by ICP-OES.

Sample ID	Total Solids Content (%)	Mean Content mg K kg^{-1}	Intra-Lab Standard Deviation S_r mg K kg^{-1}	Intra-Lab Repeatability r mg K kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg K kg^{-1}	Inter-Lab Reproducibility R mg K kg^{-1}
M 2014-D	3.15	834	8	24	95	285
M 2015-D	11.2	2160	16	48	176	528
M 2012-B	56.5	12250	56	168	1080	3324
M 2016-F	68.4	15700	72	216	1190	3570

MAP Program, 66–71 laboratories reporting.

Table M-5.2.5. Laboratory precision data for manure zinc as-received basis, by ICP-OES.

Sample ID	Total Solids Content (%)	Mean Content mg Zn kg^{-1}	Intra-Lab Standard Deviation S_r mg Zn kg^{-1}	Intra-Lab Repeatability r mg Zn kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg Zn kg^{-1}	Inter-Lab Reproducibility R mg Zn kg^{-1}
M 2014-D	3.15	15.6	0.05	0.14	1.71	5.14
M 2015-D	11.2	20.0	0.06	0.18	2.19	6.57
M 2012-B	56.5	311	0.53	59	21.8	65.3
M 2016-F	68.4	68.8	0.15	22.0	6.60	19.7

MAP Program, 45–56 laboratories reporting.

ery should be 100 ± 15% for macro elements and 100 ± 25% for micro elements or as dictated by regulatory agencies.

9.10 Reference Material (RM). An RM with a matrix similar to that of the materials tested should be obtained to assess the accuracy and overall reliability of the analytical process and analyzed with each batch. *Acceptance criteria: Results should be within specified uncertainty limits.*

9.11 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for ICP-OES of manure materials that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program.

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least

Table M-5.2.6. Laboratory precision data for manure copper as-received basis, by ICP-OES.

Sample ID	Total Solids Content (%)	Mean Content mg Cu kg^{-1}	Intra-Lab Standard Deviation S_r mg Cu kg^{-1}	Intra-Lab Repeatability r mg Cu kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg Cu kg^{-1}	Inter-Lab Reproducibility R mg Cu kg^{-1}
M 2014-D	3.15	2.54	0.01	0.03	0.41	1.24
M 2015-D	11.2	3.66	0.01	0.04	0.38	1.15
M 2012-B	56.5	194	0.40	1.19	12.9	38.7
M 2016-F	68.4	13.9	0.05	0.14	1.35	4.05

MAP Program, 52–54 laboratories reporting.

annually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. Spectrophotometric repeatability and reproducibility were calculated for phosphorus, potassium, zinc, and copper in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program coordinated by the Minnesota Department of Agriculture, in proficiency rounds conducted in 2015 and 2016 of 10 laboratories. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained (Tables M-5.2.3 to M-5.2.6).

11.0 Safety and Disposal

11.1 Laboratory Safety. Use nitric acid in a fume hood during digestion and wherever possible. During nitric acid digestion, wear safety glasses, lab coat, chemical-resistant gloves, apron, and face shield. During routine ICP-OES operation, wear safety glasses, standard nitrile gloves, closed-toe shoes, and lab coat.

11.2 Caution.

11.2.1 Concentrated nitric acid is extremely irritating to skin and mucus membranes and can cause severe burns to skin. If skin contact occurs, flush with large volumes of water.

11.2.2 Concentrated nitric acid is a Category 3 oxidizer and can cause fire when in contact with combustible material.

11.2.3 The ICP-OES plasma is extremely hot (~ 10,000 K) and radiates dangerous levels of radio frequency and ultraviolet energy. The torch and its surroundings remain hot for up to five minutes after the plasma is extinguished. Exposure can cause severe skin and eye damage. Never view the ICP-OES torch directly without protective eyewear. Never operate the plasma unless the torch compartment door is closed, with the locking lever fully latched.

11.2.4 Sample combustion during ICP-OES operation can generate toxic fumes. Ensure proper ventilation of the ICP-OES torch for chemical vapors.

11.2.5 The method involves the use of compressed gases which pose explosion hazards when heated and asphyxiation hazards in enclosed spaces. Strictly follow safety measures specific to the proper installation, storage, and handling of gas cylinders or tanks. Store in a well-ventilated place.

11.3 Reagents Disposal. Reagents should be treated as hazardous waste and disposed of according to state requirements. Reagents and spills should be neutralized with a suitable compound, e.g., sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), or limestone ($CaCO_3$).

11.4 Samples Disposal. Digests should be neutralized with a suitable compound, e.g., Na_2CO_3, $NaHCO_3$, or $CaCO_3$ before disposal. Digestates should be disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

U. S. Environmental Protection Agency. (2001). Method 200.7. Trace elements in water, solids and biosolids by inductively coupled plasma-atomic emission spectrometry. Revision 5.0.

U. S. Environmental Protection Agency. (1996). Method 6010B. Inductively coupled plasma-atomic emission spectrometry. Revision 2.

M-5.3
Phosphorus by Automated Spectrophotometry

AUTHOR: Robert O. Miller

1.0 Scope and Application

This method determines the amount of total phosphorus (P) in acid digests of liquid and solid animal manures by spectrophotometric analysis. Total P may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The total elemental content of P in acid digests as orthophosphate P (PO_4-P), which is determined by spectrophotometric analysis based on the method of Murphy and Riley (1962). Orthophosphate reacts with ammonium molybdate in acidic solution to form phosphomolybdic acid, which upon reduction with ascorbic acid produces an intensely blue complex measured at a wavelength of 660 or 880 nm. Antimony potassium tartrate is added along with heating to increase the rate of reduction. The method is adapted from US EPA Method 365.1.

2.2 Method Performance. A lab should be able to attain an MDL of 0.2 mg P L^{-1}. An MDL should be determined and reported individually by the laboratory upon completion of the MDL outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B.

2.3 Method Interferences. Spectrophotometric analysis requires clarification of samples that are colored in the absorption range (880 nm) that may interfere with the analysis of P. This can be achieved through the use of a dialyzer membrane or by filtering the sample using a smaller membrane filter (0.45 µm). Alternately, some instruments include a color blanking feature that measures the sample color with and without reagent and corrects for the absorbance effect of the sample color. Barium, Pb, Cu, and Ag interfere by forming a phosphate precipitate, but the effect is negligible in natural water (Cho and Nielsen, 2007). Arsenic as arsenate (AsO_4^{3-}) produces a color similar to phosphate (Murphy & Riley, 1962) and may cause a positive interference.

2.4 Method Reporting. P concentration is reported as a mass fraction (% or mg kg^{-1}) of the as-received sample. Results may also be reported as a mass fraction on a dry-weight basis if the total solids content is determined on a parallel subsample. Phosphorus may be reported as P_2O_5; see Appendix A for conversions.

2.5 Method Advantages. Low MDL; highly reproducible; automated instruments have high throughput.

2.6 Method Disadvantages. Linear range limited to two orders of magnitude.

3.0 Definitions

3.1 Orthophosphate (PO_4^{3-}). Inorganic phosphate or orthophosphate is an ester of orthophosphoric acid.

3.2 Spectrophotometric analysis. Determination of an analyte in a liquid matrix by the reaction with a chromophore and measurement of the amount of light absorbed at a specific wavelength, where the change in absorption is proportional to the analyte concentration.

4.0 Sample Processing, Preservation, and Storage

4.1 See Chapter M-5.1, Digestion and Dissolution for Elemental Analysis.

5.0 Apparatus

5.1 Volumetric flasks.

5.2 Pipettes.

5.3 Spectrophotometric flow injection, continuous flow, or discrete analyzer capable of performing total P analysis based on the orthophosphate reaction with ammonium molybdate, with ascorbic acid reduction (Murphy and Riley, 1962) measured spectrophotometrically at 880 nm.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I grade.

6.2 Nitric acid (HNO_3). Concentrated, trace metal grade.

6.3 Spectrophotometric analysis reagents.

6.3.1 Sulfuric acid solution, 2.5 M: Slowly add 70 mL of reagent grade concentrated H_2SO_4 to approximately 400 mL of reagent grade water. Cool to room temperature and dilute to 500 mL with reagent grade water.

6.3.2 Antimony potassium tartrate solution, 0.30 g L^{-1}: Dissolve 0.30 g $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$ in 50 mL of reagent grade water in 100 mL volumetric flask, and dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

6.3.3 Ammonium molybdate solution, 4.0 g L^{-1}: Dissolve 4.0 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 mL reagent grade water. Store at 4°C in a plastic bottle.

6.3.4 Ascorbic acid solution, 1.8 g L^{-1}: Dissolve 18 g ascorbic acid in 100 mL reagent grade water. Solution is stable for one week, if prepared with water containing no more than trace amounts of heavy metals and stored at 4°C.

6.3.5 Combined working reagent: Mix the above reagents in the following proportions for 100 mL of the mixed reagent: 50 mL of 2.5 M H_2SO_4 (Section 6.3.1), 5 mL of antimony potassium tartrate solution (Section 6.3.2), 15 mL of ammonium molybdate solution (Section 6.3.3), and 30 mL of ascorbic acid solution (Section 6.3.4), in order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until the turbidity disappears before processing. Mix after addition of each reagent, and equilibrate to room temperature. Stability of this solution is limited; it must be freshly prepared for each analytical run. Spectrophotometric instrument manufacturer may have reagent concentrations that may diverge from these concentrations.

6.4 Stock standard solutions. Stock standard solutions may be purchased from commercial sources or prepared in the lab from ultra-high purity grade chemicals (99.99–99.999% pure), reagent grade water, and 2–5% HNO_3. Purchased stock solutions are prepared at typical concentrations of 1,000 mg P L^{-1}.

6.5 Calibration standards. Working calibration standards are prepared by diluting the stock solutions in reagent grade water and 2–5% HNO_3. The calibration standards should be prepared at the same acid concentration as the sample digests in order to minimize matrix inferences. Prepare a minimum of six calibration standards, 0.5, 2.0, 5.0, 10.0, 20.0, and 40.0 mg P L^{-1}. Store calibration standards in FEP fluorocarbon bottles or unused polyethylene bottles. Refrigerate at ≤ 6 °C when not in use.

6.6 Calibration Blank (CB). Reagent water prepared in 2–5% HNO_3. Prepare a sufficient quantity to flush the system between standards and samples. Store in FEP bottle.

6.7 Calibration Verification (CV) standard. Prepare the CV solution with analyte concentrations at the mid-range of the calibration curve, from the same stock solutions as the calibration standards and at the same HNO_3 concentration. Store in an FEP bottle.

6.8 Independent Calibration Verification (ICV) standard. Prepare the ICV solution with concentrations of all elements in the range of the mid-point of the calibration curve, from a different source than that used to make the calibration standards and at the same HNO_3 concentration. Store in an FEP bottle.

6.9 Matrix Spike (MS) solution. Prepare the MS solution with concentrations of all elements from a different source than that used to make the calibration standards and at the same HNO_3 concentration. The P concentrations of the matrix spike solution should be approximately 1–5 times the background concentration of the P in the sample, or approximately 100 times the MDL.

6.10 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to that of the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Digest manure sample in accordance with the specified digestion procedures (Chapter M-5.1).

7.2 Initialize and calibrate instrument according to manufacturer instructions. Follow protocols outlined in Section 9.0 for method blanks, standards, duplicates, and matrix spikes. Transfer the filtrate to sample tubes and place on autosampler tray in preparation for analysis. Determine phosphorus concentration of unknown manure solutions.

7.3 Samples with concentrations exceeding the highest calibration standard should be diluted with matrix matched to digest solution.

8.0 Calculations and Reporting

8.1 Calculations. Instrument data is converted by the operating system to extract concentration in mg P L^{-1}.

8.1.1 Solid and semi-solid manure. Results are converted to mg P kg^{-1} on an as-received basis as follows:

$$P, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:

C = extract/digest result, mg P L^{-1}

V = extract/digest volume, mL
M = sample mass (g) used in extraction or digest
DF = additional dilution factor, when applicable

8.1.2 Liquid manure. Results are converted to mg P L^{-1} on an as-received basis as follows:

$$P, mg\ L^{-1} = C \times DF$$

where:
C = extract/digest result, mg P L^{-1}
DF = additional dilution factor, when applicable

8.2 Client reports. Report all values to 1 mg L^{-1} or mg P kg^{-1}, on an as-received basis and to three significant digits. Alternate reporting units: solid manure can be reported as P% and lbs P ton^{-1}; liquid and semi-solid manure samples can be reported as mg P L^{-1}, lb P 1000 gal^{-1}, or lb $acre\text{-}inch^{-1}$. The client report should also provide the P_2O_5 concentration in lb ton^{-1} or lb 1000 gal^{-1} on an as-received basis. See Appendix A for conversions. Any P concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of six calibration standards for every batch of 20 or fewer samples. The calibration range is specific to the analysis method, but a calibration range of 0.2–50.0 mg P L^{-1} will include most manure samples. A higher calibration in the range of 50.0–250.0 mg P L^{-1} may be necessary for some samples. Any extract concentration above the highest calibration standard should be diluted and rerun. The lowest standard should ≤LOQ. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration.*

9.2 Calibration Blank (CB). CB should be analyzed after calibration and every 10 samples. *Acceptance criteria: The matrix-matched P concentration must be lower than the MDL. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination is eliminated.*

9.3 Method Blank (MB). After the CB, analyze one MB per batch of 20 or fewer samples. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, filters or apparatus. *Acceptance criteria: Background P should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until source of contamination is eliminated.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured P concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed CCV.*

9.5 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured P concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.6 Duplicate. Analyze a minimum of one duplicate sample one per analytical batch of 20 or fewer samples and calculate the RPD. The duplicate should be separated in the batch from the first sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; RPD. *Acceptance criteria: An RPD of ≤ 10% should be routinely achievable for P when the analyte concentration is greater than the LOQ. RPD as a statistical approach to evaluating precision is sensitive to concentration and can be expected to exceed 10% for where P concentration is low (< 5 mg P kg^{-1}). The operator may select samples for duplicate analysis that are expected to exceed to the LOQ, or may consider the use of a statistical test that is less sensitive to low analyte concentrations, such as Relative Standard Deviation (RSD), also known as the Coefficient of Variation (CV).*

9.7 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch of 20 or fewer samples. Used to evaluate precision, matrix interferences, and extraction efficiency, a spike of known P concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background P, but should not exceed the calibration range. A mid-range concentration from the calibration curve is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the P spike should be thoroughly mixed into the sample matrix

Table M-5.3.1. Quality control guidelines for the spectrophotometric phosphorus method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Calibration Blank (CB)	After calibration, every 10 samples	*P < MDL*
Method Blank (MB)	Every batch	*P < LOQ*
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 10% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%
Reference Material	Every batch	Within uncertainty limits

before extractant is added. Calculate percent MSR according to Appendix A: Definitions: Matrix Spike Recovery. *Acceptance criteria: If recovery of P falls outside the control limits of 100 ± 15%, P is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.8 Reference Material (RM). An RM with a matrix similar to that of the materials tested should be obtained to assess the accuracy and overall reliability of the analytical process and analyzed with each batch. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.9 Proficiency Requirement. The laboratory shall participate in a proficiency testing program for manure P that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

9.10 Analyte carry-over. Carryover should be determined for P for the spectrophotometric method based on the procedure method outlined in Appendix A. *Perform as needed or during preventive maintenance.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) shall be determined based on the method described by the US EPA in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. No data available.

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear safety glasses with side shields, chemical resistant gloves, and lab coat or apron.

11.2 Caution. Sulfuric acid causes severe skin burns and eye damage. Antimony potassium tartrate is acutely toxic if ingested or inhaled.

11.3 Reagents Disposal. Sulfuric acid may be corrosive to metals. Storage and disposal should be in a corrosion-resistant container. May require neutralization prior to disposal. Dispose of reagents in accordance with local/state and/or federal regulations.

11.4 Samples Disposal. Dispose of digests in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

Cho, Y. H., & Nielsen, S. S. (2017). Phosphorus determination by Murphy-Riley method. *In Food analysis laboratory manual.* Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-319-44127-6_17

Gales, M.E., Jr., Julian, E.C., & Kroner, R.C. (1966). Method for quantitative determination of total phosphorus in water. *American Water Works Association Journal,* 58, 1363–1368.

Murphy J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta,* 27:31–36.

Pai, S-C., Yang, C-C., & Riley, J. P. (1990). Effects of acidity and molybdate concentration on the kinetics of the formation of the phosphoantimonylmolybdenum blue complex: *Analytica Chimica Acta,* 229:115–120.

U. S. Environmental Protection Agency. (1993). Method 365.1. Determination of phosphorus by semi-automated colorimetry. Revision 2.0.

M-6.1
Water Extractable Phosphorus, 100:1 solution to solids ratio

AUTHOR: John T. Spargo

1.0 Scope and Application

This method provides a standardized measure of water-extractable P in manure and other organic residuals (e.g., biosolids, food waste, compost) for use as an index of dissolved P runoff loss potential following recent land application.

2.0 Method Principle

2.1 Method Description. This method follows procedures described by Kleinman et al. (2007). A representative subsample of as-received manure is extracted with water at a 100:1 solution:solids ratio for 1 hr, centrifuged and, if necessary, filtered. The extract is analyzed for P by ICP-OES and results are expressed on a dry-weight equivalent basis. The method includes both water-soluble organic and inorganic forms of P.

2.2 Method Performance. A laboratory performing this method should be capable of achieving an MDL ≤ 0.05 g P kg^{-1}, dry weight equivalent. MDLs should be determined following the procedure outlined in Appendix B. See Section 10.2 for method uncertainty.

2.3 Method Interferences. Sample processing and extraction ratio have a significant influence on results. Deviations from the protocol described in this method should be avoided. If any modifications are made, they should be reported to the end-user with appropriate qualifiers.

2.4 Method Reporting. Water-extractable P is expressed as g P kg^{-1} or mass % on a dry-weight basis.

2.5 Method Advantages. The method is relatively simple and reproducible.

2.6 Method Disadvantages. Heterogeneous materials can be problematic since subsamples cannot be dried or ground. Larger aliquots may be needed to obtain a representative subsample. In this case, a larger container will be required to maintain the 100:1 solution to solids ratio.

3.0 Definitions

3.1 WEP (100:1). Water extractable P, as determined by this method.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Samples should be held at ≤ 6 °C and extracted within 21 days of sample receipt. Do not freeze samples. Sample containers should be pre-washed with phosphate-free detergents and rinsed with reagent grade water. Plastic and glass containers are both suitable.

4.2 Sample Processing. The extraction should be performed on a representative subsample that has not been dried or ground. For further information, see Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. The recommended test portion size is 2.0 g, dry-weight equivalent of the as-received sample. *Note: if solids content is less than 1%, no water is added, and a 202 g subsample of the as-received sample is carried through the procedure.*

4.4 Total Solids Content. Subsample and determine Total Solids content according to Chapter M-1.1 and record.

5.0 Apparatus

5.1 Analytical balance, 300 g capacity and minimum accuracy of 0.001 g.

5.2 Reciprocating mechanical shaker capable of 180–200 excursions per minute (epm) or orbital shaker capable of 150 (rpm).

5.3 Filters, Whatman No. #40 or equivalent.

5.4 Centrifuge bottles, 250 mL, capable of withstanding maximum RCF of 1500 × g.

5.5 Centrifuge, capable of RCF of 1500 × g.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I.

6.2 Boiling Chips. PTFE chips used for method blank and lab fortified blank (e.g., Chemware PTFE Ultra Pure Boiling Stones Part # 26397-103)

6.3. Stock Standard Solution. Purchased or prepared stock P standard (1000 or 10,000 mg P L^{-1}) in 2% nitric acid. Used to prepare lab fortified blank.

6.4 Concentrated hydrochloric acid (HCl), 12 M. ACS grade.

6.5 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program.

7.0 Procedure

7.1 Determine the as-received sample weight needed to provide 2.0 g dry solids as follows:

$$sample\ size, g = 2 \times \frac{100}{solids, \%}$$

7.2 Weigh a subsample equivalent to 2.0 g solids into a 250 mL centrifuge bottle. Record the actual weight of the subsample.

7.3 Add reagent grade water to bring to a final weight of 202 g. If the total solids content of the sample is less than or equal to 1%, use a 202 g sample and do not add additional water.

7.4 Shake samples on a reciprocating mechanical shaker at 180–200 epm or an orbital shaker at 150 rpm for 60 minutes. Remove bottles from shaker and centrifuge at 1,500 × g for 10 minutes. If particulates are present, filter the supernatant (Whatman #40 or equivalent).

7.5 Analyze supernatant for P by ICP-OES according to Chapter M-5.2. If sample extract cannot be analyzed the same day, acidify to prevent precipitation of calcium phosphates by adding 5 drops of concentrated HCl for each 20 mL of extract to lower pH to approximately 2.0 and hold at ≤ 6 °C. Acidified extracts can be held for up to three weeks before P measurement.

8.0 Calculations and Reporting

8.1 Calculations. The WEP concentration on a dry weight basis is determined as follows:

$$WEP, g\ kg^{-1} = extract\ P\ concentration, mg\ L^{-1} \times \frac{0.2}{sample\ size, g} \times \frac{100}{solids, \%}$$

8.2 Client Reports. Report as g WEP kg^{-1} or mass percent on a dry weight basis. WEP concentration below LOQ should be reported as <LOQ.

The WEP concentration is used to calculate the P Source Coefficient (PSC) by multiplying by the appropriate term.

8.2.1 The Pennsylvania PSC is calculated as follows:

$$PSC = WEP\ dry\ weight\ basis, \% \times 1.17$$

8.2.2 For other states, use the appropriate coefficient to determine the PSC.

9.0 Quality Control

9.1 Method Blank (MB). Include one MB per batch of 20 or fewer samples. Prepare the MB using 2 g of boiling chips weighed into a 250 mL centrifuge bottle and brought to a final weight of 202 g with reagent grade water. The MB must be carried through the complete procedure. *Acceptance criteria: Background P should be below the LOQ. If criteria is exceeded, re-analyze MB. If second MB fails, halt analysis until source of contamination is eliminated.*

9.2 Laboratory fortified blank (LFB). Run one LFB with each analytical batch of 20 or fewer samples. Prepare by weighing 2 g of boiling chips into a 250 mL centrifuge bottle and adding sufficient stock P solution to obtain a final concentration ≤ 10x the LOQ when brought to a final weight of 202 g with reagent grade water. For example, add 1 mL of 1,000 mg P kg^{-1} stock P standard to 2-g of boiling chips in a 250 mL centrifuge bottle and bring to final weight of 202 g for a final concentration of 5 mg P L_1 (equivalent to 0.5 g P kg^{-1}, dry-weight basis). Carry the LFB through the entire WEP procedure. *Acceptance criteria: Recovery of P must be within 100±15%. If the recovery is not within 100 ± 15%, remake the LFB and reanalyze. If the LFB fails to pass the second time, identify and correct the source of the problem and re-run samples.*

9.3 Laboratory Duplicate. Include a minimum of one duplicate sample per analytical batch of 20 or fewer samples. Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference. *Acceptance criteria: An RPD of ≤ 20% should be routinely achievable for P when the analyte concentration is greater than the LOQ.*

9.4 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program.

Table M-6.1.1. Quality control guidelines for the WEP method.

Quality Control Element	Frequency	Acceptance Criteria
Method Blank (MB)	Every batch	P < LOQ
Laboratory fortified blank (LFB)	Every batch	Recovery, 100 ± 15%
Duplicate	Every batch	RPD ≤ 20% within the documented acceptance limits

Table M-6.1.2. Laboratory precision data for manure WEP method, as-received basis.

Sample ID	Total Solids Content (%)	Mean Content mg P kg^{-1}	Intra-Lab Standard Deviation S_r mg P kg^{-1}	Intra-Lab Repeatability r mg P kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg P kg^{-1}	Inter-Lab Reproducibility R mg P kg^{-1}
M 2014-D	3.12	3736	78	235	868	2605
M 2015-D	11.2	3095	69	208	632	1896
M 2012-B	56.5	6501	99	300	2060	6190
M 2016-F	68.4	1386	16	49	282	848

MAP Program, 10–12 laboratories reporting.

9.5 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least annually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. WEP method repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program coordinated by the Minnesota Department of Agriculture, in proficiency rounds conducted in 2012 and 2016. Laboratories utilized instruments from different manufacturers and the following precision data were obtained (Table M-6.1.2).

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear appropriate personal protective equipment, including lab coat, gloves, and safety glasses.

11.2 Caution. Use considerable caution when handling concentrated HCl. Prepare under hood, wear safety glasses and gloves, and avoid contact with skin.

11.3 Reagents Disposal. Dispose of reagents in accordance with local/state and/or federal regulations.

11.4 Samples Disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

Kleinman, P., Sullivan, D., Wolf, A., Brandt, R., Dou, Z., Elliott, H., Kovar, J., Leytem, A., Maguire, R., Moore, P.,. Saporito, L., Sharpley, A., Shober, A., Sims, T., Toth, J., Toor. G., Zhang, H., & Zhang, T. (2007). Selection of a water extractable phosphorus test for manures and biosolids as an indicator of runoff loss potential. *Journal of Environmental Quality*, 36:1357–1367.

Wolf, A. M., Moore, P. A., Kleinman, P. J. A., & Sullivan, D. M. (2009). Water-extractable phosphorus in animal manure and biosolids. p. 76-80. In J.L. Kovar and G.M Pierzynski (Eds.), Methods of phosphorus analysis for soils, sediments, residuals and waters. *Southern Cooperative Series Bulletin,* #408, Virginia Tech University.

Wolf, A.M., Kleinman, P. J. A., Sharpley, A. N., & Beegle, D. B. (2005). Development of a water-extractable phosphorus test for manures: An inter-laboratory study. *Soil Science Society of America Journal,* 67:695–700.

M-7.1
Calcium Carbonate Equivalent (CCE)

AUTHORS: Kristin Hicks, Tim Hoerner, and John T. Spargo

1.0 Scope and Application

This method quantitatively determines the acid-neutralizing capacity of manure or other organic residuals (e.g., biosolids, food waste, compost) relative to pure calcium carbonate ($CaCO_3$) by reacting with acid at elevated temperature and titrating with base to a potentiometric or indicator endpoint. The test provides useful information for any material that may have significant calcium carbonate equivalence (CCE), such as lime-stabilized biosolids, layer litter, paper mill waste, and ash. The method is applicable to both solid and liquid waste materials. Land application rates should not exceed CCE loading rates necessary to optimize soil pH, as determined by soil testing.

2.0 Method Principle

2.1 Method Description. Calcium carbonate equivalence is a measure of the acid-neutralizing capacity of a waste material and is expressed as a percentage of pure calcium carbonate ($CaCO_3$). This method is adapted from the AOAC 955.01 procedure for Neutralizing Value for Liming Materials, and consists of reacting the material with 50 mL 0.5 M HCl and back-titrating with 0.25 M NaOH to a potentiometric (pH 7.0) or pH indicator (phenolphthalein) endpoint. The volume of NaOH required to attain the endpoint is inversely related to the CCE.

2.2 Method Performance. A laboratory performing this method should be capable of achieving an MDL of 0.3–0.5% CCE.

2.3 Method Interferences.

2.3.1 HCl is volatile and may be lost if the solution is boiled at too high a temperature or for too much time. This will cause an overestimation of CCE.

2.3.2 Materials containing large amounts of ferrous iron or humic matter (resulting in colored acid digest) will interfere with observation of phenolphthalein endpoint. These samples should be titrated using a potentiometric endpoint.

2.3.3 Heterogeneous materials such as manure, compost, or other waste materials can result in non-representative subsampling and poor precision. Thoroughly homogenize dried and ground samples before subsampling for analysis. A larger subsample size should be used if duplicate analysis indicates high variability.

2.3.4 Highly reactive samples may boil over during the heating process. For samples that boil over, the sample may be analyzed by 1) weighing less material (0.5 g or 5 mL instead of the recommended 1 g or 10 mL), 2) adding the acid and letting the sample rest for one hour before heating, 3) using an anti-foaming agent, or 4) a combination of these approaches.

2.3.5 Grinding the sample material will increase the specific surface area of the material. This may result in a CCE value that differs from the effective CCE of the as-received material.

2.4 Method Advantages. The method is relatively simple and does not require costly equipment or materials.

2.5 Method Disadvantages. The method is somewhat laborious and slow and involves the use of a strong acid and base.

3.0 Definitions

3.1 Calcium carbonate equivalence (CCE). An expression of the liming effectiveness of a material in terms of its equivalence value to pure calcium carbonate, given in %.

3.2 Effective calcium carbonate equivalence (ECCE). An expression of agricultural lime effectiveness based on the combined effect of the CCE and the fineness of grind. Required for product labeling purposes in some states. ECCE is also referred to as effective neutralizing value

(ENV), total neutralizing power (TNP), and effective neutralizing material (ENM).

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Due to the effect of moisture and temperature on pH, stability of the sample in storage depends on the percent solids of the material and on the storage conditions. Solid and semi-solid materials dried at 105 °C overnight or until constant weight is achieved may be stored for up to several months in sealed plastic vials or bags. If the solid or semi-solid sample is not dried upon receipt, it may be stored at ≤ 6 °C for up to 7 days. Slurry and liquid samples may be stored at ≤ 6 °C for up to 7 days.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample material and dry at 105 °C overnight or until constant weight is achieved. Longer drying times may be necessary for samples with low solids content such as sludges. The dried sample should be ground to pass a 1 mm sieve or finer. To report CCE on an as-received basis, weigh the sample before and after drying (see Section 8.0). Slurry (4–10% solids) and liquid (< 4% solids) are best analyzed as-received, without drying.

4.3 Sample Test Portion. For solid samples, the recommended test portion size is 1.0 g, dry weight equivalence. Minimum recommended test size is 0.2 g. For liquid samples, the recommended test portion size is 10 mL.

5.0 Apparatus

5.1 Drying oven, forced-air, capable of heating to 110 °C

5.2 250 mL Erlenmeyer flasks, watch glasses, 250 mL beakers

5.3 Graduated cylinder, Class A, or calibrated re-pipettor to measure 50 mL HCl

5.4 Analytical balance that reports to 0.001 g

5.5 Hotplate capable of heating to 150 °C

5.6 Stir plate with magnetic stir bars or an overhead stirrer.

5.7 50–100 mL titration burette calibrated to 0.1 mL increments

5.8 pH meter with glass and reference combination electrode (for potentiometric endpoint procedure)

6.0 Reagents and Standards

6.1 Reagent grade water, ASTM Type I or II.

6.2 NaOH, 0.25 M. Purchase certified pre-standardized 0.25 M NaOH solution OR prepare as follows:

6.2.1 Dissolve 10 g NaOH pellets in about 800 mL of reagent grade water in 1 L volumetric flask, allow to cool, and bring to volume. Store in a polyethylene bottle. Standardize as follows:

6.2.2 Dry 6–9 g potassium acid phthalate (KHP), $KH(C_8H_4O_4)$, 110 °C for 2 hours. Cool in a desiccator before weighing.

6.2.3 Weigh 1.20–1.40 g KHP into each of three 250 mL flasks and dissolve in 50–75 mL reagent grade water, warming if necessary. Record the actual weight of KHP weighed into each flask. This is equivalent to about 6–7 mmol of H+ and should require 24–28 mL 0.25 M NaOH to neutralize.

6.2.4 Rinse the burette thoroughly with the NaOH titrant and fill.

6.2.5 Add 3–4 drops of phenolphthalein indicator to each flask and titrate to the first faint pink that persists for 20 seconds. Titrate dropwise near the endpoint. Record the volume of titrant to reach the endpoint.

6.2.6 Calculate the molarity of the NaOH using the formula weight 204.22 mg $mmol^{-1}$ for KHP as follows:

$$Molarity, NaOH = \frac{KHP, mg}{\left(204.22\ \frac{mg}{mmol}\ KHP\right) \times (vol.NaOH\ to\ reach\ end\ point, mL)}$$

Ensure that all three replicates are close to 0.25 M NaOH and are within 5% of each other.

6.3 HCl, 0.5 M. Purchase certified pre-standardized 0.5 M HCl solution or prepare as follows:

6.3.1 Add 41.5 mL concentrated HCl to approximately 800 mL of reagent grade water in a 1 L volumetric flask, allow to cool, and bring to volume. Store in glass or a polyethylene bottle. Standardize as follows *(Note, by AOAC 955.01, HCl can be standardized against standardized NaOH).*

6.3.2 Dry 4.0– 5.0 g of the primary standard TRIS Base, $C_4H_{11}NO_3$, at 110 °C for 2 hours. Cool in a desiccator to room temperature before weighing.

6.3.3 Weigh 0.400–1.000 g of TRIS into three 50 mL beakers. Add approximately 20 mL reagent grade water to each and dissolve TRIS.

6.3.4 Rinse the burette thoroughly with the HCl titrant and fill.

6.3.5 Add two drops of bromocresol green/methyl red mixed indicator to each beaker with TRIS and titrate with HCl to the endpoint denoted by a color change from green to red. Record the volume of HCl titrant to reach the endpoint.

6.3.6 Calculate the molarity of the HCl by using the formula weight of 121.14 mg $mmol^{-1}$ for TRIS as follows,

$$Molarity, HCl = \frac{TRIS, mg}{\left(121.14\ \frac{mg}{mmol}\ TRIS\right) \times (vol.HCl\ to\ reach\ end\ point, mL)}$$

Ensure that all 3 replicates are close to 0.5 M HCl and are within 5% of each other.

6.4 Reagent grade calcium carbonate reference material (RM), 99.8% purity. Store in its original container, with the cap tightly closed, in a dry environment, under normal laboratory temperatures, and protected from acid fumes.

6.5 Reference pH buffer solutions (e.g., pH 4.00, 7.00, and 10.00) to calibrate and a pH 7.0 buffer used for initial and continuing calibration verification.

6.6 Phenolphthalein solution, 0.1 g in 100 mL ethanol (used to standardize NaOH and indicator endpoint procedure only).

6.7 Bromocresol green/methyl red mixed indicator (used to standardize HCl).

6.8 Potassium acid phthalate (KHP), $KH(C_8H_4O_4)$ (used to standardize NaOH).

6.9 Primary standard TRIS base (THAM), $C_4H_{11}NO_3$ (used to standardize HCl).

7.0 Procedure

7.1 Weigh 1.0 g dried sample and RM or 10 mL liquid material into a 250 mL Erlenmeyer flask. Record weight of the liquid sample and, if other than 1.0 g, the weight of the dry material.

7.2 Add 50.0 mL of 0.5 M HCl to each sample and RM.

7.3 Place a glass flask cover on each flask to aid condensation and reduce evaporation.

7.4 Place the flask on the hot plate and boil gently for five minutes. Use anti-foaming agent if sample begins to boil over. Remove from heat and allow to cool to room temperature.

7.5 For each batch, analyze RM, MB, duplicate and CCV as indicated in Section 9.0.

7.6 Potentiometric endpoint procedure

7.6.1 Calibrate meter following manufacturer's instructions with at least three standard buffer solutions that encompass the expected range of the samples during titration (e.g., 4.00, 7.00, and 10.00). Follow protocols outlined in Section 9.0 for acceptance criteria.

7.6.2 Transfer sample to a 250 mL beaker, add a magnetic stir bar, insert pH electrode, and stir at medium speed. Optional: To prevent acid loss that will result in CCE values being overestimated, this step may be performed in the original Erlenmeyer flask.

7.6.3 Using a buret, titrate the sample to pH 7.00 ± 0.05 with 0.25 M NaOH. Deliver NaOH solution rapidly to pH 5.00, then dropwise until the solution reaches pH 7.00 and remains constant for approximately 1 min while stirring. *Note: if endpoint is passed, add 1 mL or less 0.5 M HCl to bring pH to less than 7.0 and back titrate slowly to 7.00. Add the volume of additional 0.5 M HCl to the initial 50 mL for calculation of CCE.*

7.7 Indicator endpoint procedure

7.7.1 Transfer sample to a 250 mL beaker, add a magnetic stir bar, add a few drops of phenolphthalein indicator solution, and stir at moderate speed.

7.7.2 Using a buret, titrate the sample with 0.25 M NaOH. Deliver NaOH until the phenolphthalein endpoint is reached; the solution turns a faint pink color. *Note: if the endpoint is passed, add 1 mL or less 0.5 M HCl until the solution clears and back titrate slowly to the endpoint. Add the volume of additional 0.5 M HCl to the initial 50 mL for calculation of CCE.*

8.0 Calculations and Reporting

8.1 Method Reporting. Results should be reported in percent CCE on an as-received and dry-weight basis to the nearest 1%.

8.2 CCE is calculated as follows:

$$CCE, \% = 5 \times \frac{[(HCl, M\ \times\ HCl, mL) - (NaOH, M\ \times\ NaOH, mL)]}{sample\ mass, g}$$

8.2.1 Expression of results on a dry-weight basis. If the sample was dried at 105 °C prior to determining CCE, report results calculated in 8.2. If the sample was run as-received, calculate CCE on a dry weight basis as follows:

$$CCE, \%\ (dry\ weight) = \frac{[CCE, \%\ (as - received)]}{(Solids, \%\ \div 100)}$$

8.2.2 Expression of results on an as-received basis. If the sample was run as received, report results calculated in 8.2. If the sample was dried at 105 °C prior to determining CCE, report CCE on an as-received basis as follows:

$$CCE, \%\ (as\text{–}received) = [CCE, \%\ (dry\ weight)] \times (Solids, \%\ \div\ 100)$$

9.0 Quality Control

9.1 Calibration (valid for potentiometric procedure only). Calibrate pH meter on the day of use, using pH buffers bracketing unknown samples. *Acceptance criteria: Slope of calibration should be within 98–102%. If the slope is not within this range, recalibrate and check slope again. If an acceptable slope cannot be achieved, discontinue the analysis, and diagnose and address the problem.*

9.2 Continuing Calibration Verification (CCV) (valid for potentiometric procedure only). Run a pH 7.0 buffer CCV immediately after calibration (should be from a second source), after every 10 samples (can be primary or secondary source), and at the end of the batch. *Acceptance criteria: pH results must be within 7.0 ± 0.1. If not, run a second aliquot of the pH 7.0 buffer. If the second CCV fails, identify the problem, recalibrate the electrode, and reanalyze the previous 10 samples.*

9.3 Duplicate. Perform one duplicate per batch 20 or fewer samples. Calculate RPD according to Appendix A: Definitions: Laboratory duplicate; Relative Percent Difference. *Acceptance criteria: RPD of duplicates should be ≤ 20%. If the RPD exceeds 20%, this may indicate that a representative secondary subsample was not obtained. Re-homogenize the secondary sample and repeat the duplicate analysis. Evaluate laboratory homogenization procedures.*

9.4 Reference Material (RM), Laboratory Grade (99.8%) $CaCO_3$. Run one calcium carbonate RM (~0.5 g) at the beginning of each analytical batch. *Acceptance criteria: The minimum acceptable range is 97–103% CCE or within the stated uncertainty range.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). The Method Detection Limit (MDL) shall be determined based on the method described in Appendix B. Verify MDL and LOQ at least annually or when apparatus is repaired or replaced. LOQ is calculated as follows:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Precision. No data available.

11.0 Safety and Disposal

11.1 Laboratory Safety. This method involves using acid under boiling conditions on materials that may be highly reactive. When handling acids under reactive conditions, wear protective clothing, chemical-resistant gloves, and a face shield. When handling acids under non-reactive conditions, wear protective clothing, standard latex or nitrile gloves, and safety glasses.

11.2 Caution. Not applicable.

11.3 Reagents Disposal. Neutralize prior to disposal.

11.4 Samples Disposal. No special considerations needed.

12.0 Reference Documents and Additional Resources

AOAC. (1998). *Official methods of analysis* (16th ed). Method 955.01—Neutralizing value for liming materials.

M-8.1
pH

AUTHORS: John T. Spargo, Rachel Brimmer, Jason Lessl, Bryan Thayer, and Kristin Hicks

1.0 Scope and Application

This is a potentiometric method for directly measuring undiluted liquid manures or a laboratory-prepared water slurry of solid or semi-solid manures. pH is a measure of active acidity and can vary among manures depending on the species, feed, bedding, and storage and treatment practices associated with the production system. Since pH is not a measure of total acidity or alkalinity, it cannot be used to predict the effect manure will have on soil pH following application. The pH of the manure does influence ammonia volatilization potential. Ammonia volatilization can be reduced by decreasing manure pH with amendments such as alum or ferrous sulfate.

2.0 Method Principle

2.1 Method Description. For a liquid manure, pH is measured directly by electrode (adapted from US EPA 9040C). For solid or semi-solid manure, pH must be measured in a laboratory-prepared slurry (adapted from US EPA 9045D). The following methods outline manure pH measurement in liquids and in semi-solid or solid manures at a 1:2 manure/water (v/v) slurry. Hygroscopic samples may require more water to create enough slurry for proper pH electrode operation. Modifications in the manure/water ratio must be reported with the results.

2.2 Method Performance. With proper calibration, results should be reproducible within ± 0.1 pH units. Method detection limits are not applicable for pH.

2.3 Method Interferences.

2.3.1 Samples with very low or very high pH may give incorrect readings. For samples with a true pH of > 10, the measured pH may be incorrectly low. This error can be minimized by using a low-sodium-error electrode. Strong acid solutions, with a true pH of < 1, may give incorrectly high pH measurements.

2.3.2 Errors will occur when the electrode becomes coated. If an electrode becomes coated with an oily material that will not rinse free, the electrode should be cleaned per the manufacturer's instructions.

2.3.3 Temperature fluctuations will cause measurement errors.

2.4 Method Reporting. Report pH to the nearest 0.1 pH unit. For samples not analyzed as-received, indicate the ratio of manure to water. If additional water is needed to obtain a slurry consistency, the required volume should be noted.

2.5 Method Advantages. pH measurement systems range from simple bench-top meters to automated measurement systems.

2.6 Method Disadvantages. It can be challenging to create an adequate 1:2 (v/v) slurry with hygroscopic samples.

3.0 Definitions

3.1 pH. A logarithmic measure of the activity of H+ ions in solution. Solutions with a pH less than 7.0 are acidic and solutions with a pH greater than 7.0 are alkaline.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Samples should be stored at ≤ 6 °C and analyzed as soon as possible but no later than 7 days after sample receipt. Not recommended for freezing.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid manures (10–20% solids), the primary sample should be mixed and analyzed as-received (i.e., do not dry). Avoid excessive homogenization techniques as this will alter sample pH. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling, may be manually mixed immediately prior to analysis, and may be analyzed directly on the as-received sample. For further information, see Chapter 2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. For liquid samples, pH is measured on a 25 mL mixed test portion or directly on the mixed primary sample. For solid and semi-solid sam-

ples (1:2, v/v, manure:water), pH is measured on a 20.0 cm^3 test portion from the ≥ 200 cm^3 homogenized secondary sample.

5.0 Apparatus

5.1 Analytical balance, accurate to 0.1 g.

5.2 Scoop, 20 cm^3 volumetric (or other appropriate size).

5.3 Beaker, 50–100 mL, or container of similar volume.

5.4 Glass stir rod or reciprocating horizontal mechanical shaker capable of 180–200 excursions per minute (epm), or orbital capable of 150 rpm.

5.5 Dispenser, able to deliver 20–50 mL reagent grade water. Manual syringes or other appropriate dispensing equipment may be used.

5.6 pH meter with temperature compensation and reference electrode.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I or II.

6.2 Standard pH buffer solutions (purchased): pH 4.00, 6.00, 7.00, 10.00, and 12.00.

6.3 Reference Material (RM). Certified standard or internal RM with a matrix similar to the unknown samples.

7.0 Procedure

7.1 Calibrate pH meter using three standard buffer solutions according to manufacturer specifications, following additional quality control protocols outlined in Section 9.0. Allow samples to warm to room temperature (20–24 °C) prior to analysis to reduce temperature compensation. *Note: If EC is measured on the same subsample, it should be analyzed before pH measurement to prevent KCl interference.*

7.2 Liquid manure, direct measurement

7.2.1 Thoroughly mix liquid manure by shaking the bottle. Pour about 25 mL into a cup.

7.2.2 Immerse pH electrode and record the pH value when the meter has stabilized. Rinse probe with reagent grade water between measurements.

7.3 Semi-solid or solid manure, 1:2 v/v

7.3.1 Scoop 20 cm^3 semi-solid/solid manure (as-received) into a sample cup. Add 40 (1:2 ratio) mL reagent grade water. Additional dilutions are permitted if working with hygroscopic or low moisture materials. If the sample absorbs all reagent water, add water in 10 mL increments until the appropriate slurry consistency is obtained. Note the addition of water and include the final ratio on the report.

7.3.2 Stir well for 10 seconds with a glass stir rod or cover with parafilm and place on a reciprocating shaker for 5 minutes. If stirring manually, allow to stand for 30 minutes. If using a shaker, allow to stand for 15 minutes.

7.3.3 Immerse electrode probe into slurry and allow to equilibrate. When the meter has stabilized, record the pH value as pH (1:2, v/v, manure:water). Rinse probe between measurements.

8.0 Calculations and Reporting

8.1 Report manure pH to the nearest 0.1 pH unit.

8.2 Report semi-solid and solid manure results as pH (1:2, v/v, manure:water), adjusting the ratio as necessary.

9.0 Quality Control

9.1 Calibration. Calibrate pH meter on the day of use, using pH buffers bracketing unknown samples. *Acceptance criteria: Slope of calibration should be within 98–102%. If the slope is not within this range, recalibrate and check slope again. If an acceptable slope cannot be achieved, discontinue the analysis, and diagnose and address the problem.*

9.2 Method Blank. Not applicable.

9.3 Continuing Calibration Verification (CCV): Check calibration with standard buffers 6.00 and 10.00 immediately after initial calibration, after every 10 samples, and at the end of every analytical batch. *Acceptance criteria: Results within ± 0.05 units of the buffer value. If CCV is out of range, recalibrate the pH meter, and retest the 6.00 and 10.00 buffer solutions to verify calibration. Retest all samples run since last acceptable calibration verification.*

9.4 Duplicate. Perform replicate analysis on at least 5% of samples in each batch. *Acceptance criteria: An RPD of ≤ 10% should be routinely achievable for pH values 5.0–9.0. Calculate RPD according to Appendix A: Definitions: Laboratory duplicate; Relative Percent Difference.*

9.5 Matrix Spike Recovery (MSR). Not applicable.

9.6 Reference Material (RM): Manure, sludge, or soil RM may be used. Include one RM with each batch of samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.7 Proficiency testing. It is recommended that the laboratory participate in a proficiency testing program for analysis of manures such as the Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program.

10.0 Limit of Quantitation and Method Performance

10.1 LOQ, not applicable.

10.2 Method Performance: pH test repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program coordinated by the Minnesota Depart-

Table M-8.1.1. Laboratory precision data for manure pH.

Sample ID	Total Solids Content (%)	pH	Intra-Lab Standard Deviation S_r	Intra-Lab Repeatability r	Inter-Lab Reproducibility standard deviation S_R	Inter-Lab Reproducibility R
M 1414-A	3.2	7.27	0.06	0.16	0.25	0.70
M 2015-D	15.7	5.33	0.04	0.11	0.32	0.90
M 2012-B	56.5	7.07	0.03	0.10	0.51	1.27
M 2016-F	68.4	7.54	0.05	0.12	0.20	0.56

MAP Program, 41–43 laboratories reporting. Repeatability and reproducibility calculated based on ISO 5725-2:1994.

ment of Agriculture, from proficiency rounds conducted in 2015 and 2016 of 10 laboratories. Laboratories utilized instruments from different manufacturers and the following precision data were obtained (Table M-8.1.1)

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear safety glasses, standard nitrile gloves, and a lab coat to protect against exposure to manure material and calibration standards.

11.2 Caution. Not applicable.

11.3 Reagent Disposal. Not applicable.

11.4 Sample Disposal. Samples should be disposed of according to state requirements.

12.0 Reference Documents and Additional Resources

U. S. Environmental Protection Agency. (2004). Method 9040C. pH electrochemical measurement. Test methods for evaluating solid wastes, physical/chemical methods. Publication SW-846. National Technical Information Service.

U. S. Environmental Protection Agency. (2004). Method 9045D. Soil and waste pH test methods for evaluating solid wastes, physical/chemical methods. Publication SW-846. National Technical Information Service.

M-9.1
Electrical Conductivity (EC)

AUTHORS: John T. Spargo, Rachel Brimmer, Jason Lessl, Bryan Thayer, and Kristin Hicks

1.0 Scope and Application

Electrical conductivity (EC) provides a measure of the ability of an aqueous solution to carry a current. EC of an aqueous solution depends on the total concentration, mobility, and valence of ions and on the temperature of the sample. For this reason, EC measurements can be used to provide an estimate of total dissolved solids and salt loading when manure is land-applied.

For a liquid manure, EC can be directly measured using a conductivity cell and meter; for solid or semi-solid manure, however, EC must be measured in a slurry. As with soil EC, different manure-to-water ratios can be used based on characteristics of the sample and how the results will be used.

2.0 Method Principle

2.1 The following methods outline direct EC measurement in a liquid manure, without diluting (adapted from US EPA 9050A), or in a semi-solid or solid manure using either a 1:2 manure/water slurry (v/v) ratio (adapted from South. Coop. Bull 289, 2014) or a 1:5 dry weight equivalent manure:water (dw_{eq}/v) ratio (adapted from TMECC 4.10-A). Hygroscopic samples and those containing large amounts of hay or sawdust may require more water to create sufficient slurry for proper EC electrode operation.

EC of the solution or slurry is measured at room temperature (25 °C) using a self-contained conductivity meter. These slurries may also be used for pH measurement following EC determination.

2.2 Method Performance. Method detection limits are not applicable for EC.

2.3 Method Interferences. Platinum electrodes can degrade and cause erratic results. Re-platinize or replace the electrode as needed. Measured values of the EC may be influenced by contamination of the electrodes. Air bubbles or other contaminants on the electrodes may cause erratic measurements.

2.4 Method Reporting. EC is reported as dS m^{-1} at 25 °C.

2.5 Method Advantages. The method is simple and inexpensive. The direct measurement of EC on liquid samples requires minimal sample preparation. The 1:2 (v/v) ratio manure/water slurry requires simple preparation steps. The 1:5 (dw_{eq}/v) ratio is generally more reproducible for drier samples since the slurry is produced based on weight rather than volume of manure.

2.6 Method Disadvantages. Uncertainty increases with higher concentrations of salts. For drier or highly heterogeneous samples, it can be difficult to collect consistent subsamples based on volume; the precision of the 1:2 (v/v) ratio slurries may therefore be poor for these manures. Preparation of the 1:5 (dw_{eq}/v) ratio slurry requires that the solids content be determined on a parallel sample and that additional calculations be performed.

3.0 Definitions

3.1 Electrical Conductivity (EC). Conductivity is the ability of a solution to pass an electric current. The conductivity of a solution is affected by the concentration of total dissolved solids and the temperature of the solution.

3.2 Reference solution (RS). An external solution, which has been established to be fit for its intended use in a measurement process (e.g., standard KCl reference solution used for calibration and continuing calibration verification of an EC meter).

3.3 Solids, Total Dissolved (TDS). Total dissolved solids is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized, or colloidal suspended form.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Manure samples should be refrigerated at ≤ 6 °C upon receipt to retard microbial activity and volatilization losses. Refrigerated holding time should not exceed 7 days.

4.2 Sample Processing. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsa-

mpling, may be manually mixed immediately prior to EC analysis, and may be analyzed directly on the as-received sample. For solid (> 20% solids) and semi-solid manures (10–20% solids) analyzed on a 1:2 v/v ratio basis, the primary sample should be mixed and the secondary sample analyzed on an as-received basis. Avoid excessive homogenization techniques that may release ions from biomass resulting in higher apparent conductivity. For further information, see Chapter 2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. For liquid samples, EC is measured on a 25 mL mixed, test portion or directly on the mixed, primary sample. For solid and semi-solid samples (1:2, v/v, ratio), EC is measured on a 20.0 cm^3 test portion from the ≥ 200 cm^3 homogenized, secondary sample. For EC on a 1:5 (dw_{eq}/v) ratio, the test portion is 40.0 g dry weight equivalent of an as-received sample.

4.4 Total Solids Content. If measuring EC on a 1:5 (dw_{eq}/v) ratio slurry, subsample and determine Total Solids content according to Chapter M-1.1 and record.

5.0 Apparatus

5.1 Analytical balance, accurate to 0.1 g.

5.2 Scoop. 10–20 cm^3.

5.3 Beaker, 100–150 mL, glass or plastic.

5.4 Dispenser. To deliver 20 or 40 mL of reagent grade water.

5.5 Magnetic stirrer and Teflon-coated stir bar and/or glass stir rod.

5.6 Shaker. Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm), or orbital shaker capable of 150 opm.

5.7 Conductivity meter with temperature compensation and electrode.

6.0 Reagents and Standards

6.1 Reagent grade water. ASTM Type I or II.

6.2 Boiling chips, glass beads, or reagent grade sand used to prepare method blank samples.

6.3 Commercial KCl Reference Solutions: 718 and 1,413 dS m^{-1} (or other appropriate standards covering working range).

7.0 Procedure

7.1 Calibrate the conductivity meter using the standard KCl solutions, following manufacturer's instructions. Follow protocols outlined in Section 9.0 for quality control. Allow samples and standards to warm to room temperature (20–24 °C) prior to analysis to reduce temperature compensation.

7.2 Liquid manure, direct measurement.

7.2.1 Thoroughly mix liquid manure by shaking the bottle. Pour about 25 mL into a cup.

7.2.2 Immerse EC electrode and temperature probe (if applicable) and record the EC value when the meter has stabilized as dS m^{-1}. Rinse probe between measurements.

7.3 Semi-solid or solid manure, 1:2 (as-received) v/v.

7.3.1 Scoop 20 cm^3 semi-solid/solid manure into a sample cup. Add 40 (1:2 ratio) mL reagent grade water. Additional dilutions are permitted if working with hygroscopic or low-moisture materials. If the sample absorbs the reagent water, add additional water in 10 mL increments until the appropriate slurry consistency is obtained. Note the addition of water and include the final ratio on the report.

7.3.2 Stir well for 10 seconds with a glass stir rod or cover with parafilm and place on a shaker for 5 minutes. If stirring manually, allow to stand for 30 minutes. If using a shaker, allow slurry to stand for 10 minutes.

7.3.3 Immerse electrode and temperature probe into slurry and allow to equilibrate with the sample. When the meter has stabilized, record the EC value dS m^{-1} (1:2, v/v). Rinse probe between measurements.

7.4 Semi-solid or solid manure, 1:5 (dw_{eq}/v) ratio.

7.4.1 Determine the as-received sample weight needed to provide 40.0 g solids as follows:

$$Sample\ mass, g = 40 \times \frac{100}{solids, \%}$$

See Chapter M-1.1 to determine total solids content.

7.4.2 Weigh the as-received subsample equivalent to 40.0 g dry solids into a container (e.g., 500 mL screw top plastic bottle).

7.4.3 Bring the final slurry to a 1:5 dry weight, g: water, mL ratio by adding sufficient reagent grade water to bring the final weight to 240 g (assumes 1 mL of water weighs 1.0 g).

7.4.4 Place the container on a shaker for 20 min at 180 epm or opm. Allow to stand for 10 minutes.

7.4.5 Immerse electrode and temperature probe into slurry and allow them to equilibrate with the sample. When the meter has stabilized, record the EC value as dS m^{-1} (1:5, dw_{eq}/v).

Table M-9.1.1. Laboratory precision data for manure EC (1:5) method, as-received basis.

Sample ID	Total Solids Content (%)	EC Content dS m^{-1}	Intra-Lab Standard Deviation S_r dS m^{-1}	Intra-Lab Repeatability r dS m^{-1}	Inter-Lab Reproducibility standard deviation S_R dS m^{-1}	Inter-Lab Reproducibility R dS m^{-1}
M 2014-D	3.2	1.05	0.01	0.02	0.12	0.36
M 2015-D	11.2	3.30	0.01	0.04	0.33	1.01
M 2012-B	56.5	7.45	0.08	0.25	3.06	9.18
M 2016-F	68.4	3.48	0.04	0.11	0.92	2.76

MAP Program, 32–36 laboratories reporting.

8.0 Calculations and Reporting

8.1 Report EC of the as-received manure as dS m^{-1} (or mmho/cm) to the nearest 0.1 dS m^{-1}. If EC was measured on a slurry, note the ratio used and whether it was based on manure volume or mass (e.g., 1:2, v/v or 1:5, dw_{eq} w/v).

9.0 Quality Control

9.1 Calibration. After calibration and before measuring samples, verify calibration using appropriate standards covering expected range. *Acceptance criteria: ± 2% for 20 dS m^{-1} and below, and ± 5% above 20 dS m^{-1}.*

9.2 Method blank (MB). Include a MB sample with each batch. The MB should match the matrix of the sample and be carried through the entire analytical process. For liquid samples, use reagent grade water as the blank. For solid or semi-solid samples, use boiling chips, glass beads, or reagent grade sand. *Acceptance criteria: Repeat the extraction if the value of the MB exceeds 0.1 dS m^{-1}.*

9.3 Continuing Calibration Verification (CCV). Check the EC of a standard KCl solution before running any samples, after every 10 samples, and at the end of each batch. *Acceptance criteria: Solution should read ±2% for 20 dS m^{-1} and below, and ±5% above 20 dS m^{-1}.*

9.4 Duplicates. Perform one duplicate per analytical batch. Calculate RPD according to Appendix A: Definitions: Laboratory duplicate; Relative Percent Difference. *Acceptance criteria: The RPD should be ≤ 15%. If the RPD exceeds 15%, this indicates that a representative subsample was not obtained. Resample and repeat the duplicate analysis.*

9.5 Reference Solution (RS). Include at least one standard RS with each batch of samples. *Acceptance criteria: the standard RS result should be within the specified uncertainty limits.*

9.6 Proficiency testing. It is recommended that the laboratory participate in a proficiency testing program for analysis of manures, such as the Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

10.0 Limit of Quantitation and Method Performance

10.1 LOQ. not applicable.

10.2 Method Performance: EC method repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program coordinated by the Minnesota Department of Agriculture, from proficiency rounds conducted in 2015 and 2016 with 10 laboratories. Laboratories utilized instruments from different manufacturers and the following precision data were obtained (Table M-9.1.1.)

11.0 Safety and Disposal

11.1 Laboratory Safety. Follow routine laboratory safety protocols for handling manure. Wear safety glasses, gloves, and appropriate lab clothing.

11.2 Caution. Not applicable.

11.3 Reagent Disposal. Dispose of KCl standard according to state requirements.

11.4 Sample Disposal. Dispose of manure samples according to state requirements.

12.0 Reference Documents and Additional Resources

Southern Cooperative Series. (2014). Soil test methods from the southern United States. *Southern Cooperative Series Bulletin,* 419. ISBN# 1581614195.

USDA and U. S. Composting Council. (2001). Method 4.10-A. Electrical conductivity for compost, 1:5 slurry method, mass basis. In: *Test methods for the examination of composting and compost*. W.H. Thompson (Ed.).

U. S. Environmental Protection Agency. (1996). Method 9050A. Specific conductance test methods for evaluating solid waste. Publication SW-846.

M-10.1
Chloride by Spectrophotometry

AUTHOR: Robert O. Miller

1.0 Scope and Application

This method is quantitative for the concentration of chloride (Cl^-) in liquid and solid animal manure by extraction with 0.01 M $Ca(NO_3)_2$ and the subsequent spectrophotometric analysis. Cl^- may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The Cl^- content of a manure sample is determined by extraction with a calcium nitrate solution and subsequent spectrophotometric analysis based on a modification of the mercury (II) thiocyanate ($Hg(SCN)_2$) method of US EPA 9251. Chloride is displaced by thiocynate; in the presence of ferric iron, thiocynate forms a ferric thiocyanate chromophore at 480 nm which is stable and proportional to the Cl^- content.

2.2 Method Performance. The user should be able to obtain an MDL of 2 mg Cl^- kg^{-1} (as-received sample basis, instrument manufacturer dependent). An MDL should be determined and reported individually by the laboratory upon completion of the MDL outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B, Section 10.2 for method uncertainty.

2.3 Method Interferences. Spectrophotometrically clarify samples that are colored in the absorption range (480 nm) that may interfere, using a dialyzer membrane or filtering the sample using a membrane filter (0.45 µm). Alternately, some instruments include a color blanking feature that measures the sample color with and without reagent and corrects for the absorbance effect of the sample color.

2.4 Method Reporting. Chloride content is measured as mg Cl^- L^{-1} and reported on an as-received basis as mg Cl^- kg^{-1} or lbs per ton^{-1} for solid and mg Cl^- L^{-1} or lbs per 1000 gal^{-1} for liquid manures.

2.5 Method Advantages. Avoids use of concentrated acids; well-suited to automation; reliable.

2.6 Method Disadvantages. Potential contamination from filter paper and operator. Moderate precision. Mercuric thiocynate is highly toxic and requires hazardous waste disposal. Analyte may be below MDL for liquid samples.

3.0 Definitions

3.1 Chloride (Cl^-). An ion which forms inorganic, covalent bonds with one of several elements, metals and non-metals. Relatively inert toward reactions with nitrogen, oxygen, and carbon. Typically found as salts of sodium, potassium, calcium, and magnesium.

3.2 Spectrophotometric analysis. Determination of an analyte in a liquid matrix by reaction with a chromophore and measurement of the amount of light absorbed at a specific wavelength, where the change in absorption is proportional to the analyte concentration.

4.0 Sample Storage, Processing, and Test Portion

4.1 Sample Storage. Store samples in sealed plastic containers, at ≤ 6 °C, and analyze within 28 days. Do not freeze.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Recommended sample size is 1.0 g of solid or 10.0 mL of liquid. Concentration of Cl^- typically found in manure samples is sufficient to quantify at these amounts.

5.0 Apparatus

5.1 Analytical balance, capable of weighing to 0.01 g.

5.2 Extraction vessel with sealable cap, 200 or 250 mL. Polypropylene- or Cl^--free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm).

5.4 Volumetric dispenser, calibrated to 5–100 mL.

5.5 Büchner funnel filtration equipment, funnel, flask, and associated vacuum system.

5.6 Filters, paper or glass fiber; 8 µm and 0.45 µm Cl^--free. ***Note:*** *filters may have significant Cl^- contamination, and content should be verified.*

5.7 Centrifuge, high-speed, capable of RCF of 3000 × g for 50 mL centrifuge tubes.

5.8 Centrifuge tubes and caps, 50 mL capacity, compatible with centrifuge.

5.9 Spectrophotometric flow injection, continuous flow, or discrete autoanalyzer capable of performing Cl^- analysis based on mercury thiocyanate ($Hg(SCN)_2$) and subsequent quantification of azochromophore measured spectrophotometrically at 480 nm with an instrument detection limit of 1.0 mg L^{-1}.

6.0 Reagents

6.1 Reagent grade water, ASTM Type I.

6.2 Extraction solution, 0.01 M $Ca(NO_3)_2 \cdot 4H_2O$ ACS grade: dilute 2.36 g in 1.0 L final volume with reagent grade water.

6.3 Mercuric thiocyanate color reagent from US EPA method 9251.

- **6.3.1** Mercuric thiocyanate solution. Dissolve 4.17 g of ACS grade $Hg(SCN)_2$ in 500 mL methanol. Dilute to 1.0 L with methanol, mix, and filter through filter paper.
- **6.3.2** Ferric nitrate solution, 20.2%. Dissolve 202.0 g of ACS grade $Fe(NO_3)_3 \cdot 9H_2O$ in 500 mL of reagent water. Add 31.5 mL concentrated nitric acid, mix, and dilute to 1.0 L with reagent water.
- **6.3.3** Color reagent. Add 150 mL of mercuric thiocyanate solution to 150 mL of ferric nitrate solution), mix, and dilute to 1.0 L with reagent water. A combined color reagent is commercially available.

6.4 Chloride calibration standard: 200.0 mg Cl^- L^{-1}. Weigh 0.420 g of dried (~2 hours at 105 °C) ACS grade potassium chloride (KCl), dry 1–2 g of KCl in a 250 mL beaker (maximize surface area) at 105 °C for 8 hours. Allow to cool in a desiccator to room temperature before use. Dissolve in 1.0 L in reagent grade water. Prepare calibration standards, minimum six, of 0.5–50 mg Cl^- L^{-1} matrix matched in extraction reagent.

6.5 Independent Calibration Verification (ICV) solution. Prepare the ICV solution with a concentration of Cl^- near the midpoint of the calibration curve, from a different source than that used to make the calibration standards, matrix-matched to the extraction solution.

6.6 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Extract Cl^- from the manure matrix.

- **7.1.1** Semi-solid and solid waste. Weigh 1.0–5.0 ± 0.05 g of well-homogenized manure into a extraction vessel and dilute 100 mL of 0.01 M $Ca(NO_3)_2$ extraction solution from a volumetric dispenser.
- **7.1.2** Liquid or slurry waste material. Transfer 10.0 ± 0.1 mL into an extraction vessel. Add 90 mL 0.01 M $Ca(NO_3)_2$ extraction solution from a volumetric dispenser.

7.2 Cap extraction vessel and place on reciprocating mechanical shaker for 30 minutes.

7.3 After shaking, clarify extract to remove suspended particulates and turbidity using Büchner funnel filtration equipment with highly retentive filter paper or by centrifugation for 10 minutes.

7.4 Initialize and calibrate continuous flow, flow injection, or discrete analysis instrument according to manufacturer's instructions. Follow protocols outlined in Section 9.0. Determine chloride concentration of unknown manure solutions. Samples with concentration exceeding the highest calibration standard should be diluted with matrix-matched extraction solution. Record dilution factor and re-analyze.

8.0 Calculations and Reporting

8.1 Calculations. Instrument data is converted by the operating system to extract concentration in mg L^{-1}.

- **8.1.1** Solid and semi-solid manure. Results are converted to mg Cl^- kg^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:

C = extract result, mg Cl^- L^{-1}

V = extract volume, mL

M = sample mass (g) used in extraction or digest

DF = additional dilution factor, when applicable

8.1.2 Liquid manure. Results are converted to mg Cl^- L^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = C \times DF$$

where:
C = extract result, mg Cl^- L^{-1}
DF = additional dilution factor, when applicable

8.2 Client reports. Report all values to 1 mg Cl^- kg^{-1} or mg Cl^- L^{-1} as on an as-received basis to three significant digits. Alternative reporting units: solid manure as lb ton^{-1}; liquid manure as lb 1000 gal^{-1}. Any Cl^- concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of five calibration standards for every batch of 20 or fewer samples. The calibration range is specific to the analysis method, but a calibration range of 0.5–50.0 mg Cl^- L^{-1} will include most manure samples. A higher calibration in the range of 50.0–250.0 mg Cl^- L^{-1} may be necessary for some samples. Any extract concentration above the highest calibration standard should be diluted with 0.01 M $Ca(NO_3)_2$ and rerun. The lowest standard should ≤LOQ. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration, otherwise perform maintenance and recalibrate.*

9.2 Calibration Blank (CB). After calibration and every 10 samples CB should be analyzed. *Acceptance criteria: The matrix-matched Cl^- concentration must be lower than the LOQ. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination is eliminated.*

9.3 Method Blank (MB). After the CB, one MB per batch of 20 or fewer samples. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, filters, or apparatus. *Acceptance criteria: Background Cl^- should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until source of contamination is eliminated.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured Cl^- concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed.*

9.5 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured Cl^- concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.6 Duplicate. Analyze a minimum of one duplicate sample one per analytical batch of 20 or fewer samples and calculate the RPD. The duplicate should be separated in the batch from the first sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference (RPD). *Acceptance criteria: An RPD of ≤ 15% should be routinely achievable for Cl^- when the analyte concentration is greater than the LOQ. The operator may select samples for duplicate analysis that are expected to exceed the LOQ.*

9.7 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch of 20 or fewer samples. Used to evaluate precision, matrix interferences, and extraction efficiency, a spike of known Cl^- concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background Cl^- but should not exceed the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the MDL is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the Cl^- spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate percent MSR according to Appendix A: Definitions: Matrix Spike Recovery. *Acceptance criteria: If recovery of Cl^- falls outside the control limits of 100 ± 15%, Cl^- is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.8 Reference Material (RM). An RM with a matrix similar to that of materials tested should be analyzed with every batch of 20 or fewer samples. *Acceptance criteria: Results should be within specified uncertainty limits.*

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure Cl^- that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two

Table M-10.1.1. Quality control guidelines for the spectrophotometric Cl^- method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Calibration Blank (CB)	After calibration, every 10 samples	$Cl^- <$ MDL
Method Blank (MB)	Every batch	$Cl^- <$ LOQ
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 15% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%
Reference Material	Every batch	Within uncertainty limits

Table 10.1.2. Laboratory precision data for manure Cl^- as-received basis spectrophotometric method.

Sample ID	Total Solids Content (%)	Mean Content mg Cl^- kg^{-1}	Intra-Lab Standard Deviation S_r mg Cl^- kg^{-1}	Intra-Lab Repeatability r mg Cl^- kg^{-1}	Inter-Lab Reproducibility standard deviation S_R mg Cl^- kg^{-1}	Inter-Lab Reproducibility R mg Cl^- kg^{-1}
M 2014-D	3.15	280	6	15	37	100
M 2015-D	11.2	770	23	64	190	530
M 2012-B	56.5	3480	51	144	370	1040
M 2016-F	68.4	3850	130	360	220	630

MAP Program, 8–21 laboratories reporting.

rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

9.10 Analyte Carryover. Carryover should be determined for Cl^- based on the procedure outlined in Appendix A. *Perform as needed or during preventive maintenance.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) for chloride shall be determined based on the method described by the US EPA in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. Chloride spectrophotometric repeatability and reproducibility were calculated in accordance with ISO 5725-2 from results of the Manure Analysis Proficiency (MAP) Program, coordinated by the Minnesota Department of Agriculture, in proficiency rounds conducted in 20125 and 2016 for 8-21 of ten laboratories. Laboratories utilized instruments from different manufacturers, and the following precision data were obtained (Table 10.1.2)

11.0 Safety and Disposal

11.1 Laboratory and Safety. Wear safety glasses with side shields, chemical resistant gloves, and lab coat or apron.

11.2 Caution. Mercuric thiocyanate is fatal if swallowed, in contact with skin, or inhaled. Wear appropriate PPE and handle with extreme care.

11.3 Reagents Disposal. Dispose of reagents and method waste in accordance with local/state and/or federal regulations. Mercuric thiocyanate is a hazardous reagent and waste requires special disposal practices.

11.4 Samples Disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

American Public Health Association. (2012). Standard methods for examination of water and wastewater, 22nd ed. Method 4500-Cl- A.

Gavlack, Horneck, R. E. D. A., & Miller, R. O. (2005). *Soil and water reference methods for the western region* (3rd ed.). Western Regional Extension Publication. WREP-125. Oregon State University.

International Organization for Standardization. (2000). Water quality — Determination of chloride by flow analysis (CFA and FIA) and photometric or potentiometric detection. ISO Report 15682:2000.

International Organization for Standardization. (2019). Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. ISO 5725-2:2019.

U. S. Environmental Protection Agency. (2001). Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual. Chapter 2. (EPA-823-B-01-002).

M-10.2
Chloride by Coulometry

AUTHOR: Robert O. Miller

1.0 Scope and Application

This method quantitatively determines the concentration of chloride (Cl^-) in liquid and solid animal manure by extraction with an acetic acid solution and subsequent coulometric titration with a silver electrode. Cl^- may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The following procedure is adapted from Holstege, et al. (2010). The Cl^- content of a manure is determined by extraction with an acetic acid solution and subsequent analysis by coulometric titration with silver electrodes. The combination of silver and chloride ions is a quantitative reaction resulting in a precipitate of silver chloride (AgCl) at the cathode, decreasing the indicator current, and, in turn, a release of silver ions at the anode to bring the silver ion concentration back to its original level before the chloride-induced precipitation. The concentration of Cl^- in solution is proportional to the length of time current flows.

2.2 Method Performance. A laboratory should be able to achieve an MDL of 2 mg Cl^- kg^{-1} (as-received sample basis, dependent on instrument manufacturer). An MDL should be determined and reported individually by the laboratory upon completion of the MDL outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B.

2.3 Method Interferences. Bromide and iodide will combine with silver when present in concentrations exceeding 10 mg I L^{-1}, resulting in a positive interference.

2.4 Method Reporting. Chloride content is measured as mg Cl^- L^{-1} and reported on an as-received basis as mg Cl^- kg^{-1} or lbs per ton^{-1} for solid and mg Cl^- L^{-1} or lbs per 1000 gal^{-1} for liquid manures.

2.5 Method Advantages. Avoids use of concentrated acids. No hazardous reagents.

2.6 Method Disadvantages. Limited analytical range. Low throughput.

3.0 Definitions

3.1 Chloride (Cl^-). An ion which forms inorganic, covalent bonds with one of several elements, metals and non-metals. Relatively inert toward reactions with nitrogen, oxygen, and carbon. Typically found as salts of sodium, potassium, calcium, and magnesium.

3.2 Coulometric titration analysis. An analysis method that determines the amount of an analyte transformed during an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced in reaction.

4.0 Sample Storage, Processing, and Test Portion

4.1 Sample Storage. Store samples in sealed plastic containers, at ≤ 6 °C, and analyze within 28 days. Do not freeze.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Recommended sample size is 1.0 g of solid or 10.0 mL of liquid. Concentration of Cl^- typically found in manure samples is sufficient to quantify at these amounts.

5.0 Apparatus

5.1 Analytical balance, capable of weighing to 0.001 g.

5.2 Extraction vessel with sealable cap, 200 or 250 mL. Polypropylene or chloride-free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm).

5.4 Volumetric dispenser, calibrated to 5.0–50 mL.

5.5 Büchner funnel filtration equipment, funnel, flask, and associated vacuum system.

5.6 Filters, paper or glass fiber; 8 µm and 0.45 µm Cl^- free. ***Note:*** *specific filter suppliers may have significant Cl^- contamination and content should be verified.*

5.7 Centrifuge, high-speed, capable of RCF 3000 × g for 50 mL centrifuge tubes.

5.8 Centrifuge tubes, 50 mL capacity, compatible with centrifuge.

5.9 Chloridometer instrument capable of performing chloride analysis based on coulometric titration with Ag chemistry.

6.0 Reagents

6.1 Reagent grade water, ASTM Type I grade.

6.2 Extraction solution, 2.0% glacial acetic acid solution. Dilute 20 mL ACS grade acetic acid CH_3COOH (99%) in 50 mL reagent grade water and dilute to 1 L. Verify that acetic acid reagent contains < 1.0 mg Cl^- L^{-1}.

6.3 Instrument gelatin reagent (manufacturer specific) solution. Mix 0.27 g gelatin reagent (supplied by instrument manufacturer) in 50 mL reagent grade water; warm to dissolve. Store at ≤ 6 °C.

6.4 Instrument carrier solution (20% v/v acetic acid, 2.4% v/v nitric acid). Mix 200 mL reagent grade water, 200 mL ACS grade acetic acid, and 24 mL concentrated HNO_3, diluted to 1 L ml with reagent grade water.

6.5 Chloride calibration standard: 200.0 mg Cl^- L^{-1}. Weigh 0.420 g of dried ACS grade potassium chloride, dry 1–2 g of KCl in a 250 mL beaker (maximize surface area) at 105 °C for 8 hours. Allow to cool in a desiccator to room temperature before use. Dilute in 1.0 L in reagent grade water. Prepare five working standards of 2, 10, 20, 50 and 100 mg Cl^- L^{-1} matrix matched in glacial acetic acid extraction solution.

6.6 Independent Calibration Verification (ICV) solution. Prepare the ICV solution with a concentration of Cl^- near the midpoint of the calibration curve, from a different source than that used to make the calibration standards, matrix-matched to the extraction solution.

6.7 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Extract Cl^- from the manure matrix.

7.1.1 Semi-solid and solid waste. Weigh 1.0–5.0 ± 0.05 g of well-homogenized manure into a extraction vessel and dilute 100 mL of 2% acetic acid extraction solution from a volumetric dispenser.

7.1.2 Liquid waste material, transfer 10.0 ± 0.1 mL into an extraction vessel. Add 90 mL of 2% acetic acid extraction solution from a volumetric dispenser.

7.2 Seal extraction vessel and place on reciprocating mechanical shaker for 30 minutes.

7.3 After shaking, clarify extract to remove suspended particulates and turbidity using Büchner funnel filtration equipment with highly retentive filter paper or by centrifugation for 10 minutes.

7.4 Follow manufacturer instructions for operation and calibration of the chloridometer instrument. Follow protocols outlined in Section 9.0. Determine chloride concentration of unknown manure solutions. Samples with concentration exceeding the highest calibration standard should be diluted with matrix matched extraction solution. Record dilution factor and re-analyze.

8.0 Calculations and Reporting

8.1 Calculations. Instrument data is converted by the operating system to extract concentration in mg L^{-1}.

8.1.1 Solid and semi-solid manure. Results are converted to mg Cl^- kg^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:
C = extract result, mg Cl^- L^{-1}
V = extract volume, mL
M = sample mass (g) used in extraction or digest
DF = additional dilution factor, when applicable

8.1.2 Liquid manure. Results are converted to mg Cl^- L^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = C \times DF$$

where:
C = extract result, mg Cl^- L^{-1}
DF = additional dilution factor, when applicable

8.2 Client reports. Report all values to 1 mg Cl^- kg^{-1} or mg Cl^- L^{-1} on an as-received basis to three significant digits. Alternative reporting units: solid manure as lb Cl^- ton^{-1}; liquid manure as lb Cl^- 1000 gal^{-1}. Any Cl^- concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of five calibration standards for every batch of 20 or fewer samples. The calibration range is specific to the analysis method, but a calibration range of 5–50 mg Cl^- L^{-1} will include most manure samples. A higher calibration in the range of 50.0–200.0 mg Cl^- L^{-1} may be necessary for some samples. Any extract concentration above the highest calibration standard should be diluted with glacial acetic acid extraction solution and rerun. The lowest standard should ≤LOQ. *Acceptance criteria: Value of $r^2 \geq 0.99$ for linear and $r^2 \geq 0.999$ for polynomial calibration; otherwise, perform maintenance and recalibrate.*

9.2 Calibration Blank (CB). After calibration and every 10 samples, CB should be analyzed. *Acceptance criteria: The matrix-matched Cl^- concentration must be lower than the LOQ. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination is eliminated.*

9.3 Method Blank (MB). After the CB, one MB per batch should be analyzed. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, filters, or apparatus. *Acceptance criteria: Background Cl^- should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until source of contamination is eliminated.*

9.4 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured Cl^- concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed.*

9.5 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured Cl^- concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.6 Duplicate. Analyze a minimum of one duplicate sample one per analytical batch of 20 or fewer samples and calculate the RPD. The duplicate should be separated in the batch from the first sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; Relative Percent Difference (RPD). *Acceptance criteria: An RPD of ≤ 15% should be routinely achievable for Cl^- when the analyte concentration is greater than the LOQ. The operator may select samples for duplicate analysis that are expected to exceed the LOQ.*

9.7 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch of 20 or fewer samples. Used to evaluate precision, matrix interferences, and extraction efficiency, a spike of known Cl^- concentration is added to one of two identical sample aliquots. The spike

Table M-10.2.1. Quality control guidelines for the coulometric Cl^- method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$, linear $r^2 \geq 0.999$, polynomial
Calibration Blank (CB)	After calibration, every 10 samples	$Cl^- <$ MDL
Method Blank (MB)	Every batch	$Cl^- <$ LOQ
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 15% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%
Reference Material	Every batch	Within uncertainty limits

should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background Cl^- but should not exceed the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the MDL is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the Cl^- spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate percent MSR according to Appendix A: Definitions: MSR. *Acceptance criteria: If recovery of Cl^- falls outside the control limits of 100 ± 15%, Cl is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.8 Reference Material (RM). A RM with a matrix similar to that of materials tested should be analyzed with every batch of 20 or fewer samples. *Acceptance criteria: Results should be within specified uncertainty limits.*

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure Cl^- that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program. *Perform bi-annually.*

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) for chloride shall be determined based on based on the method described by the US EPA in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. No data available.

11.0 Safety and Disposal

11.1 Laboratory Safety. Wear safety glasses with side shields, chemical resistant gloves, and lab coat or apron.

11.2 Caution. Not applicable.

11.3 Reagents Disposal. Dispose of reagents in accordance with local/state and/or federal regulations.

11.4 Samples Disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

American Society of Agricultural Engineers. ASAE S292.5: Uniform terminology for waste management. (pp 656–659).

U. S. Environmental Protection Agency. (2001). Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual. Chapter 2. (EPA-823-B-01-002).

Davey, B.G., & Bembrick, M. (1969). The potentiometric estimation of chloride in water extracts of soils. S*oil Science Society of America Journal,* 33:385–387.

Holstege, D, Price, P., Miller, R. O., & Meyer, D. (2010). California analytical methods manual for dairy general order compliance – Nutrient management plan constituents. University of California, Davis Analytical Laboratory. https://anlab.ucdavis.edu/media/pdf/uc_analytical_methods.pdf

M-10.3
Chloride by Potentiometry

AUTHOR: Robert O. Miller

1.0 Scope and Application

This method quantitatively determines the concentration of chloride (Cl^-) in liquid and solid animal manure by extraction with an acetic acid solution and subsequent determination by one of two methods: (1) chloride ion selective electrode (ISE); or (2) potentiometric titration with silver nitrate ($AgNO_3$) using a silver (Ag) ion selective electrode. Chloride may be requested for nutrient management planning or regulatory compliance.

2.0 Method Principle

2.1 Method Description. The chloride content of manure is determined by extraction with a 2.0% acetic acid extraction, as described by Holstege et al. (2010), followed by analysis by either: (1) a chloride (ISE) and calomel reference electrode, adapted from US EPA method 9212; or (2) by potentiometric titration with $AgNO_3$ using a silver sensing electrode and calomel reference electrode pair, as developed by Davey and Bembrick (1969) and described by Rayment and Higgenson (2011).

2.2 Method Performance. The user should be able to obtain an MDL of 10 mg Cl^- kg^{-1} (as-received sample basis, instrument manufacturer dependent). An MDL should be determined and reported individually by the laboratory upon completion of the MDL outlined in US EPA 40 CFR 136: Definition and Procedure for the Determination of the Method Detection Limit; see Appendix B.

2.3 Method Interferences. Bromide, iodide, sulfide, cyanide, thiosulfates, and ammonia, when present in concentrations exceeding 10 mg L^{-1}, may interfere, and can be removed by lowering pH < 4. Polyvalent cations (e.g., Fe and Al) > 300 mg kg^{-1} interfere by forming complexes with chloride which are not measured by the chloride ISE, and require treatment with 2% solution EDTA, diluted 1:1. Cyanides, sulfides, and thiosulfates present should be removed by means of oxidation with H_2O_2. Organic compounds may impact ISE performance.

2.4 Method Reporting. Chloride content is measured as mg Cl^- L^{-1} and reported on an as-received basis as mg Cl^- kg^{-1} or lbs per ton^{-1} for solid and mg Cl^- L^{-1} or lbs per 1000 gal^{-1} for liquid manures.

2.5 Method Advantages: Effective with colored or turbid solutions; avoids use of concentrated acids.

2.6 Method Disadvantages: Limited analytical range. ISE requires periodic cleaning of organic coating. Manual method. Limited throughput.

3.0 Definitions

3.1 Chloride (Cl^-). An anion which forms inorganic, covalent bonds with one of several elements, metals and non-metals. Relatively inert toward reactions with nitrogen, oxygen, and carbon. Typically found as salts of sodium, potassium, calcium, or magnesium.

3.2 Potentiometric analysis. An electroanalytical technique whereby the potential between two electrodes is measured using a high impedance voltmeter during a titration.

4.0 Sample Processing, Preservation, and Storage

4.1 Sample Storage. Store samples in sealed plastic containers, at ≤ 6 °C, and analyze within 28 days. Do not freeze.

4.2 Sample Processing. For solid (> 20% solids) and semi-solid materials (10–20% solids), subsample at least 200 cm^3 of the mixed, primary sample and homogenize according to Chapter S-2.0, Laboratory Sample Processing and Storage. Slurry (4–10% solids) and liquid (< 4% solids) samples do not generally require subsampling but, where necessary, refer to Chapter S-2.0, Laboratory Sample Processing and Storage.

4.3 Sample Test Portion. Recommended sample size is 1.0 g of solid or 10.0 mL of liquid. Concentration of Cl^- typically found in manure samples is sufficient to quantify at these amounts.

5.0 Apparatus

5.1 Analytical balance: minimum resolution ± 0.01g.

5.2 Extraction vessel with sealable cap, 200 or 250 mL. Polypropylene or Cl^--free glass.

5.3 Reciprocating horizontal mechanical shaker, capable of 180 excursions per minute (epm).

5.4 Volumetric dispenser, calibrated to 5.0–100 mL.

5.5 Büchner funnel filtration equipment, funnel, flask, and associated vacuum system.

5.6 Filters, paper or glass fiber; 8 µm and 0.45 µm Cl^- free. Note, specific filter suppliers may have significant Cl^- contamination and content should be verified.

5.7 Centrifuge, high-speed, capable of RCF 3000 × g for 50 mL centrifuge tubes.

5.8 Centrifuge tubes, 50 mL capacity, compatible with centrifuge.

5.9 Magnetic stir plate and stir bar.

5.10 pH/mV meter with digital readout capable of measuring potential difference between electrodes.

5.11 $Ag/AgNO_3$ electrode with integrated temperature sensor and calomel electrode. Note: If using a Cl^- combination ISE, ensure the ISE is filled with the solution recommended by the manufacturer. Replace the solution if the ISE has not been used for a week. If using a Cl^- ISE and a separate double-junction reference electrode, ensure reference electrode inner and outer chambers are filled with solutions recommended by the manufacturer. Equilibrate electrode(s) for at least one hour in a 30.0 mg Cl^- L^{-1} calibration standard before use.

5.12 Automated titrator buret with Ag billet combination electrode (or equivalent), 100 or 250 ml beaker digital readout capable of measuring potential difference between electrodes. Note: Cl^- determination by potentiometric titration with $AgNO_3$ only.

6.0 Reagents

6.1 Reagent grade water, ASTM Type I.

6.2 Extraction reagent, 0.35 M (2.0%) glacial acetic acid solution. Dilute 20 mL ACS grade acetic acid CH_3COOH (99%) in 50 mL reagent grade water and dilute to 1.0 L. Verify acetic acid reagent contains < 1.0 mg Cl^- L^{-1}.

6.3 Chloride ISE reagents:

6.3.1 Ionic Strength Adjuster (ISA) buffer solution, 5 M $NaNO_3$. Dissolve 42.5 g of ACS grade $NaNO_3$ in sufficient reagent grade water to make 100 mL of solution. Note: 5 M $NaNO_3$ ISA buffer solution may be purchased from a commercial vendor.

6.3.2 Chloride calibration standard: 200.0 mg Cl^- L^{-1}. Weigh 0.420 g of dried ACS grade potassium chloride, dry 1–2 g of KCl in a 250 mL beaker (maximize surface area) at 105 °C for 8 hours. Allow to cool in a desiccator to room temperature before use. Dilute in 1.0 L in reagent grade water. Prepare five working standards of 10, 20, 40, 100, and 200 mg Cl^- L^{-1} matrix matched in 2.0% acetic acid solution.

6.4 Chloride potentiometric titration reagents:

6.4.1 Chloride calibration standard: 1000 mg Cl^- L^{-1}, NaCl (0.02821 M). Weigh 1.648 g of dried ACS grade sodium chloride, dry 2–3 g of NaCl in a 250 mL beaker (maximize surface area) at 105 °C for 8 hours. Add 20 mL acetic acid solution and dilute to 1.0 L with reagent grade water.

6.4.2 $AgNO_3$ titrant, 0.010 M. Dissolve 1.698 g of ACS grade $AgNO_3$ and dilute to 1.0 L with reagent grade water. 1.0 mL of solution contains 1.00 mg of Ag equivalent. Store in a brown glass bottle, away from light. Note: 0.01M $AgNO_3$ titrant may be purchased from a commercial vendor.

6.4.3 Nitric acid 0.4 M. Mix ACS grade 70% nitric acid, dissolve 25.48 mL in 250 mL reagent grade water, swirl, and bring to 1.0 L final volume.

6.5 Independent Calibration Verification (ICV) solution. Prepare the ICV solution with a concentration of Cl^- near the midpoint of the calibration curve, from a different source than that used to make the calibration standards, matrix-matched to the extraction solution.

6.6 Reference Material (RM). Certified, standard, or internal RM with a matrix similar to the samples. Sources: Minnesota Department of Agriculture's Manure Analysis Proficiency (MAP) Program, Waters Environmental Resource Associates (ERA), and Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL).

7.0 Procedure

7.1 Extract Cl^- from the manure matrix.

7.1.1 Semi-solid and solid waste. Weigh 1.0–5.0 ± 0.05 g of well-homogenized manure into a extraction vessel and dilute 100 mL of 2% acetic acid extraction solution from a volumetric dispenser.

7.1.2 Liquid waste material, transfer 10.0 ± 0.1 mL into an extraction vessel. Add 90 mL of 2% acetic acid extraction solution from a volumetric dispenser.

7.2 Place cap on extraction vessel and shake on reciprocating mechanical shaker for 30 minutes.

7.3 After shaking, remove suspended particulates and turbidity using Büchner funnel filtration equipment with a 0.45 µm filter or by centrifugation for 10 minutes.

7.4 Determine Cl^- content using ISE, method option 1:

7.4.1 Equilibrate standards and unknown samples to room temperature. Follow protocols outlined in Section 9.0 for quality control samples.

7.4.2 Calibrate the electrode. Utilizing $Ag/AgNO_3$ electrode and pH/mV meter, calibrate the ISE chloride probe using 10.0 mg Cl^- L^{-1} and 100 mg Cl^- L^{-1} calibration standards. Add 50.0 mL of calibration standard and 1.00 mL of ISA solution to a 100 mL beaker. Calibrate ISE meter in terms of Cl^- concentration following the manufacturer's instructions. Prepare a calibration curve by plotting measured potential (mV) as a function of the logarithm of Cl^- concentration of each calibration standard. The slope must be 54–60 mV per decade of Cl^- concentration. If the slope is not acceptable, the ISE may not be working properly. For corrective action, consult the ISE operation manual.

Note: During analysis of standards and unknown samples, add a PTFE-coated magnetic stir bar, place the beaker on a magnetic stir plate, and stir at slow speed (no visible vortex). Immerse the electrode tips to just above the rotating stir bar. If using a pH/mV meter, record the meter reading (mV) as soon as the reading is stable, but in no case should the time exceed five minutes after immersing the electrode tips.

7.4.3 Prior to and between analyses, rinse the electrodes thoroughly with reagent grade water and gently shake off excess water. Low-level measurements are faster if the electrode tips are first immersed for five minutes in reagent water.

7.4.4 Add 50.0 mL of sample and 1.0 mL of ISA to a 100 mL beaker. Immerse the electrode tip(s) to just above the rotating stir bar. Record the meter reading (mV or concentration) as soon as the reading is stable. If reading mV, determine chloride concentration from the calibration curve.

7.4.5 When analyses have been completed, rinse the electrodes thoroughly and store them in a 30.0 mg Cl^- L standard solution. If the electrodes are not to be used within one day, drain the internal filling solutions, rinse with reagent water, and store dry.

7.4.6 For unknown samples exceeding calibration, dilute with acetic acid extraction solution, record dilution factor, and re-analyze. Report results to the nearest 10 mg Cl^- kg^{-1}.

7.5 Determine Cl^- content by potentiometric titration with $AgNO_3$ using a silver ISE, method option 2:

7.5.1 Equilibrate standards and unknown samples to room temperature. Follow protocols outlined in Section 9.0 for quality control samples.

7.5.2 Standardization of $AgNO_3$ titrant. Pipet 1.0 mL of the 1000 mg Cl^- L^{-1} NaCl calibration standard into a clean 100 mL beaker (1.0 mL = 1.0 mg Cl^-). Add reagent grade water to the beaker to the 60 mL mark. Add 1.0 mL of 0.4 M nitric acid, and initiate automated titration with $AgNO_3$ titrant, 0.010 M. Repeat with 10 mL aliquot NaCl chloride standard. Standardize titrant weekly.

$$Molarity\ of\ AgNO_3 = \frac{KCl, mL \times M\ KCl}{t}$$

where:
t = titration volume of (mL) of $AgNO_3$ solution
Confirm autotitrator Cl^- calibration, 1.0 mL = 1.0 mg Cl^-.

7.5.3 Pipette 1.0 mL aliquot of unknown sample extract into automated titrator 60 mL beaker, add 1.0 mL of 0.4 N HNO_3, and bring to 60 mL final volume with reagent grade water. Insert $Ag/AgNO_3$ electrode and initiate automated titration. Record concentration mg Cl^- L^{-1}.

For unknown samples exceeding the calibration range, dilute with acetic acid extraction solution, record dilution factor, and re-analyze.

8.0 Calculations and Reporting

8.1 Calculations. Instrument data is converted by the operating system to extract concentration in mg L^{-1}.

8.1.1 Solid and semi-solid manure. Results are converted to mg Cl^- kg^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = \left[\frac{(C \times V)}{M}\right] \times DF$$

where:
C = extract result, mg Cl^- L^{-1}

V = extract volume, mL
M = sample mass (g) used in extraction or digest
DF = additional dilution factor, when applicable

8.1.2 Liquid and slurry manure. Results are converted to mg Cl^- L^{-1} on an as-received basis as follows:

$$Cl^-, mg\ kg^{-1} = C \times DF$$

where:
C = extract result, mg Cl^- L^{-1}
DF = additional dilution factor, when applicable

8.2 Client reports. Report all values to 1 mg Cl^- kg^{-1} or mg Cl^- L^{-1} as on an as-received basis to three significant digits. Alternative reporting units: solid manure as mg kg^{-1} and/or lb ton^{-1}; liquid manure as mg L^{-1} and/or lb 1000 gal^{-1}. Any Cl^- concentration below LOQ is reported as <LOQ.

9.0 Quality Control

9.1 Calibration. The initial calibration should include a minimum of two calibration standards daily. The calibration range is specific to the analysis method, but a calibration range of 10–200 mg L^{-1} Cl^- will include most manure samples. Any extract concentration above the highest calibration standard should be diluted and rerun. The lowest standard should ≤LOQ. *Acceptance criteria: Value of $r^2 \geq 0.99$. Otherwise perform maintenance and recalibrate.*

9.2 Calibration Blank (CB). After calibration and every 10 samples CB should be analyzed. *Acceptance criteria: The matrix-matched Cl^- concentration must be lower than the MDL. If criteria is exceeded, prepare a second aliquot and re-analyze CB. If second CB passes, calibration is verified; if second CB fails, halt analysis until source of contamination is eliminated.*

9.3 Independent Calibration Verification (ICV). ICV standards, prepared from materials obtained from a source independent of the one used for preparing the calibration standards and at concentrations at the low and high end of the calibration range, should be analyzed after calibration. Calculate the percent error (PE) between the ICV known and the as-measured Cl^- concentration according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded, re-analyze ICV. If second ICV passes, calibration is verified. Otherwise, recalibrate with new calibration standards and re-analyze all samples since last valid ICV.*

9.4 Method Blank (MB). After the CB, one MB per batch should be analyzed. This differs from the rinse samples in that the blank is analyzed as an unknown in order to test for contamination in the water, reagents, filters, or apparatus. *Acceptance criteria: Background Cl^- should be below the LOQ or documented historical acceptance limits. If criteria is exceeded, re-analyze MB. If second MB passes, calibration is verified. If second MB fails, halt analysis until the source of contamination is eliminated.*

9.5 Continuing Calibration Verification (CCV). Two CCV standards (low, ≤ 5 × MDL, and high, 80% of high calibration standard) should be analyzed at the beginning of and during the run using a calibration standard. The frequency of CCV analysis is once every 10 samples, alternating between low and high. Calculate the percent error (PE) between the CCV known and the as-measured Cl^- concentrations according to Appendix A: Definitions; Percent Error. *Acceptance criteria: Acceptable variation is ± 10 PE. If criteria is exceeded for more than one CCV or other QC, determine the cause of the failure. Recalibrate and re-analyze as needed CCV.*

9.6 Duplicate. Analyze a minimum of one duplicate sample per analytical batch and calculate the RPD. The

Table M-10.3.1. Quality control guidelines for the potentiometric Cl^- method.

Quality Control Element	Frequency	Acceptance Criteria
Calibration	Every batch	$r^2 \geq 0.99$
Calibration Blank (CB)	After calibration, every 10 samples	Cl^- < MDL
Method Blank (MB)	Every batch	Cl^- < LOQ
Continuing Calibration Verification (CCV)	After calibration, every 10 samples	Within ± 10 PE
Independent Calibration Verification (ICV)	After calibration	Within ± 10 PE
Duplicate	Every batch	RPD ≤ 15% within the documented acceptance limits
Matrix Spike Recovery (MSR)	Every batch	Recovery, 100 ± 15%

duplicate should be separated in the batch from the first sample by at least 10 unknown samples. Calculate RPD according to Appendix A: Definitions: Duplicate; RPD. *Acceptance criteria: An RPD of ≤ 15% should be routinely achievable for Cl^- when the analyte concentration is greater than the LOQ. RPD as a statistical approach to evaluating precision is sensitive to concentration and can be expected to exceed 10% where Cl^- concentration is low (< 5 mg kg^{-1}). The operator may select samples for duplicate analysis that are expected to exceed to the LOQ or may consider the use of a statistical test that is less sensitive to low analyte concentrations, such as Relative Standard Deviation (RSD), also known as the Coefficient of Variation (CV).*

9.7 Matrix Spike Recovery (MSR). The laboratory should analyze one MSR per batch. Used to evaluate precision, matrix interferences, and extraction efficiency, a spike of known Cl^- concentration is added to one of two identical sample aliquots. The spike should be added after homogenization of the primary sample but prior to sample extraction and filtration. The spike concentration should be high enough to be discernible from background Cl^- but should not exceed the calibration range. A mid-range concentration from the calibration curve or a concentration approximately 100 times the MDL is usually suitable. The ICV or CCV may be used for this purpose. Once added to the sample aliquot, the Cl^- spike should be thoroughly mixed into the sample matrix before extractant is added. Calculate percent MSR according to Appendix A: Definitions: MSR. *Acceptance criteria: If recovery of Cl^- falls outside the control limits of 100 ± 15%, Cl^- is judged outside control, and the source of the problem should be identified and resolved before continuing analyses.*

9.8 Reference Material (RM). An RM with a matrix similar to that of materials tested should be analyzed with every batch of 20 or fewer samples. *Acceptance criteria: Results should be within reference specified uncertainty limits.*

9.9 Proficiency Requirement. Laboratory shall participate in a proficiency testing program for manure Cl^- that is compliant with International Laboratory Accreditation Cooperation (ILAC) G13 guidelines, minimum two rounds per year. Example, Minnesota Department of Agriculture, Manure Analysis Proficiency (MAP) Program.

10.0 Limit of Quantification and Method Performance

10.1 Limit of Quantification (LOQ). Method Detection Limit (MDL) for chloride shall be determined based on the method described by the US EPA in Appendix B. LOQ shall be determined annually and calculated using the following equation:

$$LOQ \geq 3 \times MDL$$

An LOQ less than or equal to three times the MDL specified in Section 2.2 must be achieved prior to using this method.

10.2 Method Performance. No data available.

11.0 Safety and Disposal

11.1 Health and safety. Manure can contain pathogenic organisms and compounds which cause eye or skin irritation. Always wear appropriate lab clothing latex gloves and eye protection when handling manure.

11.2 Laboratory safety. Wear lab coat, gloves, and eye protection when handling manure and reagents.

11.3 Caution. Not applicable.

11.4 Reagents disposal. Dispose of reagents in accordance with local/state and/or federal regulations.

11.5 Samples disposal. Dispose of manure in accordance with local/state and/or federal regulations.

12.0 Reference Documents and Additional Resources

Davey, B.G., & Bembrick, M. (1969). The potentiometric estimation of chloride in water extracts of soils. *Soil Science Society of America Journal,* 33:385–387.

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SW-846 Test Method 9212: Potentiometric determination of chloride in aqueous samples with ion-selective electrode. (1996). Rev 0.

Thermo Scientific. User guide. Chloride ion selective electrode. 254808-001 Rev.A. https://assets.fishersci.com/TFS-Assets/LSG/manuals/D12671~.pdf

Standard Methods 4500-Cl- D., Chloride, Potentiometric Method. (2012). In Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (Eds.), Standard methods for the examination of water and wastewater. American Public Health Association.

Slurry manure from a dairy facility stored in an earthen basin.
Photo taken by Melissa Wilson (University of Minnesota).

Laboratory Performance Management

L-1.0
Laboratory Quality Management

AUTHORS: Robert O. Miller and John T. Spargo

Lab quality management (LQM) is the aggregation of all lab activities, including management associated with the generation of reliable analytical test measurement data. It can be considered a wider extension of the concept of "Good Laboratory Practice" (GLP), developed in the U. S. in the 1980s, which outlines principles intended to assure lab quality and integrity. An LQM system is comprised of two primary components: Quality Assurance (QA) and Quality Control (QC).

The ISO defines QA as *"the assembly of all planned and systematic actions necessary to provide adequate confidence that product, process, or service will satisfy given quality requirements."*

A QA program ensures the reliability of analytical test measurements through the application of standard operating procedures (SOPs), appropriate quality control and assessment procedures, and systematic documentation of the entirety of laboratory operation. It provides documentation of the organizational structure, personnel, training and safety practices, analytical method(s), and process monitoring, and includes an evaluation of performance and a procedure to address performance issues. A complete listing of QA plan components can be found in US EPA SW-846 (EPA, 1986).

The ISO defines QC as *"the operational techniques and activities that are used to satisfy quality requirements."* QC is a monitoring program undertaken specifically to achieve accurate and reliable analytical results, prevent errors, document the uncertainty and the ongoing performance of an analytical method, and assure reliable performance.

While an effective QA program will initially add to the overhead cost of a laboratory operation, this investment should be more than offset by improved analytical reliability, resulting in less repeat analysis and in improved performance. Most funding agencies require a documented QA program, as well as accuracy and precision statistics, as part of routine nutrient analysis of soils and biological materials. Reporting these statistics will improve client confidence in and satisfaction with the data.

The relative cost/benefit ratio of individual QC practices should be considered when implementing or modifying a QA program (Garfield, 1991). A QA program should reflect the end-use of the analytical results and be based on input from lab personnel, vendors, and clients. This chapter does not include a complete listing of all QA components, but is intended to address common operational problems and practices affecting analytical quality in an inorganic laboratory. For other viewpoints and a more extensive list of QA/QC program components, the following references are recommended:

- Guidelines for quality management in soil and plant laboratories, L.P. van Reeuwijk, 1998.
- Quality assurance of chemical measurements, J.K. Taylor, 1987.
- Quality assurance quality control guidelines for forage laboratories, Thiex, Torma, Wolf, and Collins, 1999.
- Association of American Feed Control Officials Quality assurance/quality control guidelines for state feed laboratories, Ogden, Kane, Knapp, Thiex, and Torma, 1998.

The accuracy and precision specifications listed within the methods appearing in this manual are considered acceptable for manure analysis and attainable by all laboratories. Higher standards are almost always attainable, and there should be a continuing effort to provide the highest quality analytical results possible from the laboratory resources available.

1.0 Standard Operating Procedures (SOPs)

The fundamental component of an LQM program is the laboratory SOP document. This document defines a compulsory set of instructions for regular recurring operations and laboratory processes to ensure they are carried out in a consistent manner. SOPs of importance to the laboratory include sample receipt, sample preparation, reagent preparation, instrument operation, analytical methods, safety

Table L-1.0.1. Known sources of error in manure testing.

Source of Error	Corrective Action
Variable or heterogeneous samples.	Homogenize thoroughly prior to subsampling. Use larger sample size. Run replicate analysis.
Sample carryover on digestion vessels or other apparatus.	Decontaminate with cleaning solution between uses.
Contamination of samples or equipment by lab environment.	Store samples, reagents, and equipment separately.
Samples weighed, processed, or analyzed out of order.	Verify sample IDs during subsampling. Run known reference samples at regular intervals. Cross-check related parameters.
Inaccurate calibration solution content.	Check new calibration standards against old. Run an independent check sample (initial calibration verification or ICV) to verify standards.
Mismatch between sample and calibration solution matrices.	Make up calibration solution in digestion matrix or method blank solution. Use instrument internal standard(s) if applicable and available.
Drift in instrument response.	Use frequent calibration or drift checks (continuing calibration verification or CCV).
Blank values substantially above detection limit.	Use high-purity reagents and deionized water. Decontaminate sample containers between uses.
Poor instrument sensitivity or response.	Optimize all operating parameters. Check for obstructions in sample delivery system. Reconfirm instrument detection limit.
Transcription errors, faulty data handling.	Automate data transfer, verify manual input.

precautions, data reporting, responding to client complaints, and instructions on the preparation of an SOP. An SOP should have a defined time of execution and a process for re-evaluation. van Reeuwijki (1998) provides an extensive narrative on preparation of laboratory SOPs.

Laboratory sample receipt, login, preservation, holding time, and tracking should be detailed in one or more SOPs. Other lab areas worthy of individual SOPs are labware cleaning, decontamination, storage, sample preparation, analytical procedures, reference material choice/storage/disposal, health and safety, standard solution preparation and verification, data acquisition, Laboratory Information Management System (LIMS) operation, data archiving, data validation, report generation, and sample archiving and disposal (Thiex et al., 1999).

An analytical method SOP should contain a description of all method-specific process steps, addressing sample preparation; extraction and/or digestion; instrumentation calibration, operation and maintenance; calculations; quality control; and reporting. The SOP should specify instrument detection limits (IDL), method detection limits (MDL) and limits of quantification (LOQ), analyte precision, and an expectation of repeatability on client samples. The QC section should address the use, where appropriate, of certified or standard reference materials, the use of continuing calibration verification controls, the frequency of quality control samples, control limits, and corrective action steps for quality failures. Quality failure actions should address known or common problems, which can range from contamination to poor reproducibility. There should also be provision or even a separate SOP for addressing methods exhibiting chronic QC failures. All analytical method SOPs should reference published standard methods, and demonstrate method conformity.

Alterations in analytical methods can cause substantial differences in the final results. A detailed SOP defines the scope of the method, ensures method execution, minimizes variability in results, and helps in resolving deviations in analytical results.

1.1 Method and Instrument Logs

Maintaining an internal log of method performance, reagents and standards, and instrument operation and maintenance can be invaluable in preventing or troubleshooting lab problems. A useful practice is a written log of known sources of error for each method and/or lab operation. A brief list of such sources for manure testing is found in Table L -1.0.1. An instrument log should include a performance summary, maintenance schedule, and a report on maintenance problems and service calls. Logs provide continuity within a succession of laboratory technicians over time.

2.0 QC Definitions: Error, Accuracy, Precision, Bias, and Uncertainty

A discussion of quality controls requires the use of terminology and specific meanings.

Error: "the collective term for any departure of the analytical result from the true value." Errors may be random or unpredictable deviations; systematic or predictable deviations; constant, unrelated to the analyte concentration; or proportional to the analyte concentration.

Accuracy: "the trueness or the closeness of the analytical result to the true value." Accuracy is comprised of a combination of random and systematic errors and cannot be quantified directly.

Precision: "the closeness with which the results of replicate analyses of a sample agree." Precision is the measurement of dispersion or scatter about the mean value, typically expressed as the standard deviation.

Bias: "the consistent deviation of analytical results from the true value caused by a systematic error in a method." Bias is not trueness but a measure of the deviation from the true value. Sources of bias include those linked to the sample, those associated with the analytical method, and those related to the laboratory.

Uncertainty: "an error probability estimate associated with a measurement accounting for both the accuracy and precision of the measurement."

Bias and precision are best illustrated by the four types of errors (Figure L-1.0.1). The upper right of the figure shows low bias and precise measurements (small random errors). Conversely, the lower left of the figure shows high bias and imprecise results (strong bias and large random errors).

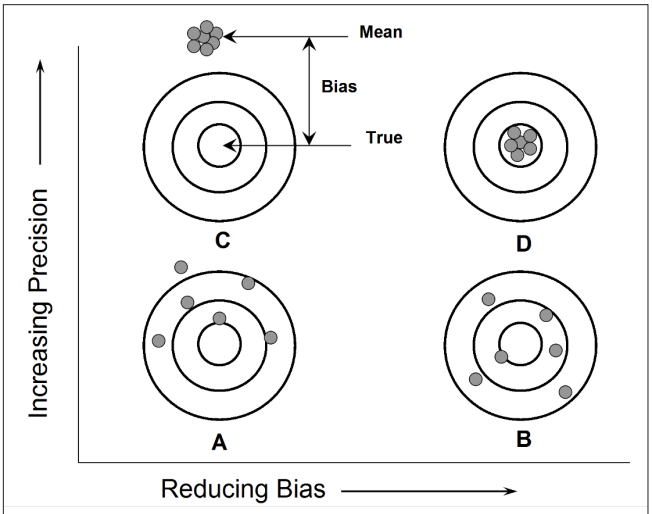

Figure L-1.0.1. An illustration of the relationship between precision and measurement bias: low precision and high bias (A); low precision and low bias (B); high precision and high bias (C); and high precision and low bias (D).

2.1 Statistics

No discussion of QC is complete without a brief discussion of statistics. The basic assumption of analytical data, obtained by repeated measurements of the same analyte under the identical conditions, results in a normal or Gaussian distribution of measurement data. The primary statistical parameters used to evaluate the data are the mean ($\bar{x}$), standard deviation (s), coefficient of variation (CV), *F*-test, *t*-test, and regression analysis. The mean represents the mathematical average of the measurements, and the standard deviation is a measure of the dispersion around the mean. For a Gaussian distribution, 68% of the measurement data falls within one standard deviation of the mean ($\bar{x} \pm s$), 95% within two standard deviations of the mean ($\bar{x} \pm 2s$), and 99.7% within three standard deviations of the mean ($\bar{x} \pm 3s$).

An evaluation of precision may be expressed as the *relative standard deviation (RSD)*, expressed as a fraction, or as the CV, calculated by dividing the standard deviation by the mean and expressed as a percentage ($s \div \bar{x} \times 100$).

Measurement uncertainty is used to assign confidence limits on the measurement mean. This is calculated by multiplying the standard error $(s/\sqrt{n})$ (where n represents the number of measurements) by the t statistic probability based on the number of replicates.

The final result of analysis is often calculated based on several measurements (weight, dilution, moisture content, and instrument calibration) and, as a result, the total error is comprised of all errors associated with the process. Propagation of errors is not a simple summation, but the square root of summation of the squared error terms for each of the components. For further discussion on calculating propagation errors, the reader is encouraged to read a discussion of experimental error (Harris, 2010).

3.0 Quality Control Analytical Practices

The objective of the SOP is to consistently produce reliable measurement data from a laboratory method. Relevant to the SOP are all aspects of the analytical method: blanks, detection and quantification limits, precision, matrix spikes, blind samples, control samples, accuracy, and instrument calibration. This differs from QC, which is undertaken to monitor the analytical process, determine measurement errors, and take corrective action for unacceptable results. Further discussion of QC monitoring is discussed in Section 5.0.

3.1 Method Detection Limit (MDL) and Limit of Quantification (LOQ)

The MDL, as defined by the US EPA, is the minimum measured analyte concentration that can be reported with 99% confidence that is distinguishable from that of the measured method blank. An estimated MDL is typically defined as 3 times the standard deviation of a method blank for an analyte. Using the estimated MDL, US EPA 821-R-16-006 describes the procedure for determining the operational MDL based on spiking, listed in Appendix B. The MDL should be verified, documented in the SOP, and updated after any change in analysis method procedures, instrumentation, or reagents. The LOQ is the lowest level at which the entire analytical system gives a recognizable and quantifiable signal and an acceptable calibration point for the analyte, and is equivalent to three times the MDL (MDL × 3) .

3.2 Calibration Blank (CB)

A calibration blank is an effective means to assess and monitor instrument calibration. It is measured at the time of instrument calibration and periodically (once every 10–15 unknown samples) thereafter, and is used to assess instrument calibration base line drift.

3.3 Method Blank (MB)

A method blank is an effective means to detect and quantify analytical bias. One or more blank samples are carried through the entire analysis method process (sub sampling, filtration, dilution, reagents, and instrumental analysis) using the same calibration. This process should be repeated on a systematic basis (with each array/batch of unknown samples) to verify that the analyte of interest is consistently below the MDL. Blanks are more likely to be significant for analytes at relatively low concentrations, as in micronutrient analyses. Blank values should be verified after any changes in analysis method procedures or reagents.

Blanks will quantify the contribution of containers, reagents, and the laboratory environment to final solution concentration. If a blank value > MDL is discovered and cannot be eliminated, the concentration should be subtracted from the values for those unknown samples associated with that batch. Blank subtraction is used to correct for systematic sources of contamination, not random ones. In this way, systematic bias can be used to improve accuracy.

3.4 Continuing Calibration Verification (CCV).

Along with the MB, a continuing calibration verification standard should be analyzed at the beginning of and periodically during an analysis batch using a mid-range calibration standard. The frequency of CCV analysis is typically either once every 10 samples or as indicated by the instrument manufacturer.

3.5 Independent Calibration Verification (ICV)

An independent calibration verification standard is prepared from a source independent of the one used for preparing the calibration standards and representing concentrations at the low and high end of the calibration range. ICVs provide an external assessment of the method.

3.6 Method Duplicate

Method precision can be documented through replicate testing of routine samples or internal lab reference samples. This involves two or more analyses of unknown samples at a specified frequency (i.e., every 10 or 20 samples). Precision control limits are based on relative percent difference (RPD) between replicates for each analyte. A relatively high frequency of replication should be used initially and reduced after valid statistics (see R-Chart in Section 5.0) and QC method precision standards are being met. Replicate analysis is useful where appropriate certified or standard reference samples are unavailable (Garfield, 1991) or when matrix and concentration range are of concern (Dellavalle, 1992).

3.7 Method Matrix Spike Recovery (MSR)

Matrix spiking can be used to assess method bias for methods measuring total analyte content. A sample is spiked with a known quantity of the analyte(s) of interest prior to digestion. Concentrations of the spiked sample are compared with that of an identical rep of the unknown sample without spiking, and the percent recovery is calculated. The technique is useful for evaluating matrix bias associated with incomplete digestion, volatilization losses, and/or analyte interferences. Spiking is *not* appropriate for methods where analyte content is measured by partial extraction and semi-quantitative analysis (Dellavalle, 1992).

3.8 Method Control Sample

Accuracy of analytical results can be verified through the use of certified reference materials (CRM), when available, or a secondary reference material (SRM). The reference sample should be similar in matrix and analyte concentration range to the unknown routine samples tested. Several SRMs can be purchased from commercial or government sources. Table L-1.0.2 lists several currently available materials, with guaranteed or provisional analyte concentrations. CRMs will have an analysis uncertainty range of the elemental content and are considered the most unbiased way to document accuracy in a laboratory QA program (Dellavalle, 1992). At present, there are no certified reference manure samples available. A reference plant tissue, soil, or sludge is an appropriate SRM for checking the accuracy of manure analyses. SRMs may be acquired from laboratory proficiency programs, such as the Manure Pro-

Table L-1.0.2. Suitable certified and standard reference materials for manure analysis.

Company or Agency	Material ID	Analytes
SCP Science 348 Route 11 Champlain, NY 12919-4816 800-361-6820 / www.scpscience.com	CP-1 – Compost	N P K Mg Ca Cu Fe Mn Zn Na pH
	BE-1 – Sewage Sludge	B Ca Cu Fe K Mg Mn Na P Zn
Environmental Research Assoc. 16341 Table Mtn. Parkway Golden, CO 80403 800-372-0122 / www.eraqc.com	Catalog # WWS-26 – Sewage Sludge	Ca Fe Mg Na Zn
	Catalog # 545 – Sludge	TKN P NH_3
	Catalog # 160 – Sewage Sludge	Ca Cu Fe Mg Na Zn K Mn
	Catalog # 542 – Soil	TKN P NH_3
Sigma Aldrich RTC 2931 Soldier Springs Rd Laramie, WY 82070 800-576-5690 / www.sigmaaldrich.com (search RTC CRM)	Catalog # RM 007-040 – Sewage Sludge	B Ca Cu Fe Mg Mn K Na Zn
	Catalog # CRM 018-050 – Sewage Sludge	B Ca Cu Fe Mg Mn K Na Zn
US Dept of Commerce **National Institute of Standards & Technology** Building 202, Room 204 Gaithersburg, MD 20895 301-975-6776 / www.nist.gov	SRM 2781 – Domestic Sludge	Ca Cu Fe Mg P K Na Zn
	SRM 2782 – Industrial Sludge	Ca Fe Mg P K Na Cu Zn

ficiency Testing (MAP) program coordinated by the Minnesota Department of Agriculture.

3.9 Method-Blind Sample

A method-blind sample is a sample of known analyte concentration inserted by the head of the laboratory or QC officer, unknown to the lab analyst, and submitted in the routine set or batch of unknown samples for the evaluation method performance. The blind sample may be an in-lab control sample of known composition and uncertainty, a CRM, or an SRM. The use of blind samples is an effective means of assessing lab bias and the effectiveness of a laboratory QA/QC program. It is often used for documenting laboratory performance audits (Taylor, 1987; US EPA, 1986).

4.0 SOP Method Validation

Validation of in-lab analytical procedures is required to assess the SOP performance. These include trueness and bias, precision, analyte working range and MDL, analyte carry-over, ruggedness, analyte recovery, interferences, and practicability. SOP method validations should be updated annually and with changes in laboratory staffing and instrumentation.

Trueness and bias. An assessment of trueness and bias may be carried out directly through assessment of CRM or SRM samples, or indirectly by comparing results of an in-house method to an official or generally accepted method. In either case, a new or alternative method can be evaluated.

Method precision. Replicate analysis is performed on a reference sample and expressed as an RSD. Values are compared to the reference materials value or values from an alternative method. Evaluate method repeatability as the agreement of a sample or reference sample results under the same conditions. Evaluate within laboratory precision from results obtained by different operators or instruments.

Analyte working range. Validation of an analytical method requires determination of both the MDL and the upper limit of quantification. The upper limit is based on specific instrument calibration ranges and/or dilution of samples that exceed the highest instrument calibration standard.

Analyte carry-over. Batch methods that utilize automated sample introduction instrumentation should be evaluated for analyte carry-over, from samples with supra-optimal concentration into ensuing samples. Carry-over can be evaluated by analyzing three high analyte standards, followed by three method blanks (Dixon, 1990).

Ruggedness testing. An analytical test method is rugged or robust if results are insensitive to laboratory method variations. Examples include temperature, extraction time, technical staff, shaking energy, pH, sample size, and time. Ruggedness testing can be evaluated using the "Youden

and Steiner factorial design" for within laboratory validation (Youden and Steiner, 1979).

Analyte recovery. Analyte recovery is the fraction of an analyte recovered after the addition of a known amount of the analyte to a sample of known concentration (i.e., a laboratory-fortified duplicate sample). Recovery is used to determine method effectiveness, and is reported as a percentage of the known addition. Acceptable values typically range between 90–100%, but vary by method, analyte, and sample matrix.

Interference. An analytical method may be susceptible to inter-element or matrix effect interferences or to interferences associated with instrumentation. In specific cases these may be quantified by replicate spiking (i.e., standard addition) or matrix blanks, or by quantification of and correction for the interfering element/compound (i.e., inter-element correction capability in many operation systems).

Practicability. The method description should include the ease or tediousness of the method as it relates to the use of specialized instrumentation, purity of reagents, cost of chemicals, time and staff constraints, and special skills / staff training.

5.0 QC Statistical Control Charts

QC statistical control charts provide an effective means of internal monitoring of "statistical control" of a laboratory process and/or method. Lab control charts are generally of two types: mean charts (also referred to as X-charts or Shewhart Control Charts) and range control charts (R-charts) for control of precision. Control charts are used by technicians and managers as decision-making tools during lab analysis to ensure method reproducibility. Separate control charts can be used for tracking multiple analytical parameters such blanks, internal controls, analyte recoveries, CRM, calibration standards, and instrument response, and can be incorporated in LIMS programming.

5.1 QC Mean Charts

QC mean charts provide a graphic interpretation of analyte measurement accuracy and bias. In each batch of test samples one or more one control samples (or internal SRM, or external CRM) is analyzed, and measurement results are recorded and plotted over a timeline. For the control sample a minimum of seven (7) measurements is needed, though a minimum of ten (10) is recommended (van Reeuwijk, 1998) for the determination of the cumulative mean and standard deviation (*s*). Superimposed on the control sample results is the SRM cumulative mean or known CRM concentration. Upper and lower warning limits (UWL & LWL) are calculated as ± 2 *s*, representing the 95% statistical confidence limits and upper and lower control limits of the mean, and UCL and LCL are calculated as ± 3 *s*, representing the 99% CL of the mean (Figure L-1.0.2).

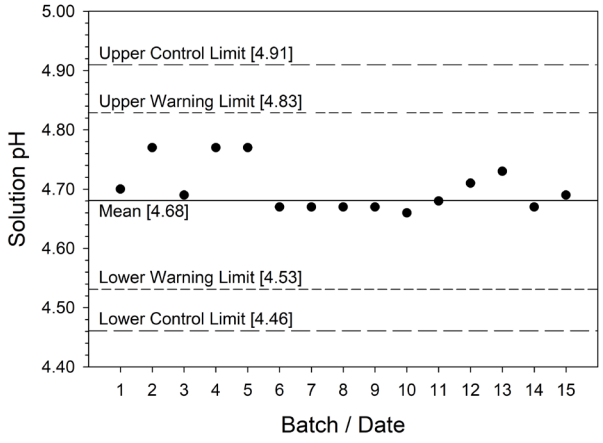

Figure L-1.0.2. Mean chart with the expected the mean and upper and lower warning and control limits.

An individual analytical measurement between UWL and LWL is considered to be within statistical control. A process is deemed to be out of statistical control if two or more measurements are between UWL and UCL and/or LWL and LCL, or if any one value is outside UCL or LCL ranges. When an analytical method is outside statistical control, all routine sample unknowns run since the last in-control check sample should be rerun. Control sample results within the warning limits, but which exhibit a trend toward the UWL or LWL, signal a potential measurement issue (Dellavalle, 1992). A full discussion of quality control chart rules and interpretation is described by van Reeuwijk (1998). X-charts are especially useful as a batch-to-batch process monitoring tool for assessing ongoing or emerging analyte measurement problems.

5.2 Range Charts

QC range (R-charts) provide graphic interpretation of precision analysis data over time. In each batch of test samples one or more control samples or SRM(s) are analyzed in duplicate (Figure L-1.0.3). A cumulative mean range is calculated and superimposed on the individual range values. Warning and control limits are calculated as 2.512 times and 3.267 times the mean range, respectively (Taylor, 1987; Garfield, 1991). Replicate ranges are absolute, with only one warning and control limit displayed (Figure L-1.0.2). R-chart data consists solely of replicate ranges, and is used only to document precision. A minimum of 15 replicated samples is recommended for producing an R-chart (Taylor, 1987).

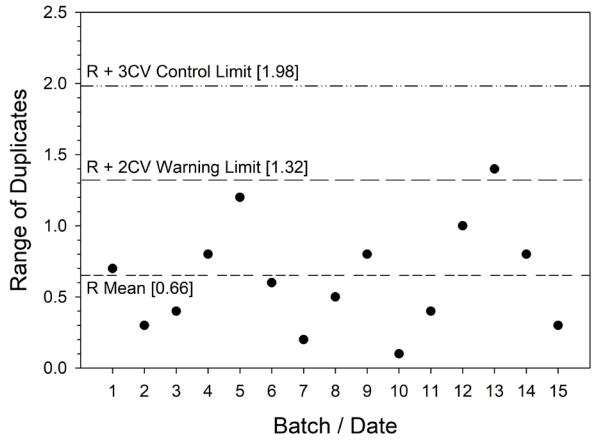

Figure L-1.0.3. Range chart of duplicates. R represents difference between duplicates.

6.0 External Quality Control

While the QC protocols discussed in the proceeding sections are restricted to lab internal quality control, individual lab bias associated with a systematic error can often go unnoticed. Two options are available for external assessment: inter-lab sample exchange and proficiency testing.

6.1 Inter-Lab Sample Exchange

It is not uncommon to have specific unknown sample analytes with questionable uncertainty or errant values from laboratory norms. Samples can be submitted to another laboratory for analysis. Three types of comparisons can be assessed: single-measurement comparison, replicate measurement comparison, and multi-sample replicate comparison. A single-measurement comparison has a wide confidence limit interval, while comparison of replicate samples provides for statistical comparison of the results. Statistically, a range of unknown samples is the most thorough and provides for both an assessment of replicate data and an evaluation of systematic bias.

6.2 Inter-Laboratory Proficiency Testing (PT)

A laboratory which proclaims it generates quality analytical data should participate in at least one inter-laboratory PT program. PT programs are generally based on single-blind assessment (known proficiency test samples, but of unknown analyte concentration) and allow for comparison of results among laboratories for the assessment of an analyte mean, standard deviation, repeatability, and reproducibility. In addition, inter-lab programs can be a useful source of secondary reference samples (SRM), which can be used internally by a laboratory participant.

Depending on the sample matrix, laboratories may follow their own method, perform a generally accepted method, or use one designated as an industry official method. Proficiency samples are submitted to participants on a periodic basis, usually semi-annually or tri-annually. Typical proficiency programs utilize two or more proficiency materials that represent a range in analyte concentrations. Analysis data is compiled and analyzed by highly skilled professionals with a background in chemical analysis and statistics. Inter-lab proficiency sample data represents statistical consensus estimates of an analyte mean and precision. Caution should be exercised when accepting PT sample mean values as "true values," since method variances and small populations may be of issue, especially on lab results with < 8 laboratory participants.

With regard to manure analysis, the Minnesota Department of Agriculture has coordinated the Manure Analysis Proficiency (MAP) program in North America since 2002; this program serves approximately 70 laboratory participants. The program evaluates three manure samples for 11 analytes semi-annually, collected from a range of manure sources. Inter-lab proficiency data is compiled, and results are generated on lab analysis performance based on consensus statistics. More information can be found by contacting the Minnesota Department of Agriculture.

7.0 Reporting

Mean and range charts of blank and control samples should be documented for each batch, reviewed daily, and evaluated on an on-going basis. Accuracy and precision statistics can be reported based on client need.

Specific projects or clients may require replicate analysis and reference sample statistics as a supplement to the analytical results. Precision is typically documented by reporting relative percent differences (RPDs) from replication of client samples. Bias can be documented by reporting SRM(s) or CRM(s), run with the analytical batch that included the client's samples.

Even when not required, a simple summary of typical precision or analytical uncertainty can be listed or sent with the report for a routine nutrient analysis of manure. A listing of 95% CL ranges for the reported analytes can be taken from the cumulative statistics for a reference sample or samples, run over the past several months or years. This simple listing of expected uncertainties can greatly improve client understanding and confidence in the quality of the analytical results.

8.0 Summary

An LQM system includes a QA program comprised of lab methods and process SOPs and a QC protocol for validating analyte accuracy and precision. SOPs provide a compulsory list of instructions of lab methods and procedures that ensure that assays are carried out in a consistent man-

ner. LQM systems include documentation of quality analytical procedures that assure method control and standards of method performance. Through the use of quality control charts, a lab method and/or process quality can be monitored and documented. Through the use of an inter-lab proficiency testing QC program, lab bias can be minimized and precision optimized.

This brief overview of LQM systems is designed to provide a basic outline. Analytical laboratories providing manure and biosolids analytical services should bi-annually review and assess their method SOPs, QC charts, and external QC proficiency reports. Laboratory analytical quality is not a destination, but a continuing journey.

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L-2.0
The Manure Analysis Proficiency (MAP) Program

AUTHORS: Jerry Floren, Robert Miller, and Larry Gunderson

Overview

In the early 1990s, the Minnesota Department of Agriculture (MDA) recognized the need for accurate manure analysis and lobbied the Minnesota Legislature to provide funds for a manure proficiency program. Funding began in 1994, and a program was developed to provide manure proficiency samples to Midwestern laboratories. The program was initiated in 1995, administered by the MDA and coordinated by Jan Jarman. Jerry Floren assumed responsibility for the program in 2001.

In 2003, through grant support from the United States Environmental Protection Agency (US EPA), the MDA manure testing program was expanded into a national program, the Manure Analysis Proficiency (MAP) Program. This support enabled the MDA to expand the number of laboratories served, purchase specialized processing equipment, archive reference manure samples, establish an advisory board, and build a method performance database. The MDA contracted with Dr. Robert Miller, Affiliate Professor at Colorado State University, to provide technical assistance.

Under the US EPA grant, the MAP Program had the following objectives:

> A national proficiency testing program would seek to ensure accurate and understandable manure test results for livestock producers. These objectives would be accomplished through: development of laboratory proficiency testing to ensure the accuracy of manure test results; identify future reference analysis methods; promote consistent recommendations for manure sampling; promote consistent reporting and interpretation of test results; and assist states, laboratories, and other key information providers in providing assistance to producers in the development and implementation of environmentally and economically sound nutrient management plans. Program oversight would be accomplished through establishment of an advisory board of stakeholders. (Floren et al., 2006, p. 6)

The program was structured to follow international standards under ISO/IEC 17025 general requirements for the coordination of a proficiency testing (PT) program. This included development of standard protocols for the preparation of manure PT samples, the use of blind sample replicates for the assessment of intra-laboratory precision, and the implementation of robust statistical measures to evaluate laboratory bias and precision.

With the exception of 2017, when the MAP Program provided 15 freeze-dried manure samples, each MAP exchange consists of three different manures with triple replicates (nine containers). From 2003 to 2006, the MAP program utilized three PT rounds per year. Since 2007, the MAP Program has utilized a biannual submission for each PT cycle.

Proficiency testing samples are collected from Minnesota farms and selected based on source animal type and a range of total solids (1–95%). Samples are ground, homogenized, packaged, and frozen prior to overnight shipping to program participants. In 2003, MAP manure analytical methods included total solids, total nitrogen using total Kjeldahl nitrogen (TKN) and nitrogen by high temperature combustion (N-C), ammonium nitrogen (NH_4-N), phosphorus (P), potassium (K), zinc (Zn), copper (Cu), and electrical conductivity 1:1 (EC). During the past 17 years, pH, nitrate-nitrogen (NO_3-N), chloride (Cl), sulfur (S), sulfate (SO_4-S), sodium (Na), and water-extractable phosphorus (WEP) methods were added to the MAP program. Electrical conductivity has expanded to include three extraction methods based on 1:1, 1:2, and 1:5 ratios. NH_4-N was expanded in 2011 to include four methods: ion selective electrode (ISE), distillation, spectrophotometric, and diffusion-conductivity.

The MAP Program compiles tabular statistical reports with each sample exchange using the open source statis-

tical program "R" (R Core Team) for all analyses. Method bias is assessed based on the inter-laboratory median and 95% confidence limits (utilizing the median absolute deviation, MAD) set at ±2.5 MAD for each PT sample. Triple replicated samples enable the evaluation of individual laboratory method precision by comparing a laboratory's relative standard deviation (RSD) – designated as "R_p" on MAP reports to the consensus intra-laboratory precision ("R_d" on the MAP reports) of all participants.

In addition to the tabular reports, the MAP Program provides unique graphical reports[1] to each participating laboratory, detailing method performance, laboratory bias, and precision for each manure sample and analysis. The graphs highlight an individual laboratory's results in one color, with results from the other participating laboratories in another color making it easy for a user to compare their results to those submitted by other laboratories.

The MDA certifies laboratories for manure testing based on a scoring system, evaluating bias and precision for total nitrogen and total phosphorus analyses. Laboratories meeting certification requirements are evaluated annually, with results posted on the MDA website. Laboratories not meeting the certification requirements may purchase a rerun sample set, are offered consultation via industry experts in laboratory analysis, and are re-evaluated.

Over the past 18 years, 55–78 laboratories have enrolled annually in the MAP program across North America. Approximately 70% are commercial laboratories, while 30% are associated with university or research laboratories. From 2003 through 2021, the MAP Program distributed 129 different manure PT samples, sourced from dairy, beef, swine, poultry, horse, sheep, and goat operations. PT samples have ranged from 1.00% to 90.6% total solids, 0.040–3.88% total nitrogen, 0.007–2.53% total phosphorus, and 0.07–0.85% NH_4^+ nitrogen.

Method Observations

Some MAP methods have high precision, while others have matrix constraints. For MAP samples evaluated from 2009–2018, the consensus intra-laboratory method precision (method relative standard deviation [RSD] within a laboratory) for total solids ranged from 2–5% for liquid manures and < 1.0% on manures with > 50% total solids. With regard to nitrogen, TKN intra-laboratory precision ranged from 1.5–4.0% for liquid manures, while that for the N-C method ranged from 4–7%. Both methods of nitrogen analysis have had similar intra-laboratory precision on manures with > 50% total solids, RSD 2–3%. Results for P and K across MAP samples generally show intra-laboratory precision from 2–5% across all manure types. A comparison of four NH_4-N analysis methods indicates intra-laboratory RSD of 4–8% on liquid manure samples and 2–4% on manures with > 50% total solids.

Generally, intra-laboratory precision for EC ranged from 1–5% across all manure matrix types, independent of the method dilution. Intra-laboratory precision values for Ca, Mg, and Na ranged from 2–7%, while those for S, Zn, and Cu ranged from 3–8%. Precision for the WEP method ranged from 3–11% and was generally 50% higher for liquid manures.

With respect to manure method inter-laboratory performance (across laboratories), several specific observations were noted. Inter-laboratory RSD was compiled for PT samples from 2012–2018. Results show consensus RSD values of 3–4% for total solids, with the exception of liquid manures < 5% total solids, which ranged from 8–10%. RSD results for TKN and N-C were 4–7% and 8–14%, respectively. For N-C for manures < 5% total solids, the inter-laboratory RSD range was 11–15%. Overall, it was noted that for manures with < 0.4% nitrogen, the combustion method resulted in inter-laboratory precision exceeding 15%, the result of concentrations approaching the N-C method limit of quantification (LOQ).

Results for P and K across MAP samples generally show inter-laboratory precision ranging from 6–11% across manure types. A comparison of four NH_4-N methods indicates intra-laboratory RSD of 6–10% on manures, with the exception of those with concentrations < 0.020% NH_4-N, which had inter-laboratory RSD ranging from 20–40% (the result of concentrations approaching the method LOQ). Generally, the ISE and distillation NH_4-N methods had higher inter-laboratory RSD values than the spectrophotometric and diffusion conductivity methods.

Overall, inter-laboratory precision for EC ranged from 6–20% across all manure matrix types, independent of the method dilution. Inter-laboratory precision values for Ca, Mg, Na, S, Zn, and Cu ranged from 8–18% across manure types, with poorer precision noted on samples with concentrations approaching the method LOQ. Precision estimates for the WEP method have ranged from 8–35%, and have been generally poorer for liquid manures. It is worth noting that inter-laboratory precision has improved in the past five years, and now averages 8–12%.

Laboratory Performance

Over the past 18 years of the MAP Program, 70–80% of participating laboratories have met the MDA certification performance standard for the key analytical methods, total N and P. While challenges with meeting MDA certification range widely, a few problem areas are regularly encountered. Specifically, some laboratories have ongoing performance issues analyzing liquid manures, likely as a result

1 See Figure L-3.0.1 in Chapter L-3.0, Evaluating Laboratory Performance.

of analyte concentrations approaching the LOQ. For other laboratories, the performance issues appear to be associated with manures that are high in total solids (> 70%), typically reflecting a lack of experience in the analysis of these types of manures. As a whole, a majority of the laboratories showing poor performance are those analyzing fewer than 1,000 samples per year.

The MDA recognizes the limitations of using MAP results for the certification of manure testing laboratories, as PT samples are "single-blind" samples — a laboratory participant knows the MDA is the sample provider, but the nutrient contents are unknown. A "double-blind" exchange, where both the manure source and the nutrient contents are unknown, is a more effective method for evaluating a laboratory for certification. In 2006, the MDA conducted a double-blind evaluation of MAP participants that greatly increased costs and presented logistical challenges. Results of the study had nearly equivalent median values, with somewhat larger MAD values on the double-blind results compared to the single-blind results (Floren et al., 2006, p. 56).

A comparison of MAP program method performance with those of other laboratory proficiency programs — the Agriculture Laboratory Proficiency (ALP) Program, which provides soil and plant analysis, and the US Compost Council (USCC) Seal of Testing Assurance Program (STA) — show parallels among the programs. Results for total N-C, P, and K quantitative methods show similar inter-laboratory and intra-laboratory precision values. Results for NH_4-N and Cl methods indicate superior intra-laboratory precision for laboratories participating in the MAP program relative to the USCC compost analysis PT program. An assessment of intra-laboratory method performance indicates that all three programs have similar RSDs for the quantitative analytical methods. However, results for soil semi-quantitative methods (extractable analysis methods) in the ALP program indicate inter-laboratory RSDs of 12–20% for extractable P, K, S, and Zn methods.

A comparison of overall laboratory performance indicates differences among the three programs. Results for the MDA and STA programs show overall performance levels of 80–90% across analysis methods, while the ALP program for plant analysis achieves only 70–80%. This discrepancy is likely because the MDA and STA programs are long-established certification programs, with required minimum standards of performance, while there is no certification of standard of performance for the ALP plant analysis PT program.

Conclusion

The MAP program is the only manure PT program in North America. It provides an external evaluation of both manure analysis test methods and the analytical quality of the laboratories conducting the tests. Through the assessment of laboratory bias and precision of multiple methods, and the use of prescribed standards of acceptable performance, laboratories are certified annually for the analysis of manure samples. By means of this evaluation, laboratories are certified by the MDA.

In summary, the MAP program provides agronomists, researchers, and agricultural producers alike with a greater level of confidence in the quality of laboratory manure analyses used in nutrient management in North America.

References

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L-3.0
Evaluating Laboratory Performance

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Overview

The Minnesota Department of Agriculture (MDA) administers the Manure Analysis Proficiency (MAP) Program – a proficiency testing (PT) program for manure analysis for North America. For each PT cycle, the MDA generates a summary report of PT statistical results and an assessment of method analytical bias and precision performance for each laboratory participant. The primary focus of this chapter is to provide insight in the assessment of laboratory method PT results, the investigation into root cause analysis of method failures, and the implementation of a corrective action.

The National Institute of Standards and Technology (NIST) defines bias as "a quantitative term describing the difference between the average of measurements made on the same object and its true value." The MAP program establishes the reference value of each measured parameter utilizing the inter-laboratory median and 95% confidence limits thereof based on 2.5 × median absolute deviation (MAD). A laboratory's analytical result (based on the mean of their three blind replicates) exceeding this acceptance range is defined as having bias and is identified as a performance failure.

MAP intra-laboratory precision (R_p) is calculated based on the three blind replicates and is used to calculate the consensus median inter-laboratory precision (R_d) of all PT participants. A laboratory R_p precision value for an analysis parameter exceeding the MAP program benchmark of 3 × R_d is identified as precision failure.

MAP Reports and Review

MAP proficiency samples are submitted biannually to laboratory participants for the evaluation of laboratory analytical performance for inorganic constituents, based on standardized methods. The PT samples represent a "single blind" evaluation of analytical performance, whereby homogenous manure materials are analyzed for the purpose of evaluating bias and precision. MAP PT samples represent a range of manure matrices submitted as blind replicates (n = 3) for inorganic analysis. MAP reports provide statistical summaries for multiple manure analysis parameters: the number of laboratory participants, minimum and maximum reported results, population median, median absolute deviation (MAD), 95% confidence limits, inter-laboratory method precision, an assessment of individual laboratory bias, and intra-laboratory method precision.

Laboratories participating in the MAP program receive additional information with more detailed descriptions of the reports than the information provided in this chapter. In addition to the graphical report described below, several tabular reports provide statistical data and even letter grades for bias and precision. If your laboratory participates in the MAP program, be sure to review the MDA description of the MAP reports.

The scatter plot in Figure L-3.0.1 has results for total N by combustion (N-C) for 37 laboratories, with their replicate means sorted from low to high. Hash marks and a circle represent the replicate and mean results, respectively, submitted by each individual laboratory. These graphs, unique for each laboratory and proficiency test sample, show the individual laboratory's results in black, and the results from the other participating laboratories in gray. The horizontal solid line represents the median, the dotted lines are 95% confidence limits, and results above and below the dashed lines are outliers. Laboratories with a large spread in their replicates are flagged for precision. Laboratories with the circle outside the dotted line are flagged for bias. The 19th laboratory (black vertical line) did not have biased results, but was flagged for precision due to the large range in its three replicate results. Laboratories 1 to 4 were flagged for low bias while laboratories 36 and 37 were flagged for high bias. The replicate means (circle) for these laboratories were outside the lower and upper control limits.

An example of MAP program laboratory PT performance is shown in Table L-3.0.1 for three manure sam-

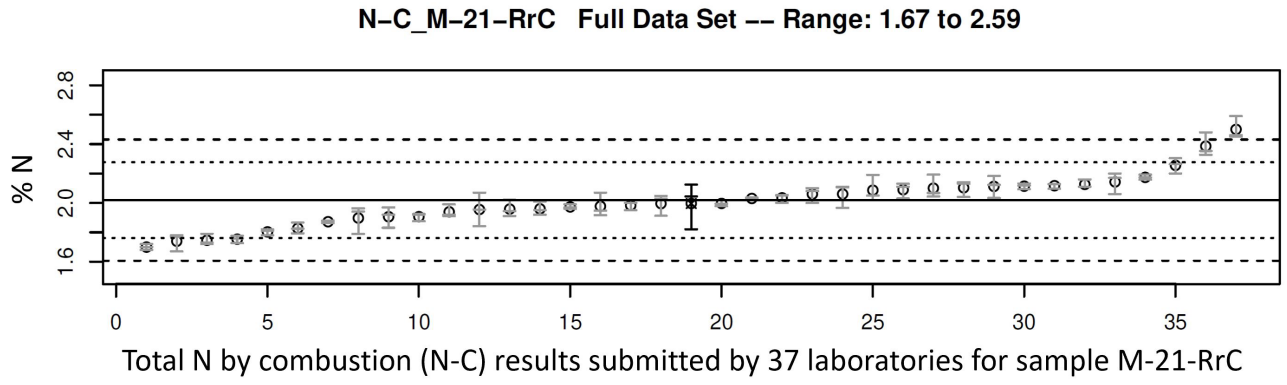

Figure L-3.0.1. Scatter plot graphs enable laboratory staff to quickly discern how their laboratory performed for each sample and analysis compared to other laboratories.

Table L-3.0.1. MAP program summary of total nitrogen by combustion (N-C) results and total phosphorus results for three manure PT samples, 2016

Analysis		MAP program results			Laboratory result		Laboratory PT performance[1]	
	Sample ID	Median	± 95% CL	R_d %	Mean	R_p %	Proficiency	Precision
N-C (%)	2016-A	0.251	0.032	2.1	0.315	0.5	fail	pass
	2016-B	0.113	0.044	2.5	0.149	1.0	pass	pass
	2016-C	0.369	0.075	2.9	0.430	1.0	pass	pass
Phosphorus (%)	2016-A	0.050	0.007	3.1	0.046	15.0	pass	fail
	2016-B	0.022	0.006	2.9	0.028	14.0	pass	fail
	2016-C	0.060	0.012	3.7	0.048	9.0	pass	pass

[1] Laboratory results exceeding 95% confidence limits of median are defined as method failure. R_p results exceeding 3 × R_d values are defined as method precision failure.

ples submitted in 2016 by a single laboratory. The laboratory result for N-C of PT sample 2016-A exceeded the 95% confidence limits of the median, had high bias, and was defined as a proficiency failure. Laboratory phosphorus results show intra-laboratory precision failures for PT samples 2016-A and 2016-B. Multiple failures for two or more PT samples, such as noted for phosphorus, often indicate a systematic performance issue. Laboratory analysis results exceeding the MAP program performance criteria for bias and intra-laboratory precision must be investigated and corrective action undertaken. MAP program method performance criteria are noted in the MDA description of reports provided to laboratories participating in the MAP Program.

Investigating PT Failures

The investigation into a method bias and/or a precision failure is defined by root cause analysis (RCA) of the laboratory's analytical processes, and can be illustrated by a "fishbone" or Ishikawa diagram (Figure L-3.0.2). RCA is the technique used to discover the cause of an issue or problem associated with nonconformity of a laboratory result and to identify appropriate solutions.

Step 1. Verify accuracy of the reported laboratory proficiency results. Was there a transcription error, was there a calculation error, was the correct method reported, was the correct reporting unit used, or was there an error in the uploaded laboratory data report format?

Step 2. Verify laboratory sample tracking. Confirm sample login ID, internal sample tracking, subsample labeling,

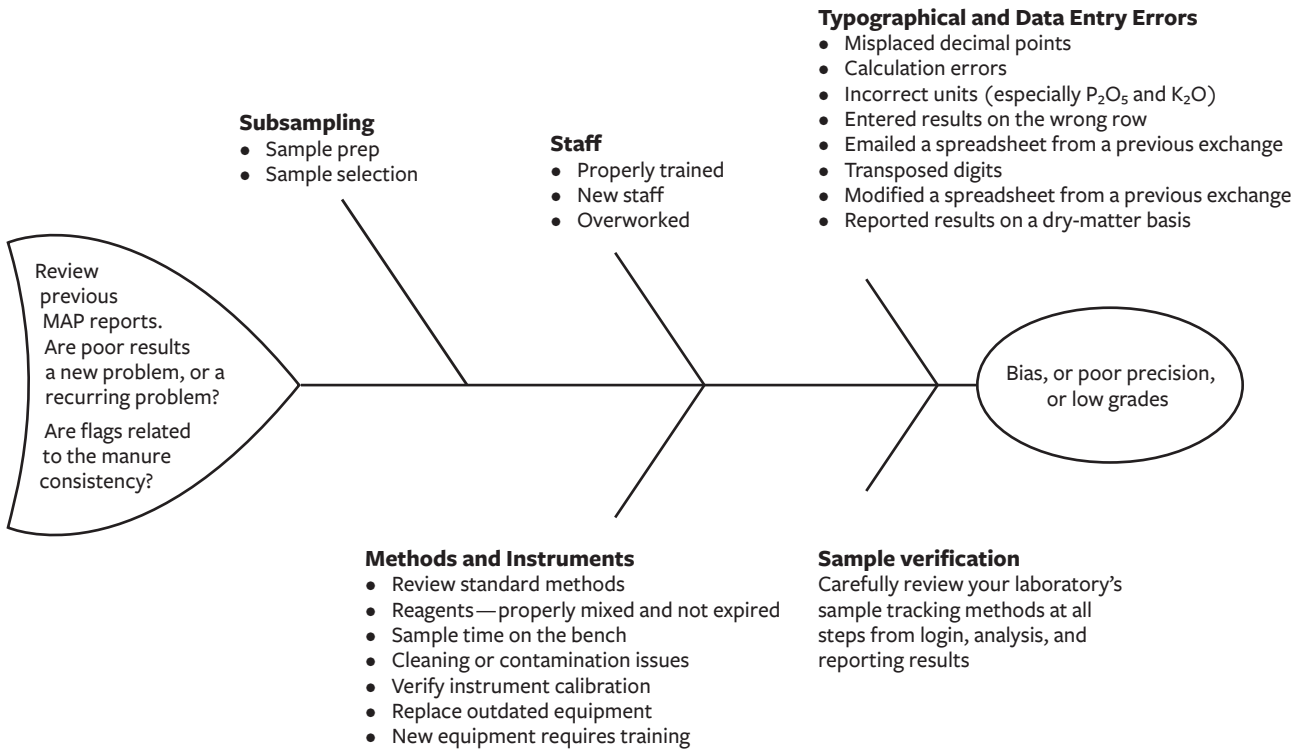

Figure L-3.0.2. This fishbone diagram highlights areas needing attention to correct bias and precision flags.
Source: The MDA description of reports provided to laboratories participating in the MAP Program.

verification of laboratory information management system (LIMS), and reporting of results. Any PT failures in login, sample tracking, and reporting are typically easily identified and resolved.

Absent data entry reporting errors and confirmation of sample IDs, analyses bias and/or precision failures likely stem from: method subsampling procedure, manure matrix issues (liquid vs. solid matrix), a nonconforming method SOP, and/or instrumentation issues. Subsampling manure is often challenging due to inclusions of bedding, feed, hair, feathers, soil, pebbles, and metal, and typically results in poor intra-laboratory precision. A laboratory with a PT precision failure should evaluate the method SOP and review alternative manure subsampling techniques which use a larger subsample or enhance sample homogenization. Liquid manure sample precision is often challenging and may be improved when a dip ladle is used, rather than a dip tube or a peristaltic pump tube.

If the RCA of a PT method failure is unresolved, the next step is the investigation of the method SOP and instrumentation.

Step 3. Did the laboratory method SOP match the method designated by the MAP program? If not, advise the MAP program coordinator of the method dis-conformity and correct or replace the laboratory SOP method with one approved by the MAP program.

Step 4. Did the analysis quality control parameters—instrument calibration, method blank (MB), Continuing Calibration Verification (CCV), duplicate precision, Matrix Spike Recovery (MSR)—meet SOP acceptance criteria? Re-verify the QC protocols and re-analyze the PT sample. Proficiency test results within the SOP quality control acceptance limits but failing PT confidence limits suggest a structural method issue, associated with method non-conformity or instrument performance. A PT sample analysis failure accompanied by results within the laboratory's SOP quality control (QC) acceptance limits is a serious problem.

Step 5. Were instrument performance issues noted prior to or following analysis of the PT samples? Re-assess instrument service schedule and address performance issues. When was the method detection limit (MDL) last verified? Bias and/or precision performance issues near the MDL imply a need to re-evaluate the MDL. Were the issues associated with staff training? Assess laboratory workload, SOP method, and staff training/qualifications.

Step 6. Confirm RCA resolution. Conduct a re-test of the failed manure analysis method using a standard refer-

ence manure in replicate, and assess that bias and precision are within acceptance limits.

Step 7. After completing the RCA and identifying and resolving any MAP method failure issue, a corrective action comment identifying the causative issues and steps taken to rectify the PT failure should be added as an addendum to the method SOP.

Annual review of PT sample performance

An annual review of MAP program results provides insight into laboratory method consistency and performance. MAP-reported laboratory analytical results that are within MAP method proficiency statistical confidence limits, but that are consistently greater than or less than the inter-laboratory median across multiple manure samples, indicate a systematic reporting bias that should be investigated. Laboratory measurement bias may be matrix-specific, e.g., noted only for samples low in total solids. Possible sources of systematic laboratory analytical bias include the following: an analyte contaminate, resulting in high bias; a sample preparation/digestion issue, resulting in low analyte recovery; an instrument standard calibration issue; an inaccurate MDL; or a nonconforming matrix matching issue. An RCA is recommended to identify causative issues.

Laboratory precision R_p results consistently showing intra-laboratory precision exceeding the MAP program R_d, but not failing precision performance (MAP program precision letter grades consistently lower than "A"), indicate issues associated with the laboratory SOP subsampling criteria for manure, analytical instrumentation stability, and/or the method MDL. Excluding manure PT samples with analyte concentrations at or approaching the laboratory MDL, R_p values for total solids, NH_4-N, TKN, N-Combustion, P, K, Ca, Mg, and S analysis parameters are typically 2–6%. R_p precision results for EC, NO_3-N, SO_4, WEP, Zn, and Cu analysis are typically 5–10%. MAP program method intra-laboratory repeatability and inter-laboratory reproducibility, as defined by ISO 5725-2 based on standard deviation, are listed by individual method in this manual. An assessment of method performance over multiple PT cycles provides insight into improving laboratory quality.

Summary

This chapter provides an overview of utilizing MAP reports to evaluate a laboratory's performance and investigate performance failures. The MDA description of MAP reports provides more extensive information on PT reports, proficiency data analysis and interpretation, performance graphs and grading, and the scoring method utilized by the MAP program to certify laboratories for manure inorganic analysis.

Reference documents and additional resources

NIST/SEMATECH e-Handbook of Statistical Methods. 2.1.1.3: Bias and accuracy. https://www.itl.nist.gov/div898/handbook/mpc/section1/mpc113.htm

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L-4.0
Reporting Results

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Laboratory client reports must meet client needs and provide the information necessary for making management decisions. To help clients make practical use of the information, these reports should be easy to understand; they must often also meet specific legal requirements. Analytical reports must provide all essential sample information and accurately reflect analytical results. Laboratories must devote the same level of care and attention to detail in reporting as they do to the accuracy and precision of analytical procedures.

Legal Requirements and Voluntary Programs

Governmental agencies may legally require specific manure analyses. These agencies often require the laboratories running these tests to participate in proficiency testing programs, such as the Manure Analysis Proficiency (MAP) Program administered by the Minnesota Department of Agriculture (MDA). The MDA also certifies laboratories based on satisfactory performance in the MAP program, and some agencies require laboratory certification by the MDA or another certifying entity.

In addition to adhering to legal requirements for manure testing, some clients participate in voluntary programs that provide cost-share funding for manure analysis or in voluntary certification programs focused on the environment. The Natural Resources Conservation Service (NRCS), for example, provides payments to farmers to develop nutrient management plans; these programs may include funding for manure analysis. In most states, to receive NRCS funding for manure analysis, clients must use a laboratory certified for manure testing by the MDA or another NRCS-approved program.

These mandatory and voluntary programs lead to regional differences in analytical needs and reporting requirements. Before accepting manure samples, ask clients if there are any required tests and ensure that your laboratory meets the qualifications (i.e., certification or participation in the MAP Program) required to run these tests.

Collecting Information from Clients

Some clients need manure analyses performed using specific methods. To generate reports meeting your customer's needs, you must ask relevant questions on the manure sample submission form. These include whether your client needs to meet any specific legal or voluntary program requirements, the tests they want completed, and their preferred units for reporting results. The most efficient way to collect this information is through the use of a sample submission form.

The minimum required identification information requested from the client must include:

1. Contact information
    a. For the person or entity submitting the sample. Include name, address, email, and phone number.
    b. For the person or entity receiving the analytical report, if different from the person submitting the sample.
2. Sample identification or location meaningful to your customer (i.e., "Dairy Barn," "Pit #2," "Outside Lot")
3. Sample collection date
4. Livestock type (i.e., dairy, beef, swine, poultry)
5. Analyses requested (this may include a "package" or combination of analyses)
6. Preferred reporting units (i.e., "Circle preferred units: *%*, *Pounds/Ton*, *Pounds/1,000 Gallons*, or *Pounds/acre-inch*.")
    a. It should be noted that Canada uses the imperial gallon (4.55 L), which is different from a U. S. gallon (3.79 L). The report should indicate if result calculations express U. S. or imperial gallons (this may only apply to labs near the Canadian-U. S. border).

Optional but useful client information:

1. Location the manure was collected (i.e., county, state, zip code)

2. Manure storage type (i.e., stockpiled, deep pit, earthen basin, stacked under a roof)
3. Manure application method
4. Requested reporting method: as-received basis, dry basis, or both.

Next, the clients will need to choose a testing package. The following questions may help them choose the analytes they want to test for:

1. Do you have any specific legal requirements? For example, does your feedlot or facility operate under a federal, state, or county permit?
2. Does your operation follow any voluntary guidelines for manure testing, or does a government program provide a payment for the costs associated with analyzing these samples?
3. Are you primarily interested in the nutrient content for crop production, or do you also have environmental concerns — for example, excess copper or salts?

Consider the following packages, but base them on your region:

1. ***Basic Package:*** Total nitrogen, phosphorus (as phosphate, P_2O_5), potassium (as potash, K_2O), and total solids; you might also add ammonium-nitrogen. This basic package includes the primary plant macronutrients to fulfill agronomic requirements. The package will likely be comparable among laboratories in a given area.
2. ***Basic Plus Package:*** The Basic package plus additional secondary nutrients — like calcium, magnesium, and sulfur — and micronutrients that may be of agronomic interest to your clients. In some areas of the country, for example, farmers may be interested in knowing how much sulfur they will apply with their manure.
3. ***Regulatory Program Package:*** The Basic package plus additional analyses needed to meet regulatory program requirements. A client may have requirements for more than one program. Requirements for a feedlot permit, for example, may differ from those for reimbursement on a voluntary, cost-share program.
4. ***Premium or Environmental Packages:*** In addition to the Basic package, include any additional analyses the client requests.

Creating Customer Reports

Excellent reports convey accurate analytical results and are easy to use. Most laboratories organize their customer reports into the following sections: Descriptive, Analytical Results, and Interpretive. Examples of generic laboratory reports for customers can be found at the end of this chapter.

DESCRIPTIVE SECTION

The purpose of this section is to allow both the customer and the laboratory to easily identify the sample. The laboratory identification system and the client reports should each contain the following information:

1. Laboratory contact information, including name, address, phone number, email, and website URL
2. Laboratory identification number assigned to the sample
3. Contact information submitted by client on the sample submission form
4. Sample information provided by the client (i.e., sample name, animal species, manure storage, and application method)
5. Four dates:
   a. sample collection date (as reported by the client)
   b. date the laboratory received the sample
   c. analysis date
   d. report date
6. Contact information for a laboratory staff member capable of answering client questions about the report or the analytical methods.
7. Optionally, if the laboratory uses a thermometer to record the sample's temperature when logged in, include the temperature in the report.

ANALYTICAL RESULTS SECTION

This section is for reporting the results of the requested laboratory tests. By knowing the units that your client prefers (and/or what is legally required of your client), you can make this section more user-friendly. It is generally recommended that, at a minimum, results should be reported on an as-received basis. In some cases, however, you may also need to include reports on a dry basis. Be sure to clearly indicate whether results are based on "as-received" manure or "dry" manure. More information about reporting basis is below. Other key points for reporting manure analysis results include:

- Always report total solids content (also called dry matter) to allow conversion of results to dry matter basis results, if desired. Reporting percent moisture, in addition to percent solids, is optional. Report to at least the nearest 0.1%.
- To maintain consistency with standardized reporting of agronomic soil fertility recommendations and commercial fertilizer grades, report total nitrogen as elemental nitrogen (N), total phosphorus expressed on an oxide basis (P_2O_5), and total potassium expressed on an oxide basis (K_2O). To main-

tain unit consistency with total nitrogen, express ammonium-nitrogen and nitrate-nitrogen on an elemental nitrogen basis.
- Report the primary agronomic nutrients in the units requested by the client.
  - If the client did not provide a preference for reporting the nutrient concentration units, providing all four reporting units (%, pounds/ton, pounds/1000 gallons, and pounds/acre-inch) is a better option than providing a single reporting unit.
- Do not report analytical results beyond the number of significant digits that are appropriate for the analytical methods and related calculations.
- Provide references and documentation, where appropriate, for calculations and methods included on the report.

More details on reporting basis: Manure results are typically reported on an as-received basis (sometimes referred to as an "as-applied" basis), even if the source is a solid manure. When a laboratory refers to a "dry" basis it means the manure has been oven-dried so that very little, if any, moisture is left. It is very rare that an oven-dried manure would be applied to a field. Reporting manure nutrient concentration on an as-received basis is most useful for field application because the client can calculate the applied nutrient rates directly from the laboratory results. In any case, it is important to clearly label how the results are reported (on a dry or as-received basis). Some reporting formats include two columns of test results, one for dry and the other for as-received. This may be the preferred method if your client did not indicate which reporting method they want to see.

Conventional nutrient management planning uses oxide equivalence for phosphorus (phosphate, P_2O_5) and potassium (potash, K_2O). To be consistent with commercial fertilizer grades, report the manure nutrient concentrations as phosphate and potash. Laboratories should use *farmer-friendly* units on the reports — i.e., the oxide equivalence, rather than the elemental concentrations for phosphorus and potassium — unless otherwise required by local guidelines. See Appendix A for appropriate unit conversions.

Some clients may request the bulk density of the manure. For liquid manure with no more than 5% total solids, the density of water (8.34 lb/U. S. gal) can be used. If the actual bulk density was determined, always provide the bulk density value used in calculations. For manure with more than 5% total solids, the bulk density of the sample submitted for analysis may not reflect the actual bulk density in the field. If bulk density is determined on such a sample, the analysis should be qualified with a statement such as "bulk density of disturbed sample."

INTERPRETIVE SECTION

A successful interpretive section can be challenging to write, as many laboratories receive samples from multiple states and regions. Each state or province often has its own crop fertility guidelines and regulations, and these may be updated every few years, making it difficult for laboratories to ensure they have accurate information. Nutrient management plan development requires far more information than can be included on a manure analysis report, and often the client does not provide enough information for estimating the available nutrients.

Unless a staff agronomist utilizes soil analysis data and other information to write the interpretive section, stating less in this section is better. If you know the state or province where the customer plans to apply manure, you could reference specific resources for crop fertilizer recommendations and availability estimates developed by the region.

The following is an example of a statement that could be included:

> "Developing a nutrient management plan requires additional information not available in this report. You may want to work with a crop consultant to determine the best manure application rates based on the manure analysis report, soil test results, estimates for nutrient availability, and the planned crop."

Finally, you may want your chief chemist, owner, or staff agronomist to sign and date the report.

Tips for Successful Reports

REPORT READABILITY

Some client reports use font sizes as small as 8 points, but such small sizes are difficult to read for people with visual impairment or declining vision. Clients will appreciate a large font size of at least 12 points. Font style is also important. When updating client reports, conduct an internet search for "most readable fonts" to see the latest font recommendations. These recommendations may suggest different fonts for printed reports, online reports, or email.

PROVIDING ACCURATE REPORTS

Analytical accuracy requires proper methods, calibrated equipment, competent staff, and appropriate quality assurance and quality control (QA/QC) procedures. Laboratory methods and QA/QC procedures are covered in other chapters of this manual; this chapter only addresses errors made in creating laboratory customer reports. Common errors include clerical (or typographical) errors, programming errors, and confusing or mixing up the samples. Lab-

oratory reports containing these errors have no value to the customer.

Errors made on client reports negate the hard work done in the laboratory. According to the MAP program, these are common errors found on laboratory client reports.

- ***Sample identification errors:*** This is the most serious error laboratories make in preparing reports. Results for incorrectly identified samples have no meaning. Thoroughly train the data entry or login staff, and review training at the beginning of each season. Send "blind" reference samples routinely through the system, beginning at the login station. Proper sample identification is critical; the next section suggests practices to reduce sample identification errors.

- ***Client identification errors:*** Make sure the person receiving the report is the person who submitted the sample. Be careful when sending out results via email. It is easy to attach the wrong file to an email, or to send an email to the wrong person.

- ***Errors related to organization of computer folders (directories) and emails:*** Consider how your laboratory organizes computer folders and sends emails. Ensure that the report sent to a client is for the current sample and not from a sample submitted in a previous year.

- ***Mailing errors:*** Placing client reports into envelopes is boring, and report mix-ups can occur. Emphasize the importance of matching the client report address with the address on the envelope.

- ***Number entry errors:*** Misplacing decimal points is a common error on client reports. Transposing digits (e.g., reporting "0.118" as "0.811") is a data entry error. All results entered by humans should be carefully reviewed to avoid misplaced decimal points, calculation errors, and transposed digits. Transposing digits on the sample ID results in sample confusion and a worthless report.

- ***Software errors:*** Use software to automatically generate reports as much as possible, but recall the adage, "To err is human, to really mess up you need a computer." After any software upgrade, carefully review a random selection of reports on multiple samples.

- ***Conversion and calculation errors:*** Double- and triple-check all conversions and calculations, including, for example, conversions of concentrations to mass per unit volume, such as parts per million (ppm) to pounds per 1,000 gallons (lb/1000 gal).

- ***Reporting unit errors:*** Ensure that calculations and reporting units are correct. A common error is confusing elemental phosphorus (P) with phosphate (P_2O_5) and elemental potassium (K), with potash (K_2O). Laboratory instruments generally produce results for elemental P and K, but customers will want P_2O_5 and K_2O conventions. Also check on the units that are reported. Report the total solids content (or moisture content) as percent. There is no unit for pH. Electrical conductivity (EC) is the most commonly misreported unit; it requires both the measure of electrical conductance and a length dimension — most commonly deciSimens per meter (dS/m) or millimhos per centimeter (mmho/cm). Some laboratories may mistakenly misspell the "mho" conductance unit (e.g., mmhhos, mmhos, mmohs) or omit the length dimension.

- ***Sample identification "enhancement" errors:*** Report sample identification information as provided by the client, but do not guess or provide extra meaning. Never guess the livestock species, as this leads to confusion. If a dairy producer receives a report for swine manure, you can expect a call from a confused customer. Even identifying a sample as solid, liquid, or slurry can lead to confusion. Try to collect this information from the client when receiving the samples.

- ***Typographical errors:*** Carefully review a selection of client reports for typographical errors. In the MAP program's double-blind study, one laboratory misspelled nitrogen as "nitorgen" on the client report. When learning of the error, the laboratory owner realized that it had been in place for over a decade.

Suggestions to reduce sample misidentification at login: Sample misidentification is one of the more common errors, and the most serious mistake a laboratory can make when reporting results. There are several ways a sample can be misidentified. Misidentification often begins at sample receiving and login — especially when a laboratory receives multiple boxes, each containing several samples.

Suggestions to improve the sample receiving and login process include the following:

- A well-trained and responsible staff member should manage the receipt of samples.
- Only work on one box of samples at a time. Never open a second box until the samples in the current

box are all correctly labeled and moved on to the next step in your laboratory's process.
- A photograph of the sample container showing the client's label provides an additional source of documentation if problems arise.
- If the login staff have questions about a particular sample, they should set it aside or flag it for further action. The initial login step is not the time, for example, to guess whether the smudged label is for "Barn 1" or "Barn 7." Contact the client if there are any questions about the sample identification. A photo of the label can help resolve these questions.
- Try to automate whenever possible, but recognize that computer-generated results need careful verification after changes are made.
- Be careful not to transpose digits when working with the laboratory ID number assigned to the sample. Incorrectly recording the sample ID as "112" rather than "121" makes the analytical results worthless. Longer ID numbers increase the possibility of transposition errors. A laboratory using a consecutive sample identification system could end up with numbers having five to six digits.
- Consider using preprinted, self-adhesive labels to identify the sample, perhaps with a barcode or even an electronic radio frequency ID (RFID) chip. A laboratory may print several identical labels for each sample. Attach labels to the client's sample container and the laboratory's sample container. Attach additional labels to other sample login documents corresponding to the sample. Several companies provide self-adhesive labels with three or four labels per row (30–40 labels per sheet).
- Sample bottles with a preprinted ID number on the label can introduce confusion. Although the intention may be to reduce errors, these bottles require someone to accurately record the sample ID number on the sample login paperwork—increasing the risk for handwriting and transcription errors. They also require a secondary system for unlabeled sample containers clients submit to the laboratory.
- For long sample ID numbers (e.g., five digits or more), consider an alphanumeric system. A numbering system starting with "AA-001" and ending with "ZZ-999" gives over 675,000 unique ID numbers. Letter combinations can indicate dates or identify different types of samples. The "M" in "AB-134-M," for example, could distinguish manure samples from other types of samples. Try to limit the identification to no more than four digits and no more than three letters.
- The MAP Program learned that a complex sample ID system significantly increases sample identification errors. When the program began in 2003, it used A, B, C, D, E, and F to identify samples, and identified replicates using "r101," "r102," and "r103." In nearly every sample exchange, at least one lab mixed up the replicates with the unknown samples. There was much less sample confusion after switching to a simpler numbering system that used only two-digit or three-digit numbers to identify samples.

Correctly use significant figures and decimal points: Some laboratory instruments report concentrations to five or 10 decimal places. State or national certification programs may require a certain number of significant digits, but the final concentration reported to the client depends on the instrument detection limit. Some customers may appreciate having the reporting limits (RL) or method detection level (MDL) included on their laboratory reports. The significance of the final reporting unit is affected by both accuracy and rounding. If the instrument detection limit is 0.1 ppm, it is inappropriate to report concentrations to two or more decimal places.

Examples of inappropriate use of significant figures in the MAP Program included soluble salts on a dry basis reported as 100,729.2 ppm and organic matter reported as 17,930.8 pounds/acre-inch. The concept of practical precision enters at this point. The application equipment may be accurate to ±100 gallons per acre or ±0.25 tons per acre. Reporting results to more than three significant digits does not practically improve nutrient management. The reality is that in the laboratory "we measure with a micrometer," and in the field "we cut with a dull axe."

Correctly use zeros and decimal points on client reports. Use a leading zero with a decimal to report concentrations below 1.0. For example, report as "0.05" rather than ".05." Use zero as a placeholder for concentrations of 10 or greater.

Decimal point placement gives accuracy of the measurement. For example,
- 5600 is accurate to the hundreds place,
- 560 is accurate to the tens place,
- 560. is accurate to the single unit place (note the decimal point), and
- 560.0 is accurate to the tenth place.

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John Johnson, Agronomist

Manure Analysis Report for:
Anderson Hog Farm
Submitted by: James M. Anderson
14353 235th Avenue
Saint Peter, MN 56082
Phone: (555) 555-1555
email: anderson.pork@andypork.com

Date Sampled: October 29, 2021 Date Analyzed: November 6, 2021
Date Received: October 29, 2021 Date Reported: November 10, 2021

Interpretation of Results

Analytical results determined on an as-received basis — Total Solids: 6.5%.

Sample Description	**Analytical Results for AR-153-M**	
Lab No: AR-153-M	*Nutrient Content in Pounds per 1,000 gallons**	
Sample ID: Finish barn #3	Total nitrogen (N) analyzed by TKN:	43.2
Manure type: Liquid swine	Ammonium Nitrogen (NH_4-N): (included in total nitrogen)	24.5
Storage type: Under barn pit	Total Phosphate (P_2O_5):	45.0
Application Method: knife injected	Total Potash (K_2O):	35.5
Incorporation: Immediate		

Developing a nutrient management plan requires additional information not available in this report. You may want to work with a crop consultant to determine the best manure application rates based on the manure analysis report, soil analysis report, estimates for nutrient availability, and the planned crop.

John Johnson — John Johnson
11.10.2021 — Date

Example 1: Basic report, Version 1.

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Manure Analysis Report for:
Anderson Hog Farm
Submitted by: James M. Anderson
14353 235th Avenue
Saint Peter, MN 56082
Phone: (555) 555-1555
email: anderson.pork@andypork.com

Date Sampled: October 29, 2021
Date Received: October 29, 2021
Lab No: AR-153-M
Sample ID: Finish barn #3
Manure type: Liquid swine
Storage type: Under barn pit

Date Analyzed: November 6, 2021
Date Reported: November 10, 2021
Application Method: Knife Injected
Incorporation: Immediate
Preferred Units: Pounds per 1,000 U. S. gallons

Interpretation of Results

	Results (as-received basis)		Analysis Method
	%	Pounds/1,000 gallons*	
Ammonium-nitrogen	0.52	43.9	RMMA M-4.2
Organic nitrogen	0.17	14.4	Calculation
Total Kjeldahl nitrogen (TKN)	0.69	58.3	RMMA M-3.2
Phosphate (as P_2O_5)	0.23	19.4	RMMA M-5.2
Potash (as K_2O)	0.46	38.9	RMMA M-5.2
Total solids	5.20	439	RMMA M-1.1
Moisture	94.8	—	Calculation

* Based on manure density of 8.34 pounds per gallon.

Developing a nutrient management plan requires additional information not available in this report. You may want to work with a crop consultant to determine the best manure application rates based on the manure analysis report, soil analysis report, estimates for nutrient availability, and the planned crop.

John Johnson — John Johnson
11.10.2021 — Date

Example 2: Basic report, Version 2.

Imaginary Agricultural Laboratory
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John Johnson, Agronomist

Manure Analysis Report for:
Anderson Hog Farm
Submitted by: James M. Anderson
14353 235th Avenue
Saint Peter, MN 56082
Phone: (555) 555-1555
email: anderson.pork@andypork.com

Date Sampled: October 29, 2021
Date Received: October 29, 2021
Lab No: AR-153-M
Sample ID: Dad's place
Manure type: Dairy
Total solids content: 12%

Date Analyzed: November 6, 2021
Date Reported: November 10, 2021

Interpretation of Results

Laboratory analysis for:*	**%**	**ppm**	***lb/ton***	***lb/1,000 gallons***	***lb/ac-in***
Total Nitrogen, N	0.34	3,400	6.8	28	770
Organic-nitrogen, N	0.31	3,100	6.2	26	702
Ammonium-nitrogen, NH_4-N	0.03	300	0.6	2.5	68
Phosphate as P_2O_5	0.25	2,500	5.0	21	566
Potash as K_2O	0.31	3,100	6.2	26	702

* Reported on an as-received basis

Developing a nutrient management plan requires additional information not available in this report. You may want to work with a crop consultant to determine the best manure application rates based on the manure analysis report, soil analysis report, estimates for nutrient availability, and the planned crop.

John Johnson — John Johnson
11.10.2021 — Date

Example 3: Basic report, Version 3.

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website: https://imaginaryag.com
John Johnson, Agronomist

Manure Analysis Report for:
Ferguson Dairy Farm
Submitted by: Ronald L. Ferguson
17256 365th Avenue
Saint Peter, MN 56082
Phone: (555) 555-1551
email: ron@fergusondairy.com

Date Sampled: October 29, 2021 Date Analyzed: November 6, 2021
Date Received: October 29, 2021 Date Reported: November 10, 2021

Interpretation of Results

Sample Description
Lab No: AR-172-M
Sample ID: Main feedlot
Manure type: Solid beef
Storage type: Open feedlot
Application Method: Broadcast and incorporation: within 24 hours
Units: Pounds per ton
Analytical basis: as-received

Developing a nutrient management plan requires additional information not available in this report. You may want to work with a crop consultant to determine the best manure application rates based on the manure analysis report, soil analysis report, estimates for nutrient availability, and the planned crop.

continued on next page

Example 4: A more detailed report. This one includes a cover page, and presents the analytical results on the second page. Note: The second page duplicates the laboratory's sample ID number, date sampled, and important customer identification information.

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website: https://imaginaryag.com
John Johnson, Agronomist

Manure Analysis Report (page 2) for:
Ferguson Dairy Farm
Submitted by: Ronald L. Ferguson
17256 365th Avenue
Saint Peter, MN 56082
Phone: (555) 555-1551
email: ron@fergusondairy.com

Date Sampled: October 29, 2021 Date Analyzed: November 6, 2021
Date Received: October 29, 2021 Date Reported: November 10, 2021

MANURE ANALYSIS RESULTS FOR SAMPLE AR-172-M

Ron Ferguson's Main Feedlot / Solid dairy manure sampled on 10/29/2021

Laboratory analysis for:	Results Percent or PPM "as rec'd" basis	Nutrient Content lb/ton	Estimated availability* lb/ton	Laboratory analysis for:	Results Percent or PPM "as rec'd" basis	Nutrient Content lb/ton	Estimated availability* lb/ton
Total Nitrogen, N	1.84%	36.7	13.1	Zinc, Zn	324 ppm	0.6	0.6
Organic-nitrogen, N	1.81%	36.1	12.6	Iron, Fe	7,908 ppm	15.8	15.8
Ammonium-nitrogen, NH_4-N	0.03%	0.6	0.6	Manganese, Mn	482 ppm	0.9	0.9
Phosphate as P_2O_5	2.25%	45.1	45.1	Copper, Cu	56 ppm	0.1	0.1
Potash as K_2O	1.81%	36.3	36.3	Boron, B	18 ppm	< 0.1	< 0.1
Sulfur, S	0.41%	8.1	2.8	Total solids	90.5%	—	—
Calcium, Ca	2.59%	51.9	51.9	Moisture	9.5%	—	—
Magnesium, Mg	0.79%	26.5	26.5	Non-volatile solids (ash)	60.1%	—	—
Sodium, Na	0.22%	4.3	4.3	Volatile solids	30.4%	—	—

* Assumes 35% of organic nitrogen and sulfur will become available during first crop year after application. Assumes 100% of ammonium-nitrogen will be available, but adjust the final rate for potential storage losses or field losses at application site. Assumes other nutrients will be 100% available during the first year following application. (Reference: Unknown Extension Service, Bulletin M-0987)

John Johnson — John Johnson

11.10.2021 — Date

Example 4: *Continued.*

Appendices

APPENDIX A
Common definitions and conversions needed for manure analyses

Manure sample classification

Table Appendix A.1. Manure sample classification

Designation*	Estimated Total Solids Content (%) as Received
Liquid	1–4%
Slurry	4–10%
Semi-Solid	10–20%
Solid	> 20%

* Adapted from Lorimor et al., 2004.

Definitions and Acronyms

ACS grade. Chemical reagents that meet or exceed purity standards set by the American Chemical Society (ACS). This grade is acceptable for food, drug, or medicinal use, and can be used for general procedures that require stringent quality specifications and a purity of ≥ 95%.

Air-dry. Sample material air-dried in a forced air oven at 36 °C for 48–72 h.

Ammonium nitrogen (NH_4-N). The nitrogen present in the form of ammonium, an inorganic salt of the ionized form of ammonia.

Analyte carry-over. A potential source of inaccuracy that may occur with some automated analyzers when residual analyte from one sample carries over into the next sample. Carryover effects are largest when a low concentration sample follows a high concentration sample. The effect can be measured by analyzing three high concentration standards (a1, a2, a3) followed by calibration blanks (b1, b2, b3). Calculate carryover as follows:

$$k, \% = \frac{(b1 - b3)}{(a3 - b3)} \times 100$$

where:

k = carryover, %
a3 = 3rd high concentration standard
b1 = 1st method blank
b3 = 3rd method blank

Analytical batch. A set of 20 or fewer unknown samples. For example, eight samples analyzed equals one batch. Thirty samples are considered two batches.

As-received. Sample material that is analyzed and/or reported at the moisture content in which it was initially received at the laboratory, either by direct measurement on samples in as-received condition, by moisture correction on samples dried prior to analysis and reported as-received, or by total solids correction on samples analyzed as-received and reported on a dry-weight basis .

Calcium carbonate equivalence (CCE). An expression of the liming effectiveness of a material in terms of its equivalent value to pure calcium carbonate, given in %.

Calibration blank (CB). A zero standard consisting of a volume of reagent water that is used for initial and continuing calibration of the analytical process. Where applicable, the CB should be matrix matched—e.g., for ICP-OES analysis, the CB contains 2–5% nitric acid in addition to reagent water, and for spectrophotometric analysis of NO_3-N and NH_4-N, the carrier is matrix–matched to the KCl extraction solution.

Carbon, total (TC). The sum of organic and inorganic carbon as determined by oxygen combustion analysis.

Carbon, total organic (TOC). A measure of total organic C, determined by various methods including TC, where samples have been pre-treated to remove inorganic C. TOC can also be estimated by determining total volatile solids.

Chloride (Cl^-). An ion which forms inorganic, covalent bonds with one of several elements, metals and non-metals. Relatively inert toward reactions with nitrogen, oxygen, and carbon. Typically found as salts of sodium, potassium, calcium, and magnesium.

Composite sample. Collection of samples at a liquid or solid manure storage, or during handling of manure at a storage, that are deemed appropriate in number to accurately represent the bulk of manure in the storage. The samples are placed together into a container and thoroughly mixed. From the mixed volume a single subsample is collected for laboratory analysis to represent all manure in the storage.

Continuing calibration verification (CCV). The CCV is a calibration standard or reference material that is prepared from the same solutions or materials used in the initial calibration and is analyzed in order to validate the ongoing calibration of the analytical system at the start of and during the run. Also known as the calibration verification solution or instrument performance check.

Coulometric titration analysis. An analysis method that determines the amount of an analyte transformed during an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced in reaction.

Diffusion conductivity analysis. Determination of an inorganic analyte via gas diffusion across an in-line hydrophobic membrane followed by electrical conductivity measurement, where the change in conductivity is proportional to the inorganic analyte concentration.

Distillation/titration analysis for NH_4^+. Distillation converts NH_4-N to NH_3 (gas) when treated with an alkali solution and is recovered as NH_4-N in a boric acid trap, with subsequent determination by titration with a standardized NaOH solution.

Effective calcium carbonate equivalence (ECCE). An expression of agricultural lime effectiveness based on the combined effect of the CCE and fineness of grind. Required for product labeling purposes in some states. ECCE is also referred to as effective neutralizing value (ENV), total neutralizing power (TNP), or effective neutralizing material (ENM).

Electrical conductivity (EC). Conductivity is the ability of a solution to pass an electric current. The conductivity of a solution is affected by the concentration of total dissolved solids and the temperature of the solution.

Holding time. The time between sample collection and analysis.

Homogenization. Mixing of manure material, either manually or by mechanical means, until physical, chemical, and/or biological homogeneity of the sample is achieved.

Independent calibration verification (ICV). A solution of method analyte(s) used to detect contamination or other errors that affect the accuracy of the calibration standards. The ICV is prepared from a source other than the one from which the calibration standards are prepared. Also known as an initial calibration verification solution.

Inductively coupled plasma-optical emission spectroscopy (ICP-OES). An analytical technique used for the simultaneous spectrophotometric detection and quantification of multiple chemical elements in an aqueous solution.

Instrument detection limit (IDL). The smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank. The IDL is the smallest amount that can be detected, but not quantified, by the instrument.

Internal standard (IS). Pure analyte added to all samples, standards, and quality controls in a known amount in order to correct for matrix interferences in the ICP-OES method. The responses of other method analytes are referenced to the internal element. The internal standard must be an analyte that is not present in the unknown sample. Also known as an internal reference (IUPAC).

Inter-element correction (IEC). A technique used in ICP-OES to determine the relationship between an interfering element concentration and response at the wavelength of the analyte of interest. IEC factors are determined on the individual instruments, and may be used within the tested concentration ranges to compensate for the effects of interfering elements. See US EPA 200.7.

Interference check solution (ICS). A solution containing known concentrations of the primary interfering elements in ICP-OES analysis, used to verify the accuracy of the correction factors for inter-element interferences. Also known as a spectral interference check solution. See US EPA 200.7.

Ionization buffer. A solution such as cesium or lithium introduced in-line to suppress ionization interference in ICP-OES.

Appendix A / Common definitions and conversions needed for manure analyses

Laboratory control sample (LCS). A laboratory-prepared solution with a known quantity of total and volatile solids, used to assure that the results produced by the laboratory remain within the criteria specified in the method for precision and bias.

Laboratory duplicate. A second aliquot or subsample of an unknown sample brought through the entire sample preparation and analytical process and used to evaluate method precision. The measured concentrations of the analyte of interest are compared between the two samples, and the method precision is determined by calculating the relative percent difference (RPD).

Laboratory fortified blank (LFB). A method blank to which known quantities of the analyte of interest are added. The purpose is to determine whether the laboratory is capable of making unbiased measurements, as determined by the % recovery (R) of the analytes. Also referred to as a spiked blank.

Laboratory fortified matrix (LFM). See matrix spike recovery (MSR).

Limit of quantitation (LOQ). The lowest level at which the entire analytical system gives a recognizable and quantifiable signal and an acceptable calibration point for the analyte. Equivalent to three times the MDL, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. Also termed minimum level or reporting limit.

Manure analysis proficiency (MAP). Refers to the Manure Analysis Proficiency Program coordinated by the Minnesota Department of Agriculture.

Matrix spike recovery (MSR). Also known as laboratory fortified matrix (LFM). Used to evaluate sample matrix interference. One MSR should be included with each analytical batch of client samples. A representative duplicate subsample is fortified with a known quantity of the analyte(s) and processed and analyzed along with the un-fortified sample. Recovery is compared to method- or laboratory-specific performance criteria to determine if matrix interference exceeds control limits. The MSR is calculated as follows:

$$MSR, \% = \frac{C_s - C}{S} \times 100$$

where:

MSR = matrix spike recovery, percent
Cs = analyte concentration in spiked sample
C= analyte concentration in unspiked sample
S = analyte concentration added to spiked sample

Method blank (MB). A laboratory sample with a matrix similar to the associated samples (e.g., reagent water, boiling chips, glass beads), known to be free of the analyte of interest, and taken through all preparation and analytical steps in the method. Used to determine if analytical contamination or other interferences are present in the laboratory environment, reagents, or apparatus.

Method detection limit (MDL). The minimum measured concentration of an analyte that can be reported with 99% confidence that is distinguishable from a method blank result. The MDL is determined according to procedures described in Appendix B.

Near infrared reflectance (NIR). A spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from 700–2500 nm).

Nitrate nitrogen (NO_3-N). The nitrogen present in the form of the nitrate ion (NO_3^-); a salt or ester of nitric acid. Note that nitrate is reported differently in different regions of the world, either as nitrate (NO_3) or nitrate-N (NO_3-N). The nitrate form is reported as the weight of all nitrogen and oxygen present. The nitrate-N form is reported solely as the nitrogen present. In the United States, the nitrate-N form is preferred to track the amount of nitrogen loading to a system, whether it be soil, water, or the atmosphere. The conversion between the two can be found in the Conversions section of this Appendix.

Nitrogen, total (TN). The sum of organic and inorganic N as determined by oxygen combustion analysis.

Nitrogen, total Kjeldahl (TKN). The sum of N contained in organic substances plus the nitrogen contained in the inorganic compounds ammonia and ammonium (NH_3 + NH_4^+).

Orthophosphate (PO_4^{3-}). Inorganic phosphate or orthophosphate is an ester of orthophosphoric acid.

Oven-dry. Sample material dried in a forced air oven at 70 ± 5 °C for 18–24 h, or until sample weight is constant and moisture losses diminish to nil (weight loss from second drying is less than 1% of initial weight loss).

Percent error (PE). A statistical test used to evaluate the accuracy of a measured value relative to a known value. Calculated as follows:

$$PE = \frac{known\ value - measured\ value}{known\ value} \times 100$$

pH. A logarithmic measure of the activity of H+ ions in solution. Solutions with a pH less than 7.0 are acidic, and solutions with a pH greater than 7.0 are alkaline.

Potentiometric analysis. An electroanalytical technique whereby the potential between two electrodes is measured using a high-impedance voltmeter during a titration.

Primary sample. The representative field sample submitted to the lab for analysis.

Reagent grade water. Water that is suitable for use in a specified procedure such that it does not interfere with the specificity, accuracy, and precision of the procedure, meeting either of the following:

ASTM Type I grade, with a resistance greater than 18 MΩ cm^{-1}, conductivity < 0.056 µS cm^{-1} at 25 °C, and < 50 ppb of total organic carbon (TOC).

ASTM Type II grade, with a resistance greater than 1 MΩ cm^{-1}, conductivity < 1.0 µS cm^{-1} at 25 °C, and < 50 ppb of total organic carbon (TOC). If not specified in the method, reagent grade water should at least meet this level.

Recovery. A statistical test used to measure accuracy, calculated as:

$$R, \% = \frac{C_s}{C} \times 100$$

where:

R = the recovery of analyte from the sample, expressed in percent
Cs = analyte concentration in the sample
C = analyte concentration in the QC solution

Reduction efficiency verification (REV). A mid-calibration range nitrite standard used to test reaction efficiency in the spectrophotometric NO_3-N method by comparison to a nitrate standard of the same concentration.

Reference material (RM). An external material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (ISO Guide 30:2015).

Certified references material (CRM). Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability (ISO Guide 30:2015).

Standard reference material (SRM). A CRM issued by NIST that also meets additional NIST-specific certification criteria and is issued with a certificate of analysis that reports the results of its characterizations and provides information regarding the appropriate use(s) of the material (NIST SP 260-136) (ISO Guide 30:2015).

Reference solution (RS). An external solution which has been established to be fit for its intended use in a measurement process (e.g., standard KCl reference solution used for calibration and continuing calibration verification of an EC meter).

Relative percent difference (RPD). A comparative statistic used to calculate the precision or random error between duplicate measurements. Defined as the absolute difference between two values divided by their mean, expressed as a percent:

$$RPD, \% = \frac{C_1 - C_2}{\left(\frac{C + C_2}{2}\right)} \times 100$$

where:

C_1 = analyte concentration in 1st sample aliquot
C_2 = analyte concentration in 2nd sample aliquot

Relative standard deviation (RSD). The ratio of the standard deviation to the mean, expressed as a percent. Provides a normalized expression of precision between multiple measurements, e.g., in developing internal reference material acceptance criteria and in evaluating acceptance for analytes at low concentrations. Also known as the Coefficient of Variation (CV). RSD is calculated as:

$$RSD, \% = \frac{s \times 100}{X}$$

where:

s = standard deviation of one or more data points
X = mean of one or more data points

Repeatability, intra-laboratory (S_R). The estimate of the within-laboratory repeatability as calculated across all laboratories taking part in the proficiency testing program which remain after outliers have been removed. Calculated from intra-laboratory standard deviation from the MAP program, three times the intra-laboratory standard deviation. Adapted from section 4.3 of ISO 2725-2:1994.

Reproducibility, inter-laboratory (S_R). The estimate of the reproducibility variance across all laboratories taking part in the proficiency testing program which remain after outliers have been removed. Calculated from inter-laboratory standard deviation from the MAP program, three times the

inter-laboratory standard deviation. Adapted from section 4.3 of ISO 2725-2:1994.

Secondary sample. The subsample collected from the primary sample for downstream analysis and archiving.

Solids, fixed. The residue of total solids remaining after heating the same sample to 500 °C, expressed as a mass percent (%) on an as-received or dry-weight basis.

Solids, total. The residue remaining after evaporation and subsequent oven-drying to constant weight at 103–105 °C.

Solids, total dissolved (TDS). A measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized, or colloidal suspended form. Method is not included in this manual.

Solids, volatile. The mass of total solids lost on ignition at 500 °C, expressed as a mass percent (%) on an as-received or dry-weight basis.

Spectrophotometric analysis. Determination of an analyte in a liquid matrix by reaction with a chromophore, and measurement of the amount of light absorbed at a specific wavelength, where the change in absorption is proportional to the analyte concentration.

Standard operating procedure (SOP). A detailed, written set of instructions that explains the steps to be performed during an experimental procedure and includes information about how to use and manage hazardous chemicals, processes, and procedures to prevent or minimize health and safety concerns.

Storage time. The time between sample receipt in the laboratory and analysis.

Test portion. The volume or weight of the secondary sample used in each downstream analysis.

Water-extractable phosphorus (WEP), 100:1 soln:solids. Standardized measure of water-extractable P in manure and other organic residuals (e.g., biosolids, food waste, compost) for use as an index of dissolved P runoff loss potential following recent land application.

Table Appendix A.2. Conversions

Unit	Multiply by	Equals
%	83.4	Pounds per 1,000 US gallons
%	20	Pounds per US ton
%	2,265	Pounds per acre-inch
%	10,000	ppm
ppm	0.00834	Pounds per 1,000 US gallons
ppm	0.002	Pounds per US ton
ppm	0.2265	Pounds per acre-inch
US ton	2,000	Pounds
US ton	0.907	Metric ton
US gallon	8.34	Pounds of water
US gallon	0.8327	Imperial gallon
US gallon	3.785	Liter
Imperial gallon	4.546	Liter
Nitrate nitrogen (NO_3-N)	4.43	Nitrate (NO_3)
Ammonia nitrogen (NH_3-N)	1.22	Ammonia (NH_3)
Ammonium nitrogen (NH_4-N)	1.29	Ammonium (NH_4)
P	2.29	P_2O_5 equivalent
K	1.20	K_2O equivalent

APPENDIX B
Procedure for the Determination of the Method Detection Limit (MDL)

AUTHOR: Robert O. Miller

1.0 Introduction

The MDL procedure described here is adapted from US EPA 821-R-16-06. It has been modified as per methods for the analysis of manure samples for the following analytes: total solids, N by combustion, TKN, NH_4-N, NO_3-N, WEP, P, K, Ca, Mg, Na, Zn, Cu, Mn, Fe, B, and Cl. It is not applicable to pH and EC measurements.

2.0 Definition

The method detection limit (MDL) is defined as the minimum measured concentration of an analyte that can be reported with 99% confidence as distinguishable from a method blank result. It is not to be confused with the Instrument Detection Limit (IDL) and the Limit of Quantification (LOQ).

3.0 Scope and Application

The MDL procedure is designed to determine the analyte detection limit for well-defined chemical methods used in the analysis of manure as described in this manual. The procedure should include all sample processing steps associated with the method. It is lab-specific and should be determined by an individual lab for each reported analyte. Values that fall below a concentration 3 × MDL (defined as the LOQ) should not be included on client reports and should be reported as <LOQ.

4.0 Procedure

1. *Estimate Method Detection Limit (MDL_e) using one or more of the following:*
   A. The mean determined concentration plus three times the standard deviation of a set of five method blanks: $MDL_e = Xb + (3 \times Sb)$.
   B. The concentration equivalent to three times the standard deviation of a minimum of five replicate instrumental measurements of spiked blanks: $MDL_e = 3 \times Ssb$.
   C. Instrumental limitations. It is recognized that the analyst experience is important to this process, but an MDL_e should include one or all of the above considerations in the initial estimate of the instrument detection limit (IDL).

   Note: The initial MDL is used when the laboratory does not have adequate data, when a new method is implemented, or when a method is used that has been rarely used in the last 24 months.

2. *Determine the initial Method Detection Limit (MDL_i)*
   A. Select a spiking level, typically 2–10 times the estimated MDL_e described in Section 1 above. Spike levels in excess of 10 times the MDL_e may be required for analytes with very poor recovery.
   B. Process a minimum of seven spiked samples and seven method blank samples through *all* steps of the method. The samples used for the MDL *must* be prepared and analyzed in at least three batches on three separate calendar dates (preparation and analysis may be on the same day). Existing lab analysis data may be used, if compliant with the requirements for at least three batches and generated within the last 24 months. Statistical outlier removal procedures should not be used, as the purpose of the MDL procedure is to capture routine method variability. However, documented instances of gross failures (e.g., instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations, provided that at least seven spiked samples and seven method blanks are available. Rationale for removal of specific outliers must be

documented and maintained on file with the MDL results.

i. For multiple instruments assigned the same MDL, the sample analyses used in the MDL determination must be distributed across all of the instruments.

ii. A minimum of two spiked samples and two method blank samples prepared and analyzed on different calendar dates is required for each instrument. Each analytical batch may contain one spiked sample and one method blank sample run together. A spiked sample and a method blank sample may be analyzed in the same batch.

iii. The same prepared spike may be analyzed on multiple instruments so long as the minimum requirement of seven preparations in at least three separate batches is maintained.

C. Evaluate spiking level: If any result for any individual analyte from the spiked samples does not meet the method qualitative identification criteria or does not provide a numerical result greater than zero, repeat the spiked samples using a higher concentration. Qualitative identification criteria are a set of rules or guidelines for establishing the identification or presence of an analyte using a measurement system. Qualitative identification does not ensure that quantitative results for the analyte can be obtained.

D. Complete computations as specified in the analytical method and express the final results in the method-specified reporting units.

i. Calculate the sample standard deviation(s) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

ii. Compute the MDL_s (the MDL based on spiked blank samples) as follows:

$$MDL_s = t_{(n-1,1-\alpha=0.99)} \times S_s$$

where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha=0.99)}$ = the Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom. See Table B-1.

Ss = sample standard deviation of the replicate spiked sample analyses.

iii. Compute the MDL_b (the MDL based on method blanks) as follows:

a. If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) such as a missing peak, commonly observed in chromatographic analysis.

b. If some but not all of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set the MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where n ≥ 100, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are … 1.5, 1.7, 1.9, 5.0, and 10, 164 × 0.99 = 162.36, which rounds to the 162nd method blank result. Therefore, MDL_b is 1.9 for n = 164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

c. If all method blanks for an individual analyte give numerical results, calculate the MDL_b as:

$$MDL_b = x_b + t_{(n-1,1-\alpha=0.99)} \times S_b$$

Table Appendix B.1. Single-Tailed 99th Percentile t Statistic.

Number of Replicates	Degrees of Freedom (n-1)	t (n-1, 0.99)
31	30	2.457
32	31	2.453
48	47	2.408
50	49	2.405
61	60	2.390
64	63	2.387
80	79	2.374
96	95	2.366
100	99	2.365

where:
MDL_b = the MDL based on method blanks
Xb = mean of the method blank results (use zero in place of the mean if the mean is negative)
$t_{(n-1, 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom. See Appendix B-1.
Sb = sample standard deviation of the replicate method blank sample analyses.

Note: If 100 or more method blanks are available, as an option, MDL_b may be set to the concentration that is greater than or equal to the 99th percentile of the method blank results, as described in Section (2)(d)(iii)(B).

E. Select the greater of MDL_s or MDL_b as the initial MDL_i.

3. *Determination of MDL — Examples*

A. Total nitrogen MDL_e by combustion analyzer was determined using a spiked Tris buffer solution in accordance with Sections 1(b) (MDL_e) and 2(b) (MDL_i), above.

i. MDL_e: Total nitrogen replicate results of five laboratory spiked blanks

Results (n = 5)	N %
Mean	0.03328
Stdev	0.00122
MDLe (3 × Stdev)	0.00366

ii. MDL_i: Seven spiked blanks were prepared based on 3 × MDL_e of 0.0037, a concentration 0.0148 N % in accordance to Section 2(b). Seven spiked blanks and seven unspiked blanks were analyzed. Data was tabulated and MDL_i determined as follows:

Results (n = 7)	Spiked Blank		Blank	
	Xsb	***Ssb***	***Xb***	***Sb***
N %	0.01504	0.00151	0.0010	0.0050

Student's *t*-value n = 7, single-tailed 99th, t = 3.143.

$MDL_s = 3.143 \times 0.00151 = 0.0047$
$MDL_b = 0.0010 + (3.143 \times 0.0050) = 0.0017$
$MDL_s > MDL_b$
Total nitrogen $MDL_i = 0.0047$ N %

B. Total phosphorus MDL_e by ICP-OES was determined using a liquid spiked solution in accordance with Sections 1(b) (MDL_e) and 2(b) (MDL_i), above.

i. MDL_e: Total phosphorus replicate results of five laboratory spiked blanks

Results (n = 5)	P, mg L^{-1}
Mean	0.0334
Stdev	0.281
MDLe (3 × Stdev)	0.843

ii. MDL_i : Nine spiked blanks were prepared based on 3 × MDL_e of 0.281 concentration of 0.843 mg L^{-1} in accordance with section 2(a). Nine spiked blanks and nine unspiked blanks were analyzed. Data was tabulated and MDL_i determined as follows:

Results (n = 9)	Spiked Blank		Blank	
	Xsb	***Ssb***	***Xb***	***Sb***
P, mg L^{-1}	0.777	0.371	0.021	0.210

Student's *t*-value n = 9, single-tailed 99th, t = 2.896.

$MDL_s = 2.896 \times 0.371 = 1.07$
$MDL_b = 0.021 + (2.896 \times 0.210) = 0.608$
$MDL_s > MDL_b$
Total phosphorus $MDL_i = 1.07$ mg L^{-1}

C. Ammonium Nitrogen (NH_4-N) MDL_e by Diffusion-Conductivity was determined using a spiked NH_4Cl solution in accordance with Sections 1(b) (MDL_e) and 2(b) (MDL_i), above.

i. MDL_e : NH_4-N replicate results of five laboratory spiked blanks.

Results (n = 5)	NH_4-N, mg L^{-1}
Mean	10.01
Stdev	0.23
MDLe (3 × Stdev)	0.70

ii. MDL_i : Seven spiked blanks were prepared based on 3 × MDL_e of 0.23 mg L^{-1}, a concentration of 0.70 mg L^{-1} in accordance with Section 2(a). Seven spiked blanks and seven unspiked blanks were analyzed. Data was tabulated and MDL_i determined as follows:

Results (n = 7)	Spiked Blank		Blank	
	Xsb	***Ssb***	***Xb***	***Sb***
NH_4-N, mg L^{-1}	0.752	0.013	0.087	0.006

Student's *t*-value n = 7, single-tailed 99th, t = 3.143.

$MDL_s = 3.143 \times 0.013 = 0.041$
$MDL_b = 0.087 + (3.143 \times 0.006) = 0.106$
$MDL_s < MDL_b$
NH_4-N $MDL_i = 0.106$ mg L^{-1}

4. *Ongoing MDL Data Collection*
   A. During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked samples on each instrument, in separate batches, using the same spiking concentration used in Section 2. If any analytes are repeatedly not detected in the quarterly spiked sample analyses, or do not meet the qualitative identification criteria of the method (see Section 2(c) of this procedure), this is an indication that the spiking level is not high enough and should be adjusted upward. Note that it is not necessary to analyze additional method blanks together with the spiked samples; the method blank population should include all of the routine method blanks analyzed with each batch during the course of sample analysis.
   B. Ensure that at least seven spiked samples and seven method blanks are completed for the annual verification. If only one instrument is in use, a minimum of seven spiked samples is still required, but they may be drawn from the last two years of data collection.
   C. At least once per year, re-evaluate the spiking level.
   D. If more than 5% of the spiked samples do not return positive numerical results that meet all method qualitative identification criteria, the spiking level must be increased and the initial MDL re-determined following the procedure in Section 2.
   E. If the method is altered in a way that can be reasonably expected to change its sensitivity, re-determine the initial MDL according to Section 2, and restart the ongoing data collection.
   F. If a new instrument is added, a new MDL must be calculated for the instrument.

5. *Annual MDL Verification*
   A. At least once every 12 months, re-calculate MDL_s and MDL_b from the collected spiked samples and method blank results using the equations in Section 2 and document.
   B. Include data generated within the last 24 months, but only data with the same spiking level. Only documented instances of gross failures (e.g., instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations. The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination. If the laboratory believes the sensitivity of the method has changed significantly, the most recent data available may be used.
   C. Include the initial MDL spiked samples, if the data were generated within 24 months.
   D. Use only data associated with acceptable instrument calibrations and batch QC. Include all routine data, with the exception of batches that are rejected and the associated samples reanalyzed. If the method has been altered in a way that can be reasonably expected to change its sensitivity, use only data collected after the change.
   E. Ideally, use all method blank results from the last 24 months for the MDL_b calculation. The laboratory has the option to use only the last six months of method blank data or the 50 most recent method blanks, whichever criteria yields the greater number of method blanks.
   F. The verified MDL is the greater of the MDL_s or MDL_b. If the verified MDL is within 0.5–2.0 times

Table Appendix B.1. Single-Tailed 99th Percentile t Statistic.

Number of Replicates	Degrees of Freedom (n-1)	t (n-1, 0.99)
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	21	2.528
26	26	2.485
31	30	2.457
32	31	2.453
48	47	2.408
50	49	2.405
61	60	2.390
64	63	2.387
80	79	2.374
96	95	2.366
100	99	2.365

the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5–2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

Addendum: Determination of the MDL for a Specific Matrix

The MDL may be determined in a specific sample matrix as well as in reagent water.

1. Analyze the sample matrix to determine the native (background) concentration of the analyte(s) of interest.
2. If the response for the native concentration is at a signal-to-noise ratio of approximately 5–20, determine the matrix-specific MDL according to Section 2 but without spiking additional analyte.
3. Calculate MDL_b using the method blanks, not the sample matrix.
4. If the signal-to-noise ratio is less than 5, the analyte(s) should be spiked into the sample matrix to obtain a concentration that will give results with a signal-to-noise ratio of approximately 10–20.
5. If the analyte(s) of interest have signal-to-noise ratio(s) greater than approximately 20, the spike concentration is too high and needs to be reduced.

5.0 Reference Document

U. S. Environmental Protection Agency. (2016). Definition and procedure for the determination of the method detection limit. Revision 2. EPA 821-R-16-006.