IMPROVEMENT AND STANDARDIZATION OF LABORATORY QUALITY ASSURANCE AND QUALITY CONTROL FOR MEHLICH III SOIL TEST METHODOLOGY: PHASE I and II.

FINAL REPORT
TSSWCB PROJECTS 01-22 and 06-04

Prepared By:
Sam Feagley, Tony Provin, Mark McFarland, and Frank Hons, Soil and Crop Sciences,
Texas A&M University, 2474 TAMU, College Station, TX 77843-2474

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Executive Summary

The exhaustive analysis of potential intra-laboratory methodology differences yielded a number of identifying practices that could skew laboratory data for the Mehlich III phosphorus (P) determination. This same analysis also confirmed the relative robustness of the method to provide relatively uniform results in spite of modest or even extreme changes in laboratory procedures or extraction conditions. A common and overarching assessment is that much of the reported variability between laboratories conducting the Mehlich III method as their primary analytical soil testing method are likely due to non-homogenous samples being submitted to two or more different laboratories. This project was never designed or intended to review differences in data due to non-homogenous samples, but only the impact of common deviations observed between agronomic soil testing laboratories and/or protocols used by agronomic laboratories which differ significantly from the environmental laboratory community.

The Mehlich III extractant, when performed by trained technicians in high volume laboratories is a reliable and reproducible test for the assessment of agronomic soil P levels. Whether scooping or weighing is used by the laboratory, key steps must be preformed correctly to insure reliable and repeatable data. The added expense of weighing of soil samples does eliminate the error associated with changes in soil sample bulk density, however this minor difference has little agronomic value or influence of fertilizer recommendations and will unlikely be implemented by commercial agronomic laboratories. The minor improvement in accuracy at the highly elevated soil P levels may be of interest to the regulatory authorities, however it must also be pointed out that across a selection of laboratories, the standard deviation for Mehlich III P will likely be 3-7% in the typical enforcement soil P range. This range is modest in comparison to the variability in soil sample collection differences previous reported by other investigators. Regulatory authorities should utilize these reported standard deviations when considering the use of absolute values for soil test P, typically 200 ppm.

Other factors not examined in this project, but known by medium and large agronomic laboratories, are the short shelf life of the extractant, development of standards, calibration of ICPs to avoid matrix interferences created by the extractant reagents and laboratory design to insure quick and accurate extraction of samples. These factors are often laboratory by laboratory specific and not always conveyable in generic SOPs.

Introduction

Both state and federal regulatory and resource management agencies rely on agronomic soil testing data as a prerequisite for participation in cost sharing programs, issuance of land use permits, and compliance monitoring. Soil testing and the nutrient recommendations based upon the soil tests are some of the most efficient ways of insuring that nutrients are being recommended and applied at agronomic rates and thus protection of surface water bodies and groundwater is being implemented through the best management practice (BMP) of soil testing. This BMP is important to protecting water quality across the state and nation. For example, prior to collecting soil samples numerous producers that have applied manures were also applying complete commercial fertilizers (contained N, P, and K), not realizing that they did not need most, if not all the commercial fertilizer. Producers that have started soil testing have found, in many cases that they need less fertilizer to produce the same or more yield due to build up of nutrients in the soil. Thus, by managing nutrients through soil testing, the potential for the reduction of over fertilization, surface runoff, and leaching of excess nutrients.

The use of and associated demands on agronomic soil testing have significantly changed since the early 1990's. In Texas, and in large part throughout the United States, initial compliance requirements placed upon agronomic soil testing laboratories and sample submitting entities were limited or non-existent. For example, initial state of Texas regulatory requirement for extractable soil P from concentrated animal feeding operations (CAFOs) was a P level below 200 parts per million (ppm). Unfortunately, the regulatory limit of 200 ppm P was applied to all soil test P (STP) methodologies, even though various methodologies used by Texas and regional soil testing laboratories extracted varying quantities of P.

The USDA/Natural Resources Conservation Service (NRCS) in Texas addressed this issue in the late 1990's by using calculations to transform P values from four widely used soil testing methods to a predicted Texas A&M University (TAMU) method value. Subsequent fertility recommendations, based on TAMU fertilizer recommendations, were determined and used for associated cost-sharing programs. In 1999, the Texas Commission on Environmental Quality (TCEQ) limited the acceptable P extraction methodologies to the method recommended by Texas Cooperative Extension (Texas AgriLife Extension Service, now) or the Mehlich III method. In January, 2004, the Texas AgriLife Extension Service Soil, Water and Forage Testing Laboratory (SWFTL) formally adopted the Mehlich III method as its official STP method. That same year, TCEQ modified the CAFO rule to limit the STP methodology to Mehlich III by inductively coupled plasma optical emission spectrophotometer (ICP). In the summer of 2005, new Texas NRCS Code 590 practice standard guidance were developed which specified the use of Mehlich III for soil test P analyzed by ICP. As a result of SWFTL's adoption of the Mehlich III, TCEQ rule changes, and the Texas NRCS practice standard changes, the only recognized soil testing method for extractable P available to producers is Mehlich III analyzed by ICP if samples are sent to the SWFTL or producers are participating in NRCS or TCEQ programs. Therefore, it is imperative to determine the variation in results between and within laboratories for Mehlich III and determine the error associated with minor/major changes in the standardized protocol based upon the literature method of Mehlich III.

Prior to the adoption of a single regulatory agronomic P extractant, absolute extractable soil P concentrations varied as much as two orders of magnitude. This resulted in the need for greater uniformity between the various service laboratories, with regard to both absolute extractable nutrient levels and the agronomic fertilizer/biosolids/manure/litter application rates recommended by the laboratories. As a result of the adoption of a single recognized soil test extractable P method, it was assumed the absolute values received would be similar if individual fields were re-sampled, or if field samples were split and distributed to multiple laboratories. Best professional judgment of several Texas A&M University soils faculty, based on professional experience and publications from other land grant universities, suggested that the variability of field-split samples could exceed 35% for extractable P. Furthermore, variability of P results between samples collected at different times and/or by different individuals may result in 50% variability, particularly if a field has received manure, litter, or biosolids.

Two projects funded by TSSWCB and USDA addressed field sampling variability within four individual fields. These projects, led by Dr. Sam Feagley, Texas AgriLife Extension Service, are evaluating existing sampling techniques and will ultimately lead to the development of formal sampling SOPs for agronomic soils. Additionally, the results of these projects will assist in segregating the relative errors associated with field sampling and those associated with current laboratory protocols for Mehlich III P.

Texas now requires all samples used for regulatory monitoring to be analyzed in certified or accredited laboratories. This process is not formalized for agronomic samples. The North American Proficiency Testing program (NAPT) is being proposed as the intra-laboratory protocol for all laboratories analyzing agronomic samples for concentrated animal feeding operations and NRCS Code 590 Nutrient Management Plans. The Western States Laboratory Proficiency Testing Program, the precursor to the NAPT program found intra-laboratory precision/error up to 25% on 2-mm pulverized and mixed samples. As a result of this significant error associated with the samples themselves, the program recommended finer grinding of samples. Upon grinding to approximately 0.45 mm, the intra-laboratory error decreased to less than 2 percent.

The overall NAPT program grew out of the Western States program, multiple state proficiency programs, and other regional/sector programs. This program, with over 180 current laboratory participants, utilizes blind sample analysis and statistical ranging of results relative to the data mean. This program is used by Nebraska to determine regulatory proficiency of agronomic soil testing laboratories, and licensing to conduct business within the state is dependent on meeting proficiency standards. Participation in the program is required by NRCS in Texas and a number of western states for soil analyses conducted for any NRCS program. The program effectively insures overall laboratory practices are in accordance with other laboratories, and that statistically similar results can be reproduced on the prepared media.

The NAPT program, because of the finely-ground nature of the media samples, does not allow for direct comparison of laboratory performance on true pulverized <2-mm diameter agronomic samples. As a result, minor differences in laboratory protocols and methodologies can have a profound influence on the reproducibility and comparability of routine analysis results for extractable soil P. The accuracy and precision of extractable soil P within a laboratory can also be influenced by shaker type, shaking speed, sub-sampling for analysis (including both weighing and volumetric methods), sample size, and the type of filter paper used. These potential differences, as well as alteration of the basic Mehlich III testing protocol by some laboratories, has resulted in some regulatory agencies suggesting that multiple Mehlich III testing methods exist. The protocols used by agronomic testing laboratories have evolved from research activities at Land Grant Universities, and/or alterations for convenience, speed, economic, and other undocumented reasons. Regulatory and monitoring agencies require assurance that reported data are of acceptable quality. The overlying challenge is improving reliability and data quality while minimizing the impacts of analytical cost on the agricultural industry.

The primary objectives of these projects were: 1) Evaluate intra- and inter-laboratory precision of Mehlich III extractable P from <2-mm agronomic soil samples; and 2) develop, evaluate and document improved procedures to reduce laboratory errors associated with Mehlich III extractable soil P. The detailed objectives to accomplish the primary objectives were: 1) A diverse group of soil samples were collected that represent multiple nutrient application scenarios, including animal wastes and commercial fertilizer application fields, and a range in soil texture to provide needed project samples and soil for the NAPT program. 2) Assess the current inter-laboratory relative error associated with Mehlich III extractable P from <2-mm diameter pulverized soil samples. 3) Identify and document laboratory protocols that enhance the precision and accuracy of Mehlich III extractable P soil test method. 4) Evaluate and document improvements of precision and accuracy of Mehlich III extractable P by agronomic soil testing laboratories using the SOPs developed by this project. 5) Provide SOPs for regulatory, governmental, educational, and testing laboratory use, and increase regional participation in NAPT program or similar identified program(s) and use of appropriate testing protocols.

Materials and Methods

Soil Collection

Twelve agricultural soils were identified for collection. Criteria for selection of these twelve soils included: 1) soil texture, 2) geographic location, 3) representativeness of soils in geographic location, 4) soil pH and 5) estimated nutrient status of soil. Collection of each soil was conducted by hand using shovels. The soil from each site was excavated from a 2.44 m by 2.44 m area to a depth of 15 cm. When possible, plant tissue was screen on site to avoid later issues with contamination of the soil with decomposing plant tissue. The entire volume of soil from the excavated site was transported to College Station and laid on poly tarps over concrete slabs to air dry. The collection of soils during the summer of 2007 was complicated by abnormally high rainfall patterns throughout the entire state of Texas. With the exception of the Oreila and Pullman soil series, all soils were collected in wet to saturated conditions. The soil samples were turned every two to three days while fans were used to accelerate air drying. All references to soils from this table forward will be based on the soil number listed in Table 1.

Table 1. Soil collected and used in the projects.

Soil Series	County	Vegetation/Land Use	Soil Number
Oreila	San Patricio	Tilled harvested canola	1
Lake Charles	Liberty	Bahiagrass pasture	2
Darco	Smith	Bahiagrass pasture	3
Hiladgo	Hiladgo	Tilled sugarcane	4
Amarillo	Bailey	Tilled corn	5
Pullman	Deaf Smith	Tilled harvested wheat	6
Tillman	Wilbarger	Bermudagrass pasture	7
Branyon	Williamson	Tilled harvested wheat	8
Burleson	Williamson	Tilled corn	9
Shipps	Burleson	Tilled harvested corn	10
Windthorst	Erath	Bermudagrass pasture	11
Hockley	Harris	Bermudagrass pasture	12

Soil Preparation

Upon completion of air drying, soil samples were processed via multiple methods, depending on the clay content and size of the air dried peds. For the sandy and friable soil samples, the soil was transferred to a 12 mm screen where all foreign materials and rocks were removed by hand. The soils with extremely large and hard clods were processed over a 25 by 100 mm opening steel grate. Each clod was inspected for foreign material and rocks prior to being forced through the grate. Following the coarse grate, the clayey soils were forced through the 12 mm screen prior to further processed by a well worn Custom Laboratory DynaCrusher. Due to the condition of the DynaCrusher, these soils received sub-optimal pulverization. Multiple grab samples of the sub-optimal pulverized soil were collected from soils 1, 2, 6, 7, 8, 9, and 10. These samples were used for the soil recovery impact study. All of the remaining sub-optimized soils and samples 3, 4, 5, 11 and 12 were further pulverized using an AgVise Soil Pulverizer fitted with a shaker sieve maintained in near new condition. Soil that was retained and separated by the sieve was returned to the pulverizer until 100% recovery was achieved. Each soil was then mixed approximately 350 kg batches starting with an initial 4 sub-batches. The mixing was performed in a new 1500 lb capacity cement mixer for 30 minutes each batch. The individual sub-batches were split into two and mixed with other split sub-batches until a minimum of 24 sub-batch mixings were preformed. The mixed soils were then stored in poly-supersaks prior to a portion of most soils being submitted to the NAPT Soil Testing Proficiency Program. Approximately 110 liters of each soil was transferred to a sealable poly-container for use during the study. Seven liters of each soil was removed from each container and further processed using a plate mill set to 0.5 mm placing. This further processing was to insure as uniform soil as possible to assess the impact of laboratory analyses methods on data integrity. The seven liters of soil was then mixed in a new small format mortar mixer and stored in two 4 liter poly containers.

The retained sub-optimal pulverized soils from the retention study were weighed and hand sieved through a 2mm sieve. The mass of the retained passed soil was recorded. Both fractions for each soil were then processed through the AgVise pulverizer as discussed above. Each fraction was then mixed in the large cement mixer as described above. A small portion of each fraction was retained and further processed and mixed as described above.

Each of the 12 soils was analyzed for pH and salinity (2:1/DI water:soil), Mehlich III K, Ca, Mg, Na and S, DTPA extractable Fe, Zn, Cu and Mn, and soil texture using the settling hydrometer method outlined by Day, 1949. These properties are listed in Tables 2A and 2B.

An internal check sample, represented by sample number 13, was developed using acid soils 1, 2, 3, 7, 9 and 12. This sample was also sent to the external laboratories.

Soil Properties

Table 2A. Soil Texture of processed soils.

Soil	Sand	Silt	Clay
	---------------------%----------------------		
1	68	6	26
2	40	34	26
3	82	14	4
4	60	12	28
5	88	6	6
6	46	34	20
7	26	52	22
8	32	32	36
9	23	42	35
10	11	44	45
11	71	22	7
12	77	14	9

Table 2B. Selected soil test properties of soils used in the projects.

Soil	pH	Cond.	NO_3-N	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
		umhos cm^{-1}	mg kg^{-1}									
1	6.1	198	3	246	2182	674	19	238	18.3	1.18	37.68	0.85
2	5.5	319	4	1398	3363	391	19	285	55.5	1.40	33.88	1.11
3	4.8	110	42	64	433	58	13	129	30.9	1.44	30.52	0.14
4	8.0	414	32	717	7511	467	69	235	6.8	0.92	7.75	0.95
5	6.8	631	206	1029	1927	356	68	222	9.1	4.50	15.08	0.38
6	7.6	912	128	2380	4881	922	131	417	7.9	4.53	43.61	1.09
7	5.5	257	21	498	1580	426	20	129	22.7	0.58	96.51	0.92
8	8.1	599	14	101	243	36	23	297	9.5	0.92	7.46	0.72
9	6.4	453	16	97	490	45	9	214	18.9	1.37	40.62	0.83
10	8.1	521	4	567	12531	481	17	178	9.3	0.47	11.80	1.13
11	8.0	124	5	162	1015	189	19	141	4.0	1.40	6.10	0.25
12	6.1	235	16	474	825	148	45	175	116.1	5.31	19.13	0.37

Soil Recovery Project Protocol

The seven soils (Table 3) used in this part of the project were dominated by clay and clay loam textures. Each soil, with the exception of soils 1 and 6 were collected in very wet conditions and required significant air drying before processing. The easily recoverable soil was defined as the soil recovered by the well worn DynaCrusher. This fraction varied by soil and is listed in Table 3. Four recovery percentages of soil were produced by adding reprocessed soil from the initial recovery was added to achieve three additional samples with the highest soil recovery of 100%.

Table 3. Recovery of <2 mm soil upon initial pulverization.

Soil	Initial Recovery	2^{nd} Recovery	3^{rd} Recovery	4^{th} Recovery
	%			
1	74.96	83.30	91.65	100
2	59.99	73.30	86.65	100
6	72.83	81.89	90.94	100
7	54.01	69.34	84.67	100
8	49.06	66.04	83.02	100
9	59.10	72.74	86.37	100
10	56.93	63.75	81.88	100

Additionally, both the initial recovered and retained soil fractions for each soil (Table 4) were analyzed by the weighed Mehlich III method listed below and soil texture using the settling hydrometer method outlined by Day, 1949.

Table 4. Soil particle size distribution of retained and captured soil samples upon initial pulverization.

Soil	Retained Sand	Retained Silt	Retained Clay	Initial Captured Sand	Initial Captured Silt	Initial Captured Clay
	--	---------------------%	--	--	--	--
1	65	10	25	69	6	25
2	30	43	27	25	46	29
6	43	32	25	35	38	27
7	23	56	21	23	52	25
8	31	34	35	31	32	37
9	23	42	35	27	42	31
10	5	52	43	9	48	43

Mehlich III Project Protocol

The Mehlich III procedure shall be used for the determination of P, K, Ca, Mg, Na and S in soil samples. The Mehlich III extract is composed of 0.2 N acetic acid-0.25 N NH_4NO_3-0.015 N NH_4F-0.013 N HNO_3-0.001 M EDTA with the pH adjusted to 2.5 plus or minus 0.1 pH units. For the purpose of this project, the standard protocol from which all data is compared is a 2 gram weighed sample, placed in a 5 ounce disposable plastic cup, shaken on a 200 rpm orbital shaker with a 1 inch throw for exactly 5 minutes and filtered through a No. 2 Whatman filter equivalent cellulose filter paper. Modifications of this protocol are described for each project protocol. No modifications were studied with regard to changes in solution chemistry or decomposition of the extractant with time. Four replications of each soil were conducted for each test modification unless otherwise noted.

Scooping vs. Weighing Protocol

Most agronomic soil testing laboratories utilize volumetric sample scoops to transfer known amounts of soil from the bulk pulverized sample storage to the extracting cup or flask. Common scoop sizes are 1, 2, 5 and 10 grams, based on the assumption of a ground soil sample density of 1.10 g cm^{-3}. This density will change depending on sample pulverization fineness and particle density. A 2 gram scoop was used during this project. A single technician was instructed to scoop the sample, strike the scoop's metal shaft three times with a 6 inch metal spatula and then scrape off the excess sample volume by running the flat edge of the spatula perpendicular across the top of the scoop. Each soil sample was scooped four times. The weighing protocol involved weighing 2 grams of each soil using a two digit top-loading balance. The samples were then extracted and analyzed according to the above described Mehlich III project protocol.

Shaking Speed Protocol

Two shaker types were used along with two speeds for each shaker. An inline shaker with a 1 inch throw was set at 200 and 250 epm. The use of inline shaker required the use of 100 ml

Erlenmeyer flasks to prevent sloshing of the extractant:soil mixture from the extraction vessel. The orbital shaker was operated at both 200 and 250 rpm. All samples were shaken for 5 min and filtered using Whatman No. 2 filter paper.

Filter Paper Protocol
The fineness retention capacity of filter paper and its influence on Mehlich III soil test P was evaluated through the testing of Whatman No. 1 and 2 filter papers. Whatman 1 filters have a mean retention of 11μm and larger while Whatman 2 filters retain 8 μm and larger particles. All other parameters remain the same as the standard protocol.

Shaker Type Protocol
This protocol directly mirrored the Shaking Speed Protocol, however it was repeated using the inline shaker operating at 200 epm and orbital shaker operating at 200 rpm. As discussed in the Shaking Speed Protocol, the 100 ml Erlenmeyer flasks were substituted for 5 ounce disposable cups. All other parameters remain constant.

Soil:Solution Ratio Protocol
The soil solution ratio protocol was designed to evaluate the impact of particle and bulk density of soil on the absolute concentrations of P extracted by the Mehlich III extractant. Six extracting ratios were used including 1:7, 1:8, 1:9, 1:10, 1:11 and 1:12. All other parameters remain the same as the standard protocol.

Sample Size Protocol
The mass of a soil sample extracted is normally considered a factor in precision and accuracy of analysis. Five sample weights were studied and included 1, 2, 3, 4, and 5 grams of soil while maintaining a 1:10 soil to extractant ratio. Each sample was weighed to 0.05 grams of the target weight. All other parameters remain constant.

Shaking Time Protocol
The concentration of nutrients extracted from soil is often related to shaking time, specifically when shaking times are less than 15 minutes. The shaking time was altered to include times of 5, 6, 7, 8, and 9 minutes of actual shaking. All other parameters remained constant.

Volumetric vs. Sample Weighing Protocol
This protocol is very similar to the Scooping vs. Weighing Protocol described above, however, it employs a separate factor of scooped sample weigh. As sample sizes increase, the relative error associated with volumetric sampling is normally considered to decrease. This protocol evaluated both weighed and scooped 2 and 5 gram samples. All other parameters remained constant.

Technician Volumetric and Mass Repeatability Protocol
This protocol explored the precision and accuracy associated with both scooping and weighing 2 and 5 gram samples by four different laboratory technicians. All other parameters remind constant.

External Laboratories

Six external laboratories that conduct the Mehlich III method on a routine basis were selected. Three Land-Grant universities and three private laboratories were selected. These laboratories in alphabetic order were: Great Lakes A&L Laboratories, Louisiana State University, Oklahoma State University, Servi-Tech Laboratory (Amarillo), Servi-Tech Laboratory (Fort Dodge), and University of Kentucky. Each laboratory was sent 39 blind samples (12 project samples and the check sample X 3 replicates) for scooping and 39 weighed 2 gram samples (12 project samples and the check sample X 3 replicates).

Results and Discussion

Phosphorus Concentrations in Recovery Samples

The analytical results for Mehlich III P are initially predicated on the recovered soil during the pulverization process. The hypothesis was that if poor soil recovery during pulverization occurred, the sample which was analyzed would be skewed. The skewing would be a result of differences in soil separates (soil texture) which would likely cause a significantly higher level of sand and silt content. As observed in Table 4, only minor differences were observed in the absolute concentration of soil separates. The reason for minor differences in soil texture, considering the pronounced differences in soil recovery in the higher clay content soils remains. A review of Table 4 data re-enforces the findings of actual P recovery listed in Table 5. No significant differences were observed at any level of soil recovery for any of the 7 soils studied. While this project found significant difference in P values based on soil recovery, these finding should not be construed as to suggest that laboratories should not attempt to recover nearly 100% of the true <2 mm fraction. The initial preparation of these soils resulted in well mixing of dried peds of <12 mm diameter. Most laboratories do not preprocess soil samples prior to drying and pulverization to the extent of the steps performed in this project.

Modern agronomic soil testing laboratories analyze thousands of samples annually. Some laboratories exceed 3 million soil samples annually. Historically, since the 1920's, agronomic soil testing laboratories have relied on volumetric soil scoops to quantitatively transfer pulverized and mixed soil from storage bottles or boxes into extracting flasks or sample analyses containers. These scoops are designed to provide an open flat surface where the laboratory technician forces the scoop through the soil sample perpendicular to the soil surface and twists the scoop upward. The scoop is then rapped multiple times with a striker, generally three times, to compact the sample. The striker is used to level the surface using a simple scraping sweep across the surface of the scoop. The historic assumption is that the soil density will be approximately 1.25 g cm^{-3}. While on average, this assumption is reasonable, differences in particle density of sand and clay minerals will result in marked variability of this number. Additionally, the fineness of the sample pulverization will cause significant differences in the ability of the sample to compact.

Table 5. Influence of percent recovery on Mehlich III phosphorus.

Soil	Recovery	Mean P Conc.	SD of Recovery	SD for all soil Recoveries
	----%---	--mg P kg^{-1}---		
1	74.96	72.09	2.88	
1	83.30	72.05	4.64	
1	91.65	73.60	4.98	
1	100	72.21	2.86	
1		72.09		3.61
2	55.99	13.93	4.64	
2	73.30	13.90	4.98	
2	86.65	13.88	2.86	
2	100	13.96	0.97	
2		13.92		1.05
6	72.83	655.5	9.38	
6	81.89	652.4	23.71	
6	90.94	653.0	4.51	
6	100	645.3	8.58	
6		651.5		12.8
7	54.01	37.03	2.81	
7	69.34	37.29	1.99	
7	84.67	34.42	2.45	
7	100	34.32	0.95	
7		35.76		2.42
8	49.06	76.82	2.81	
8	66.04	75.53	3.00	
8	83.02	76.62	3.35	
8	100	77.72	1.57	
8		76.67		2.60
9	59.10	70.00	1.25	
9	72.74	69.91	1.34	
9	86.37	71.08	2.08	
9	100	69.08	1.33	
9		70.02		1.56
10	56.93	44.22	1.99	
10	63.75	43.87	1.98	
10	81.88	44.45	2.68	
10	100	44.35	2.22	
10		44.22		2.01

Statistically, several soils including soils 1, 5, and 12 should have significantly lower Mehlich III P levels in the weighed samples as to when the soils were scooped. The only weighed soil to show significantly higher Mehlich III P levels in the weighed samples was soil 8. The majority of the soils, illustrated in Table 6A, had very little agronomic differences between weighing and scooping. When viewed from an agronomic P availability factor (Table 6B), only soils with very high Mehlich III P levels showed any significant differences in P values between scooping and weighing. Across all soils, weighing of soil samples resulted in significantly lower Mehlich III soil test P levels. While significant, the absolute difference across all soils was only 4.05%. The labor costs coupled with decreased sample throughput associated with weighing samples verses scooping samplings does not appear to be justified based on the limited improvement.

Table 6A. Scooped verses weighed 2 gram samples.

Soil	Weighed	Scooped
	----------------mg P kg^{-1}------------------	
1	76.0^{a*} (2.7)	81.3^{b} (1.8)
2	10.5^{a} (0.8)	10.5^{a} (0.4)
3	17.4^{a} (0.9)	19.6^{a} (0.7)
4	51.8^{a} (1.1)	54.1^{a} (1.3)
5	403.7^{a} (15.0)	492.3^{b} (32.1)
6	706.6^{a} (19.8)	740.3^{a} (17.8)
7	36.4^{a} (1.4)	37.5^{a} (0.5)
8	86.0^{a} (1.0)	82.8^{b} (1.0)
9	71.5^{a} (1.4)	69.9^{a} (1.7)
10	48.5^{a} (1.3)	46.5^{a} (1.7)
11	30.2^{a} (0.8)	30.4^{a} (0.2)
12	41.2^{a} (1.8)	44.6^{b} (1.3)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 6B. Scooped verses weighed 2 gram samples segregated in phosphorus availability classes.

Soil test procedure	M3 -P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	mg P kg^{-1}			
Weighed	131.4[a*]	23.6[a]	62.0[a]	555.2[a]
Scooped	142.5[b]	24.5[a]	63.2[a]	616.3[b]

†Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

All soil extractant based samples are mixed in the same manner to allow for adequate soil to extractant contact. Shaker speed has historically been considered an important factor in determining the relative amount of a nutrient that is extracted. In Table 7A, significant differences were observed between shaking speed and shaker type/shaking speed. While within a given soil differences are observed, collectively (Table 7B) found no significant difference between shaker type/speed across all soils or within a given agronomic P range. The Texas AgriLife Extension Service has historically utilized an orbital shaker (1" throw) at 200 rpm for its routine samples and all research samples and will strongly suggest that all laboratories analyzing Texas soils use similar methods. Using the same shaking type and speed will insure data generated by other laboratories can be properly evaluated and utilize Texas AgriLife Extension Service research based fertility recommendations.

Table 7A. Shaking speed and shaker type influence on extractable phosphorus.

Soil	Inline 200 epm	Inline 250 epm	Orbital 200 rpm	Orbital 250 rpm
	---------- mg P kg^{-1} ----------			
1	80.1[a*] (1.9)	82.4[a] (4.3)	70.7[b] (5.5)	78.4[b] (2.9)
2	12.1[a] (0.6)	9.3[b] (1.0)	11.5[ab] (1.2)	10.5[ab] (1.3)
3	18.4[a] (1.0)	17.1[ab] (0.6)	17.2[ab] (0.4)	16.3[b] (0.8)
4	49.5[a] (0.4)	48.7[a] (1.2)	50.5[a] (1.1)	55.8[b] (2.5)
5	453.8[a] (42.7)	416.0[a] (26.3)	397.0[a] (22.5)	380.0[a] (9.8)
6	656.1[ab] (13.7)	664.3[ab] (19.8)	704.9[a] (33.8)	643.4[b] (8.4)
7	34.6[a] (1.0)	36.1[a] (1.1)	39.3[b] (1.0)	33.8[a] (1.4)
8	76.2[a] (1.4)	76.2[a] (1.5)	82.7[b] (0.7)	82.3[b] (0.9)
9	68.2[a] (1.6)	76.3[b] (3.1)	71.5[c] (2.2)	72.1[c] (2.6)
10	34.9[a] (6.6)	40.9[b] (0.4)	47.3[c] (1.1)	48.9[c] (1.8)
11	35.1[a] (6.5)	30.2[b] (0.9)	30.7[b] (2.0)	29.8[b] (0.6)
12	36.8[a] (0.3)	37.4[ab] (0.4)	39.8[b] (0.8)	40.7[b] (0.9)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 7B. Shaking speed segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	---------- mg P kg^{-1} ----------			
Inline 200 epm	129.7[a*]	23.8[a]	60.1[a]	549.0[a]
Inline 250 epm	127.3[a]	23.2[a]	59.1[a]	540.2[a]
Orbital 200 rpm	130.3[a]	24.7[a]	60.4[a]	550.9[a]
Orbital 250 rpm	124.3[a]	22.6[a]	63.1[a]	511.7[a]

†Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

All soil samples are filtered following shaking and prior to ICP analyses. Two common filter paper specifications are used by laboratories. These include Whatman No. 1 and 2. Whatman No. 1 is a coarser filter paper with a higher flow rate than the more traditional Whatman No. 2, thus providing faster filtration and quicker sample throughput. Concern has been raised as to the potential for small particulates passing through the coarser filter and thereby creating skewed results. Based on this project, no significant differences were observed between filter paper type for any given soil (Table 8A), across all soils or defined agronomic P availability ratings (Table 8B). While this project found no differences between Whatman No. 1 and 2 filter papers suggesting filtration is a minor issue, laboratories should insure strict compliance to Whatman No. 1 and/or 2 specifications when purchasing filter paper as significant differences in filter efficiency exists between manufactures and distributors of filter paper.

Table 8A. Effect of filter paper on Mehlich III extractable phosphorus.

Soil	Whatman No. 1	Whatman No. 2
	--------------mg P kg^{-1}---------------	
1	70.3[a*] (1.1)	70.3[a] (2.9)
2	11.1[a] (0.9)	10.9[a] (0.8)
3	17.3[a] (0.6)	17.4[a] (0.3)
4	50.5[a] (0.4)	52.3[a] (1.8)
5	404.3[a] (5.0)	401.6[a] (17.8)
6	684.9[a] (6.0)	710.1[a] (38.6)
7	33.8[a] (0.5)	36.0[a] (1.9)
8	79.7[a] ((2.8)	83.8[a] (1.8)
9	68.0[a] (1.8)	70.1[a] (2.0)
10	46.5[a] (1.0)	45.1[a] (2.8)
11	29.3[a] (1.3)	29.1[a] (0.8)
12	40.0[a] (1.0)	40.2[a] (1.5)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 8B. Filter paper segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	mg P kg^{-1}			
Whatman No. 1	130.8[a*]	23.9[a]	60.1[a]	544.0[a]
Whatman No. 2	130.6[a]	23.3[a]	60.3[a]	555.8[a]

†Soil with less than 50 mg P kg^{-1} Mehlich III P
‡Soils with 50-200 mg P kg^{-1} Mehlich III P
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The shaker type protocol is a repeat of the more expanded protocol outlined above. No significant differences were observed between shaker types for individual soils (Table 9A), or all soils and defined agronomic P availability ranges (Table 9B).

Table 9A. Effect of shaker type on Mehlich III extractable phosphorus.

Soil	Inline Shaker	Orbital Shaker
	--------------mg P kg^{-1}-------------	
1	70.0^{a*} (4.4)	69.7^{a} (2.4)
2	10.9^{a} (0.9)	10.7^{a} (0.2)
3	16.8^{a} (0.9)	17.5^{a} (1.7)
4	48.3^{a} (1.2)	52.1^{b} (1.0)
5	418.5^{a} (24.8)	408.1^{a} (16.9)
6	695.9^{a} (23.1)	695.7^{a} (12.8)
7	35.6^{a} (0.8)	34.2^{a} (1.3)
8	69.7^{a} (2.8)	82.1^{b} (2.9)
9	67.7^{a} (2.7)	72.1^{a} (2.2)
10	41.1^{a} (1.2)	42.3^{a} (1.1)
11	31.3^{a} (2.4)	29.8^{a} (1.5)
12	39.6^{a} (0.3)	41.5^{a} (1.1)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 9B. Shaker type segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	--------------------------------mg P kg^{-1}------------------------------------			
Inline Shaker	128.8^{a*}	23.7^{a}	56.1^{a}	557.2^{a}
Orbital Shaker	129.7^{a}	23.0^{a}	60.0^{a}	551.9^{a}

† Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The variation in soil density effectively creates differences in dilution ratios. While the protocol specifies a 1:10 soil to extractant ratio, changing dilution ratios can easily result in 1:9 to 1:11 soil to extractant ratios for scooped soil samples. As expected, changing the dilution ratio does significantly alter the analytical P extracted concentrations for the Mehlich III. Historically, the soil testing program at the University of Arkansas utilized a 1:7 soil to Mehlich III extractant ratio. This ratio was used as it provided nearly identical P values as the colorimetric Bray P1, the extractant used prior to the adoption of the Mehlich III. In more recent years, Arkansas viewed the positive benefits of using a standardized methodology. Their findings are similar to those illustrated in Table 10A and 10B, in than the amount of P extracted is not directly proportional to the soil to extractant ratio. For example, when reviewing all soils (Table 10B), the absolute difference between a 1:7 and 1:10 extraction ratio was 21.2% not 30% as the ratio differences would suggest. This difference is likely due to the inability of the extract to adequately flow and mix with the soil during the 5 min. extraction period, an issue observed during the sample size study. Furthermore, most of the scooping data suggests that lighter samples are scooped, resulting in higher soil to extractant ratios. A comparison of the 1:10 and 1:11 ratios indicated no significant differences between these two ratios. This lack of significant difference further strengthens the fact little differences are observed between scooped and weighed samples.

Table 10A. Effect of soil:solution ratio on Mehlich III extractable phosphorus.

Soil	1:7	1:8	1:9	1:10	1:11	1:12
	--mg P kg^{-1}--					
1	67.8^{a}*(1.0)	67.3^{a} (0.9)	69.9^{a} (2.8)	70.9^{a} (3.9)	69.6^{a} (2.9)	71.4^{b} (1.6)
2	8.8^{a} (0.5)	9.7^{a} (0.5)	10.0^{a} (0.5)	11.0^{a} (0.6)	10.7^{a} (0.4)	10.9^{a} (0.5)
3	15.3^{a} (0.4)	15.3^{a} (0.4)	15.9^{a} (0.7)	17.2^{a} (0.9)	17.0^{a} (0.7)	16.6^{a} (1.2)
4	40.0^{a} (0.5)	44.5^{b} (0.7)	49.1^{c} (1.5)	52.3^{d} (2.3)	52.3^{d} (2.3)	55.2^{d} (0.6)
5	417^{ab} (19.3)	418^{ab} (23.4)	442^{ab} (21.0)	406^{a} (16.5)	451^{ab} (26.8)	463^{b} (11.5)
6	557^{a} (4.8)	587^{b} (16.1)	631^{c} (10.3)	718^{d} (24.8)	695^{d} (13.3)	722^{4d} (11.5)
7	29.0^{a} (0.8)	29.6^{a} (0.8)	31.0^{a} (1.1)	34.1^{b} (0.7)	33.3^{b} (0.7)	33.3^{b} (1.5)
8	52.8^{a} (1.9)	61.9^{b} (1.7)	67.2^{c} (0.8)	83.8^{d} (1.3)	80.9^{d} (2.4)	83.4^{d} (1.1)
9	55.9^{a} (1.8)	$59.6a^{b}$ (0.5)	61.7^{b} (1.8)	71.0^{c} (2.2)	68.9^{c} (1.6)	71.0^{c} (0.9)
10	31.2^{a} (1.3)	36.6^{b} (0.6)	40.0^{c} (1.3)	42.8^{c} (0.9)	49.6^{d} (1.5)	53.3^{e} (0.9)
11	27.3^{ab} (0.3)	28.0^{ab} (1.3)	29.4^{b} (0.6)	30.2^{b} (0.9)	30.1^{b} (1.5)	25.3^{a} (0.9)
12	33.3^{a} (1.3)	36.6^{b} (1.4)	38.7^{b} (1.4)	42.3^{c} (2.8)	42.0^{c} (1.0)	43.5^{c} (1.1)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 10A. Soil:Solution ratio segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	---------------------------------mg P kg^{-1}---------------------------			
1:7	114.9^{f*}	18.9^{c}	52.5^{c}	494.2^{f}
1:8	121.3^{e}	20.6^{bc}	54.8^{c}	521.7^{e}
1:9	131.6^{d}	22.8^{bc}	60.4^{b}	562.7^{d}
1:10	145.2^{cd}	23.8^{bc}	60.4^{b}	596.8^{c}
1:11	145.2^{b}	25.8^{b}	63.6^{ab}	628.5^{b}
1:12	159.3^{a}	28.7^{a}	67.1^{a}	697.0^{a}

† Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Increasing sample mass of the extracted sample is traditionally considered an easy method of improving precision. While a 2 gram sample is the traditional sample size for most laboratories analyzing the Mehlich III extract on ICPs, some laboratories extract 1 gram samples. The laboratories extracting 1 gram samples are generally limited to areas dominated by very sandy soils, as the filter paper and soil will absorb several milliliters of water. In this project, sample sizes of 1 through 5 grams were extracted using 1:10 soil to extractant ratio (Tables 11A and 11B). The relative differences between using 1,2,3 or 4 gram samples was minimal, however when the 5 gram sample was utilized, reduced concentrations of Mehlich III extractable P occurred (Table 11B). The reduction in extractable P for the 5 gram sample (Table 11A) is due to the inability of an orbital shaker with a 1 inch throw to keep the entire mass of sample in an agitated model. Technician observations indicated that a portion of the soil remained in the bottom of the extraction/shaking cup in a small wetted clump. This project did not review the impact of longer throw orbital shaking, as most shakers are unable to be adjusted to a throw more than 1 inch. Across all samples, the standard deviation, and estimate of precision, varied considerably between soil and sample sizes resulting in no significant improvement in precision amongst the 1-4 gram sample sizes. The precision for soils 5 and 6, the soils with very high P, was significantly better for the 4 gram sample size than for the 1-3 gram sample size. This improvement is likely due to better representation of these historically manured soils that likely contain very small aggregates or highly enriched P soil or organic matter.

Table 11A. Effect of sample size on Mehlich III extractable phosphorus.

Soil	1 gram	2 gram	3 gram	4 gram	5 gram
	mg P kg^{-1}				
1	70.6^{a*} (4.1)	70.2^{a} (2.6)	69.4^{a} (2.9)	75.7^{b} (1.1)	56.9^{c} (1.7)
2	11.4^{a} (0.5)	10.6^{a} (0.6)	10.6^{a} (0.4)	11.9^{a} (0.5)	10.3^{a} (0.5)
3	17.7^{a} (0.1)	17.0^{a} (1.2)	16.7^{a} (1.1)	18.8^{a} (0.3)	16.5^{a} (0.6)
4	53.1^{a} (1.7)	52.9^{a} (1.7)	53.1^{a} (1.4)	54.1^{a} (0.4)	46.3^{b} (0.6)
5	414.2^{a} (22.3)	411.4^{a} (20.8)	392.1^{a} (22.3)	410.3^{a} (7.6)	373.4^{b} (12.2)
6	682.1^{a} (20.8)	720.8^{b} (29.4)	677.8^{a} (22.6)	740.4^{b} (6.8)	645.4^{a} (31.8)
7	32.8^{a} (2.0)	35.8^{ab} (0.5)	32.4^{a} (0.9)	36.7^{b} (1.6)	31.0^{a} (0.3)
8	80.7^{a} (0.5)	85.9^{b} (0.6)	75.4^{c} (1.7)	78.7^{c} (2.9)	73.2^{c} (2.8)
9	66.5^{a} (3.1)	73.1^{b} (3.4)	64.2^{ac} (1.5)	68.0^{a} (3.4)	61.8^{c} (1.1)
10	47.7^{a} (0.4)	44.5^{a} (1.3)	48.0^{a} (3.0)	47.6^{a} (2.0)	37.4^{b} (0.8)
11	31.1^{a} (3.4)	28.6^{a} (1.2)	29.3^{a} (0.7)	30.8^{a} (1.2)	25.7^{b} (0.9)
12	42.2^{a} (1.9)	42.0^{a} (0.6)	37.2^{b} (1.0)	38.8^{b} (1.0)	37.7^{b} (0.4)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 11B. Sample size segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	mg P kg^{-1}			
1 gram	129.2[a*]	23.4[a]	60.1[a]	548.2[a]
2 gram	132.7[a]	23.0[a]	61.3[a]	566.1[b]
3 gram	125.7[b]	22.3[ab]	58.1[a]	535.3[c]
4 gram	134.3[a]	24.6[a]	60.5[a]	575.3[d]
5 gram	117.9[c]	20.8[b]	52.2[b]	509.4[e]

†Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The influence of shaking time has been thought to have a dramatic influence on the extractability of soil P. Since the amount of P extracted by the Mehlich III method is dependent on the level of cations extracted, it is considered an equilibrium extractant. In previous research, conducted by the SWFTL on a previously used soil nutrient extractant, concentrations of extracted P actually declined as the shaking time significantly exceeded the method described shaking interval. Similar results were observed in this project, however, in Soil 1, a significant increase in extractable P occurred with added time and then declining at the final time interval (Table 12A and 12B). Numerically, this occurred in multiple soils, however the differences were not statistically significant. The probable cause of this decline in extractable P is the precipitation of P compounds in the slurry matrix and the re-precipitation of P onto the soil. These reactions will be occurring anytime the extractant is in contact with the soil, thus accurate and expedient processing of the samples is required for this relatively short timeframe extract.

The protocol used in this project did not examine the impact of prolonged time between dispensing of the extractant solution and shaking or the time frame required for the samples to be removed from the shaker and transferred to the filtering apparatus. Based on this data, laboratories should not attempt to shake batches of samples greater than what can be fully dispensed and filtered in 8 minutes.

Table 12A. Effect of shaking time on Mehlich III extractable phosphorus.

Soil	5 min.	6 min.	7 min.	8 min.	9 min.
	-----------	-----------	mg P kg^{-1}	-----------	-----------
1	71.1[a*] (1.4)	78.2[b] (5.5)	84.7[c] (1.3)	83.0[c] (3.3)	76.8[b] (7.7)
2	11.1[a] (0.2)	11.7[a] (1.0)	12.2[a] (0.9)	12.3[a] (1.2)	10.5[a] (0.7)
3	17.3[a] (0.4)	19.4[a] (1.6)	19.3[a] (1.4)	18.6[a] (0.4)	17.6[a] (1.1)
4	52.1[ab] (1.43)	54.7[a] (1.7)	55.2[a] (2.4)	54.0[a] (1.8)	49.4[b] (0.9)
5	404.8[a] (32.3)	420.1[a] (56.2)	425.7[a] (12.2)	408.9[a] (14.4)	414.1[a] (20.3)
6	718.9[a] (28.2)	690.4[a] (28.4)	706.7[a] (26.6)	689.0[a] (23.5)	678.4[a] (14.8)
7	35.9[a] (0.7)	36.4[a] (2.0)	37.5[a] (1.7)	38.4[a] (1.7)	36.0[a] (3.6)
8	83.4[a] (0.9)	85.9[a] (2.3)	89.7[a] (2.7)	86.2[a] (0.9)	75.4[b] (1.6)
9	73.7[a] (2.6)	73.4[a] (2.6)	75.7[a] (1.0)	75.0[a] (1.9)	68.1[b] (2.6)
10	47.5[a] (1.7)	49.4[a] (2.3)	48.9[a] (2.2)	47.5[a] (0.6)	46.1[a] (0.7)
11	28.8[a] (1.5)	30.2[a] (1.3)	30.9[a] (2.2)	31.7[a] (1.6)	29.3[a] (1.4)
12	40.5[a] (0.5)	41.1[a] (0.8)	41.2[a] (1.6)	39.5[ab] (1.1)	37.0[b] (0.7)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 12B. Shaking times segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
minutes	-----------	mg P kg^{-1}	-----------	-----------
5	132.1[a*]	23.3[a]	61.4[a]	561.8[a]
6	132.6[a]	24.4[a]	63.8[a]	555.3[b]
7	135.6[a]	25.0[a]	65.9[a]	566.2[a]
8	128.5[b]	25.2[a]	57.3[b]	549.0[bc]
9	128.2[b]	23.3[a]	58.8[b]	546.3[c]

†Soil with less than 50 mg P kg^{-1} Mehlich III P
‡Soils with 50-200 mg P kg^{-1} Mehlich III P
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The volumetric weight verses technician weighing protocol is essentially an expanded version of the weighing verses scooping protocol discussed previously. In this section, the 5 gram sample weight was also studied. As mentioned previously, overall, the scooping of soil samples resulted in significantly higher extractable P levels (Tables 13A and 13B). A closer examination of the data indicates that much of this significance is due to the significant differences between scooping and weighing observed in Soil 5 of the two soils studied that had very high P levels. From an agronomic standpoint, the difference is of little value as this soil had 8 to 9 times greater soil extractable P than the crop critical levels require. Individually, the significantly higher extractable P from the 2 gram scooping was also noted in the sandy soils number 1, 4, 11 and 12. While the extractable P level was significantly different, the absolute numerical differences were generally small and have limited to no effect on fertilizer recommendations. The sandy soils likely had a significantly higher particle density and pulverized bulk density, thus resulting in greater mass of soil being scooped than when the sample was weighed. Numerically, the sandier soils (Soils 1, 3, 4, 5, 11 and 12) all had higher extractable P when scooped. This trend was repeated for the 5 gram sample sizes, however the relative difference between scooping and weighing was less, likely due to the inefficient ability to shake these larger sample sizes.

Table 13A. Effect of volumetric weight verses technician weighed samples on Mehlich III extractable phosphorus.

Soil	2 gram weighed	5 gram weighed	2 gram scooped	5 gram scooped
	-------------------------------------mg P kg^{-1}	--		
1	70.9^{a*} (3.4)	53.5^{b} (4.2)	82.0^{c} (0.8)	57.3^{b} (4.5)
2	10.9^{a} (0.4)	9.4^{a} (0.8)	11.0^{a} (0.6)	10.1^{a} (0.5)
3	16.9^{a} (0.9)	17.7^{a} (2.0)	18.7^{a} (0.6)	17.6^{a} (1.0)
4	51.6^{a} (1.1)	44.6^{b} (1.9)	55.5^{c} (0.6)	46.7^{b} (2.2)
5	407.8^{a} (30.3)	398.0^{a} (9.3)	517.5^{b} (12.5)	458.5^{c} (22.3)
6	712.1^{a} (34.8)	658.5^{b} (32.4)	737.8^{a} (12.3)	645.4^{b} (26.3)
7	35.9^{a} (1.0)	30.7^{b} (0.7)	36.7^{a} (1.1)	29.9^{b} (1.6)
8	83.6^{a} (1.9)	71.9^{b} (1.2)	84.4^{a} (3.7)	72.5^{b} (1.3)
9	74.2^{a} (1.6)	59.4^{b} (3.3)	76.3^{a} (2.6)	60.5^{b} (1.3)
10	47.9^{a} (1.1)	40.3^{b} (0.5)	45.9^{a} (0.9)	42.0^{b} (1.8)
11	30.0^{a} (0.9)	26.4^{b} (0.5)	36.1^{c} (1.3)	28.8^{b} (0.7)
12	39.2^{a} (0.6)	36.3^{b} (0.6)	44.7^{c} (0.5)	39.1^{a} (0.8)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 13B. Volumetric weight verses technician weighed samples segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	----------mg P kg^{-1}----------			
2 gram weighed	131.8[a*]	23.4[a]	61.2[a]	560.0[a]
5 gram weighed	120.6[b]	21.1[b]	51.0[b]	528.3[b]
2 gram scooped	145.6[c]	25.6[a]	64.8[a]	627.7[c]
5 gram scooped	125.7[d]	21.6[b]	53.0[b]	552.0[a]

† Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The use of scooping involves several factors which can significantly alter the actual mass of soil transferred for soil analysis. Table 14A illustrates the relative differences in extractable Mehlich III P due to technician biases. Biases may be due to a host of factors including: 1) how hard the technician strikes the scoop handle, 2) the location on the handle the technician strikes the scoop handle, 3) the force to which the technician pulls the scoop through the soil box, 4) the angle at which the technician pulls the scoop through the soil box, and 5) the angle to at which the technician scraps off the excess soil from the scoop. From a precision standpoint, individual technicians maintain a good repeatability within replicates of a given soil sample. The relative differences between technicians also appear to be somewhat predictable based on the 2 gram samples results in Table 14A. For example, Technician 1 statistically was responsible for higher extractable P levels, while Technicians 2 through 4 were nearly always achieved statistically similar results. The data from the 5 gram scooping shows more variability by these same technicians, however due to the physical extraction issues discussed earlier, this data is likely of very limited value for further discussion.

When technician performance was compared within defined agronomic P ranges, there were no statistical differences in the Mehlich III P levels between technicians (2 gram scoop) for the low-medium and high agronomic ranges (Table 14B). For the very high range, Technician 1's apparent higher mass sampling resulted in overall statistical differences for this agronomic P range. Collectively, across all soils and all agronomic P ranges, each technician achieve significantly different results (Table 14B), however the numeric differences were extremely small between Technicians 2, 3 and 4. The relative differences were 4.95% between the

Technician 2, 3 and 4. Two key points should be made regarding relative differences between the scooping technicians: 1) the relative difference also contains potential errors not associated with technician scooping, although every attempt to minimize external errors were made, and 2) Technician 1 was not an individual the Soil, Water and Forage Testing Laboratory utilizes for scooping of soil samples. These data strongly suggest laboratories that use volumetric sampling, scooping, must develop good and continuing training protocols for the technicians who are tasked with sample scooping. This is evident by Technician 1 being high most of the time when the 2 g scoop is used and low when the 5 g scoop is used.

Table 14A. Effect of technician scooped mass repeatability on Mehlich III extractable phosphorus.

Soil	Tech 1, 2g	Tech 2, 2g	Tech 3, 2g	Tech4, 2g	Tech 1, 5g	Tech 2, 5g	Tech 3, 5g	Tech 4, 5g
	-------------------------------------mg P kg^{-1}---							
	89.1^{a*}	89.7^{a}	81.1^{b}	82.4^{b}	54.3^{c}	64.0^{d}	52.2^{c}	67.6^{d}
1	(2.6)	(2.2)	(0.9)	(3.4)	(4.3)	(2.1)	(4.5)	(1.5)
	12.2^{a}	13.8^{a}	12.0^{a}	12.0^{a}	8.0^{b}	8.5^{bc}	8.8^{bc}	10.0^{c}
2	(0.3)	(1.8)	(1.1)	(0.4)	(0.2)	(0.5)	(1.0)	(0.6)
	21.7^{a}	19.2^{a}	20.2^{a}	19.5^{a}	14.8^{b}	15.4^{b}	14.0^{b}	15.8^{b}
3	(1.2)	(1.4)	(1.2)	(1.5)	(0.7)	(0.7)	(0.5)	(0.8)
	58.5^{a}	54.8^{b}	56.6^{ab}	50.3^{c}	38.8^{d}	53.0^{bc}	42.5^{d}	46.7^{d}
4	(0.3)	(2.4)	(1.9)	(2.0)	(2.6)	(9.5)	(1.9)	(1.0)
	503.0^{a}	482.6^{ab}	461.9^{bc}	434.2^{c}	372.2^{d}	396.0^{d}	379.3^{d}	388.8^{d}
5	(15.8)	(37.0)	(17.5)	(25.2)	(22.6)	(29.0)	(12.2)	(10.2)
	747.0^{a}	674.2^{b}	675.6^{b}	661.6^{b}	621.5^{bc}	635.8^{bc}	579.8^{c}	644.8^{b}
6	(17.9)	(19.3)	(11.0)	(24.0)	(5.3)	(18.1)	(49.7)	(25.0)
	37.9^{a}	34.7^{ab}	34.9^{ab}	32.3^{b}	27.8^{c}	26.9^{c}	28.7^{c}	27.3^{c}
7	(0.8)	(2.1)	(1.1)	(1.5)	(0.5)	(2.1)	(1.2)	(2.6)
	86.2^{a}	81.2^{b}	82.6^{b}	81.4^{b}	59.9^{c}	65.1^{c}	69.2^{c}	67.1^{c}
8	(1.2)	(2.2)	(2.0)	(2.1)	(4.3)	(1.7)	(0.5)	(1.7)
	75.6^{a}	67.7^{b}	69.4^{b}	70.0^{b}	46.3^{c}	54.4^{d}	52.8^{d}	56.1^{d}
9	(1.9)	(2.4)	(2.3)	(0.9)	(6.6)	(2.9)	(1.7)	(1.8)
	48.1^{a}	44.4^{b}	48.6^{a}	43.8^{b}	41.5^{b}	40.4^{bc}	41.5^{bc}	38.3^{c}
10	(1.4)	(0.4)	(0.9)	(1.3)	(4.9)	(2.6)	(2.4)	(1.1)
	37.1^{a}	34.0^{b}	34.4^{b}	32.1^{b}	28.3^{c}	25.9^{c}	27.9^{c}	26.4^{c}
11	(0.7)	(1.9)	(1.9)	(1.4)	(1.8)	(2.0)	(0.8)	(0.4)
	45.9^{a}	42.9^{a}	43.6^{a}	40.3^{b}	35.6^{c}	32.5^{c}	35.3^{c}	34.1^{c}
12	(0.7)	(2.2)	(1.6)	(2.1)	(1.0)	(0.8)	(0.8)	(1.0)

*Means with different letters between technicians within soils are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 14B. Technician scooped mass repeatability segregated in phosphorus availability classes.

Soil test procedure	Mehlich III P mean values in			
	all soils(12)	low-med (4)†	high(6)‡	v. high(2)¶
	mg P kg^{-1}			
Tech 1, 2g	146.9[a*]	27.2[a]	67.3[a]	625.1[a]
Tech 2, 2g	136.6[b]	25.4[a]	63.5[a]	578.4[b]
Tech 3, 2g	135.1b[c]	25.4[a]	63.7[a]	568.8[c]
Tech 4, 2g	130.0[d]	24.0[a]	61.4[a]	547.9[d]
Tech 1, 5g	112.3[e]	19.8[c]	46.1[b]	496.8[e]
Tech 2, 5g	118.2[fh]	19.2[c]	51.5[c]	515.9[f]
Tech 3, 5g	111.0[g]	19.9[c]	48.9[b]	479.6[g]
Tech 4, 5g	118.6[h]	19.9[c]	51.6[c]	516.8[fh]

†Soil with less than 50 mg P kg^{-1} Mehlich III P.
‡Soils with 50-200 mg P kg^{-1} Mehlich III P.
¶Soil with greater than 200 mg P kg^{-1} Mehlich III P.
*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

The analysis of project soil samples by external laboratories provides a strong validation of the Mehlich III method's robustness to provide similar analytical data by laboratories than by the very nature of conducting these analyses more than 30,000 times a year, have developed significant expertise. At the onset of this project, the North American Proficiency Testing Program, administrated by Soil Science Society of America, has observed that modest and larger sample throughput laboratories that conduct the Mehlich III method, as their primary or sole nutrient assay for P and K, routinely show very good proficiency, while laboratories performing the test for limited clientele often have difficulty achieving representative results.

The external laboratories data mirrors that of the internal laboratory's observation that scooping of soil samples in general increased the extractable P values (Tables 15A and 15B). The individual laboratory precision was generally very good within soil and across the entire sample set. A review of laboratory methodologies indicated that laboratories 1, 2 and 6 utilized orbital shakers, while laboratories 3, 4 and 5 used inline shakers. A review of the weighed data suggests a small trend toward lower P values for soil extracted with orbital shakers, however this was not statistically significant. No discernable differences in other protocols, including reported calibration of dispensers, ICP analyses/instrument protocols, shelf life, elemental standards or scooping protocols was significantly correlated to the mean differences within a given soil or across the entire sample set.

Overall, extractable P recovery between scooped and weighed samples was significantly different, however the relative differences were modest. Furthermore, the standard deviation of analysis for a given soil analyzed by all six external laboratories was directly correlated to the level of extractable P. From a regulatory standpoint, the two soil samples with greater than 200 ppm Mehlich III P, samples 5 and 6, had the lowest standard deviation of analyses, when expressed as a percentage of the mean (Table 15C).

Table 15A. External laboratory Mehlich III phosphorus scooped samples.

	Laboratory					
Soil	1	2	3	4	5	6
	mg P kg^{-1}					
1	71.1^{a*} (1.7)	79.3^{b} (2.9)	79.3^{b} (1.5)	83.7^{bc} (2.3)	88.3^{c} (4.9)	91.0^{c} (1.3)
2	10.2^{a} (0.5)	12.0^{b} (0.3)	14.3^{c} (0.6)	13.0^{bc} (1.7)	20.0^{abcd}(9.6)	14.0^{c} (0)
3	17.5^{a} (0.4)	20.6^{b} (0.4)	22.3^{c} (0.6)	21.3^{bc} (1.2)	23.0^{c} (1.0)	23.7^{d} (0.6)
4	48.4^{a} (0.9)	51.4b (0.9)	$57. 7^{c}$ (3.2)	53.7^{bc} (2.5)	60.7^{c} (7.4)	60.2^{c} (0.3)
5	403^{a} (19)	429^{ab} (12)	428^{ab} (6)	450^{c} (8)	446^{b} (13)	484^{d} (10)
6	646^{c} (11)	624^{ab} (14)	621^{a} (22)	640^{c} (7)	625^{ab} (8)	687^{d} (11)
7	30.3^{a} (0.9)	33.9^{b} (0.4)	41.0^{c} (3.6)	38.0^{c} (0)	44.0^{cd} (10)	39.3^{c} (1.6)
8	75.4^{a} (0.6)	74.8^{a} (2.6)	80.3^{b} (2.1)	80.7^{b} (1.2)	82.0^{b} (0)	84.3^{c} (0.8)
9	61.9^{a} (1.1)	72.5^{bc} (3.3)	69.0^{b} (2.6)	72.7^{c} (0.6)	73.0^{c} (1.0)	77.0^{d} (0.5)
10	44.9^{bc} (1.4)	46.3^{c} (1.3)	41.7^{a} (1.5)	44.0^{b} (1.7)	44.0^{b} (0)	48.0^{d} (2.5)
11	28.2^{a} (0.4)	32.4^{b} (0.6)	35.0^{c} (1.0)	33.3^{b} (0.6)	35.0^{c} (1.0)	37.8^{d} (0.8)
12	40.1^{a} (0.8)	45.2^{b} (0.5)	47.0^{c} (1.0)	48.7^{cd} (2.1)	49.0^{cd} (2.6)	50.5^{d} (0.9)
13	38.9^{a} (1.0)	43.0^{b} (1.1)	44.7^{b} (0.6)	45.0^{b} (1.7)	44.7^{b} (0.6)	49.7^{c} (0.8)

*Means with different letters between laboratories within soils are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 15B. External laboratory Mehlich III phosphorus 2 gram weighed samples.

Soil	Laboratory					
	1	2	3	4	5	6
	mg P kg^{-1}					
1	62.9^{a*} (0.8)	73.8^{c} (2.0)	72.7^{bc} (5.7)	76.3^{bc} (5.0)	75.3^{c} (6.7)	69.3^{b} (2.1)
2	10.4^{a} (0.1)	13.3^{c} (0.9)	14.0^{c} (0)	12.7^{abc}(2.9)	15.0^{cd} (1.4)	12.0^{b} (0)
3	14.7^{a} (0.1)	18.1^{c} (0.3)	19.3^{cd} (0.6)	20.7^{d} (1.5)	20.0^{cd} (1.7)	17.3^{b} (0.3)
4	44.3^{a} (0.7)	53.3^{c} (1.4)	54.7^{c} (2.5)	55.3^{c} (4.0)	61.7^{d} (8.5)	49.0^{b} (0.5)
5	356^{a} (5)	390^{c} (7)	365^{ab} (15)	399^{c} (12)	378^{b} (5)	352^{a} (10)
6	630^{c} (5)	637^{bc} (18)	580^{a} (3)	614^{b} (21)	606^{b} (15)	601^{b} (8)
7	29.0^{a} (0.6)	36.3^{c} (0.3)	39.0^{cd} (3.0)	38.0^{cd} (3.5)	45.0^{c} (7.6)	32.8^{b} (0.3)
8	71.9^{a} (1.2)	78.7^{b} (2.5)	74.0^{ab} (2.6)	79.3^{b} (4.0)	79.3^{b} (0.68)	76.8^{ab} (0.6)
9	60.4^{a} (0.4)	71.8^{cd} (4.7)	62.0^{b} (1.0)	67.7^{c} (1.5)	67.7^{c} (2.1)	67.5^{c} (1.3)
10	43.5^{b} (0.8)	46.9^{c} (1.4)	40.0^{a} (2.0)	46.7^{c} (1.5)	47.0^{c} (0)	46.7^{c} (2.0)
11	23.2^{a} (0.2)	$28.4b^{c}$ (1.6)	28.7^{c} (0.6)	29.3^{c} (1.5)	29.0^{c} (1.0)	27.2^{b} (0.3)
12	36.7^{a} (0.4)	42.2^{c} (0.5)	40.7^{b} (0.6)	46.3^{c} (4.0)	44.0^{c} (2.0)	41.7^{bc} (0.6)
13	36.0^{a} (0.7)	41.9^{b} (1.4)	39.7^{b} (0.6)	40.3^{b} (1.5)	40.7^{b} (0.6)	40.7^{b} (1.3)

*Means with different letters are significantly different at 0.05 levels. Standard deviations appear in parentheses.

Table 15C. Relative differences in Mehlich III extractable phosphorus between scooped and weighed soil from six external laboratories.

Soil	Scooped	SD		Weighed	SD	
	P kg^{-1}		%	mg P kg^{-1}		%
1	82.12	7.12	8.67	71.72	5.90	8.23
2	13.93	4.60	33.01	12.77	1.85	14.48
3	21.40	2.17	10.13	18.36	2.19	11.95
4	55.33	5.51	9.96	53.04	6.52	12.29
5	439.90	27.46	6.24	373.28	19.49	5.22
6	640.58	25.82	4.03	611.40	22.40	3.66
7	37.75	5.86	15.51	36.68	5.98	16.32
8	79.46	3.79	4.77	76.69	3.49	4.55
9	71.01	5.07	7.14	66.17	4.41	6.66
10	44.81	2.44	5.44	45.12	2.95	6.53
11	33.64	3.09	9.19	27.61	2.33	8.46
12	46.75	3.71	7.94	41.92	3.45	8.23
13	44.32	3.38	7.62	39.87	2.09	5.24

Conclusions and Recommendations

The exhaustive analysis of potential intra-laboratory methodology differences yielded a number of identifying practices that could skew laboratory data for the Mehlich III P determination. This same analysis also confirmed the relative robustness of the method to provide relatively uniform results in spite of modest or even extreme changes in laboratory procedures or extraction conditions. A common and overarching assessment is that much of the reported variability between laboratories conducting the Mehlich III method as their primary analytical soil testing method are likely due to non-homogenous samples being submitted to two or more different laboratories. This project was never designed or intended to review differences in data due to non-homogenous samples, but only the impact of common deviations observed between agronomic soil testing laboratories and/or protocols used by agronomic laboratories which differ significantly from the environmental laboratory community.

The Mehlich III extractant, when performed by trained technicians in high volume laboratories is a reliable and reproducible test for the assessment of agronomic soil P levels. Whether scooping or weighing is used by the laboratory, key steps must be preformed correctly to insure reliable and repeatable data. The added expense of weighing of soil samples does eliminate the error associated with changes in soil sample bulk density, however this minor difference has little agronomic value or influence of fertilizer recommendations and will unlikely be implemented by commercial agronomic laboratories. The minor improvement in accuracy at the highly elevated soil P levels may be of interest to the regulatory authorities, however it must also be pointed out that across a selection of laboratories, the standard deviation for Mehlich III P will likely be 3-7% in the typical enforcement soil P range. This range is modest in comparison to the variability in soil sample collection differences previous reported by other investigators. Regulatory authorities should utilize these reported standard deviations when considering the use of absolute values for soil test P, typically 200 ppm.

Other factors not examined in this project, but known by medium and large agronomic laboratories, are the short shelf life of the extractant, development of standards, calibration of ICPs to avoid matrix interferences created by the extractant reagents and laboratory design to insure quick and accurate extraction of samples. These factors are often laboratory by laboratory specific and not always conveyable in generic SOPs.

The conclusions drawn directly from the laboratory data generated in this project are:

1) The relative percent recovery of soil from a well mixed soil sample submitted to a laboratory should have no effect on the resulting Mehlich III extractable P results.

2) Modest differences in Mehlich III extractable P are observed between scooping and weighing of soil samples, however the relative difference is dependent on the percent of sand comprising the soil sample, with higher clay content soils having greater extractable P when scooping is used. Statistically, these differences are only observed in very high Mehlich III P soils. Technician training and avoidance of using technicians not fully trained and tested in the volumetric protocols for the scooping of soil is fundamental in the assurance of accurate and reproducible Mehlich III P data.

3) Both Whatman No. 1 and 2 filter types are suitable for Mehlich III P analyses.

4) Shaker type has no effect on extractable P, however increasing shaking speed can result in higher P recovery.

5) The soil:extractant ratio can profoundly influence the amount of extractable Mehlich III P, however when ratios are keep between 1:9 to 1:11 (method standard of 1:10), minimal differences in extractable P are observed.

6) The sample size used for extraction does not significantly alter precision or accuracy of analyses, however if sample size is increased beyond 4 grams in a 1:10 soil:extractant ratio, most orbital laboratory shakers will be unable to adequately keep the soil:extractant in a moving slurry.

7) The modest allowance of shaking beyond the stated 5 min shaking time will have limited influence of Mehlich III extractable P, however extended shaking time will result in reduced recovery of P. Laboratories should limit the time allowed for dispensing of extractant, the 5 min shaking period, and decanting into the filter apparatus to less than 9 min to avoid potential recovery problems.