Watershed Watch Nutrient Field Kit Evaluation Report

April 2019

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Kentucky Water Resources Research Institute

WATERSHED WATCH IN KENTUCKY
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Kentucky Geological Survey

Title: Watershed Watch Nutrient Field Kit Evaluation Report

Date: April 2019

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Acknowledgements:

Special thanks are extended to the following individuals and groups, without whose involvement the project would not have been possible:

- Jason Backus, Kentucky Geological Survey, University of Kentucky
- Andrea Conner, Kentucky Geological Survey, University of Kentucky
- Dale Booth, Kentucky Division of Water, Watershed Watch in Kentucky Science Advisors Chair
- Watershed Watch in Kentucky, kywater.org
- Virginia Environmental Endowment, www.vee.org
- The many student volunteers and citizen scientists that gave their time to this project

Authors' Note:

The findings and conclusions in this document are those of the authors and do not necessarily represent the views of the University of Kentucky or Watershed Watch in Kentucky.

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List of Acronyms and Definitions

cm	Centimeter
HAB	Harmful Algal Bloom
JTU	Jackson Turbidity Unit
KDOW	Kentucky Division of Water
KGS	Kentucky Geological Survey
mg/L	Milligrams per liter
mL	Milliliter
n	sample size
NH3	Ammonia
NH3-N	Ammonia-nitrogen or ammonia as nitrogen
NO3	Nitrate
NO3-N	Nitrate-nitrogen or nitrate as nitrogen
NTU	Nephelometric Turbidity Unit
ppb	Parts per billion, equivalent to µg/L
ppm	Parts per million, equivalent to mg/L
PO4	Phosphate
PO4-P	Phosphate-phosphorus or phosphate as phosphorus
R2	Coefficient of determination
SCIAD	Science Advisory Committee
WWKY	Watershed Watch in Kentucky

accuracy – how close a measured value is to the true value

aliquot – an exact measurement of a sample or reagent.

ampoule – a sealed glass capsule containing a liquid.

bias – tendency of a measurement process to over- or under-estimate the value of a population parameter.

coefficient of determination – also simplified as R^2 is a number from 0 to 1 which indicates how close the data are to a fitted regression line. The closer the value is to 1, the better the fit.

concentration – amount of a substance per a defined volume.

correlation – measure that indicates the extent to which two or more variables fluctuate together.

discrete – a measurement that can only be expressed in certain defined number values, as opposed to continuous measurements which include any value over a range.

experienced volunteer – volunteer with four or more years of experience with field-testing.

intercept – value at which the fitted line crosses the y-axis. The variance from zero indicates increasing error at low concentrations.

linear regression – a statistical method that allows for the evaluation of the relationship between two variables. It is often expressed by the equation $y = ax + b$, where y is the measured variable, x is the known variable, a is the slope, and b is intercept of the best fit line.

moderate volunteer – volunteer with one to two years of experience with field-testing or graduate students using field sampling in their thesis.

new volunteer – volunteer with no experience with field-testing.

orthophosphate – also known as reactive phosphate, it is the form of phosphorus readily available to the biological community and typically the most abundant form of phosphorus present in water.

parameter – water quality measurement for a chemical substance or physical or chemical attribute.

percent difference – difference between two values divided by the average of the two values, expressed as a percentage.

percent recovery – amount of substance measured in a sample divided by the known amount of the substance added to a sample without that substance present, expressed as a percentage.

precision – how close measured values are to one another.

reagent – a substance or mixture for use in chemical analysis or other reactions.

slope – the ratio between the known standard solution concentration and the measured concentration.

spectrophotometer – an apparatus for measuring the intensity of light in a specific part of the color spectrum

standard deviation – measure that is used to quantify the amount of variation or dispersion of a set of data values.

turbidity – measure of the degree to which the water loses its transparency due to the presence of suspended particulates.

1.0 Background

Watershed Watch in Kentucky (WWKY) is a statewide, non-profit, citizen monitoring effort to improve and protect water quality. This effort is made through raising community awareness and by supporting implementation of the goals of the Clean Water Act and other water quality initiatives [1]. WWKY's purpose is to coordinate and advise the eight basin groups across the state (Big Sandy, Four Rivers, Kentucky River, Licking River, Salt River, Tradewater / Lower Green River, Upper Cumberland, and Upper Green River) such that consistent tools are used by citizen scientists for collection and interpretation of data.

In November 2017, the Science Advisory Committee (SCIAD) of WWKY met to discuss the parameters and analysis methods being used by individual basins across the state. As part of the core monitoring program, all basins across the state routinely sample for *E. coli*, water temperature, dissolved oxygen, conductivity, and pH using standard methodology. However, individual basin teams have pursued other parameters based on the unique land uses and geology of each basin as well as funding and interest of each basin team. According the Watershed Watch Data Portal [2], only four of the basins sampled nutrient parameters in either 2016 or 2017 and only two of the basins sampled nutrient parameters in both years. The cost of laboratory analysis was indicated by the committee to be the primary impediment to additional nutrient sampling, despite the recognized importance of these parameters.

Since nutrient enrichment is the second most common cause of impairment for Kentucky waterways [3], there is a statewide need for routine citizen monitoring to evaluate the impacts on the waterways. According to the 2016 list of impaired streams in Kentucky, nutrient / eutrophication biological indicators are the main cause of impairment for 1,753 miles of stream and river [3]. Algal blooms and eutrophication are two of the most commonly known issues associated with unbalanced nutrient levels in waterbodies [4]. Harmful algal blooms (HABs) occur when algae colonies grow out of control, producing toxic conditions [4]. These toxic conditions can be from the algae itself producing toxins or simply from the enormous amounts of algae decaying and consuming all the oxygen available in the water. Both situations cause widespread illness and fatalities in fish, birds, mammals, and sometimes even humans if recreational and potable water sources become contaminated [4].

In FY 2017, the Kentucky Division of Water (KDOW) and partners responded to 17 reports of algal blooms or illnesses possibly related to HABs and three reservoirs received advisories [4]. In 2017, WWKY worked with the KDOW to develop a Lakes Monitoring Program with the intention "to focus resources and bring awareness to the ever-increasing issues associated with harmful algal blooms across the state" [5].

With this backdrop, SCIAD determined WWKY would benefit from performing nutrient analyses statewide. However, due to the numerous low-cost, nutrient field-testing products that have been introduced in recent years, SCIAD expressed the need to determine which field kits are most accurate, cost effective and accessible to volunteers. This report details an evaluation of the data quality, ease of use, time duration of analysis, and other factors associated with these field kits.

2.0 Methods

Thirteen kits were selected to evaluate four different parameters: ammonia, nitrate, phosphorous, and turbidity. The goal of this study was to evaluate each field kit for precision, accuracy, bias, ease of use, and overall performance. Table 1 details the kits evaluated during this study.

Table 1: Field Test Kits Evaluated

MANUFACTURER	KIT TYPE	PRODUCT NAME	MODEL NO. / CODE
AMMONIA			
Chemetrics	CHEMets Kit	Ammonia	K-1510
Hach	Test Kit	Ammonia, Mid Range	NI-8
Hanna Instruments	Checker	Ammonia Low Range	HI700
NITRATE			
Chemetrics	CHEMets Kit	Nitrate	K-6904
Hach	Test Kit	Nitrate	NI-14
LaMotte	Test Kit	Nitrate Nitrogen Low Range Comparator	3615-01
PHOSPHORUS			
Chemetrics	CHEMets Kit	Phosphate	K-8510
Hach	Test Kit	Phosphate, Ortho- / Meta-	PO-23
Hanna Instruments	Checker	Phosphate Low Range	HI713
Hanna Instruments	Checker	Phosphorus High Range	HI706
LaMotte	Test Kit	Low Range Phosphate	3121-02
LaMotte	Insta-Test Analytic	Phosphate Test Strips, Low Range	3021-G-ENV
TURBIDITY			
LaMotte	Test Kit	Turbidity	7519-01

2.1 Study Design

For each parameter, two field kits were purchased. Three volunteers were sought to evaluate each kit. Volunteers for the study included undergraduate and graduate students, university staff, and watershed watch volunteers. All study participants were provided copies of the manufacturer's instructions for each kit and one short walkthrough of these instructions. Since the objective was to evaluate how well the manufacturer's instructions alone assisted the volunteers, more thorough training was not provided. Volunteers were instructed to estimate values between two concentration intervals if they deemed appropriate.

The Kentucky Geological Survey (KGS) prepared known standard solutions of each concentration using deionized reagent grade water spiked with the parameter of interest. Sufficient volume was prepared to allow all analyses to use the same solutions. Typically, the measurements made for each parameter was analyzed in two four-hour sessions, one for repeated sample analysis of a single concentration to evaluate precision, and one to evaluate a range of concentrations to evaluate bias and accuracy over the range of collected in prior WWKY studies.

2.1.1 Repeated Concentration

Each of the three volunteers performed four replicates using each kit (two per manufacturer) for concentrations between the detection limit and an expected action limit. A total of 24 analyses were performed per kit manufacturer, except where reagents limited the amount of testing that could be performed. Because many field kits are non-continuous, effort was made to test a concentration near an incremental step value common across all kits. The kit replicate results were then compared to four laboratory tests for that parameter run from the same concentration.

2.1.2 Range of Concentrations

Each of the three volunteers performed five tests using each kit (two per manufacturer) on a range of values likely to be encountered in the field. A total of 30 analyses were performed per kit manufacturer, except where reagents limited the amount of testing that could be performed. The range of testing values was determined by querying the Watershed Watch Data Portal. For field kits that had incremental steps in the results, effort was made to find a concentration near those values across all kits. Results were compared to ten laboratory tests (two at each concentration) and two blanks for that parameter.

The concentrations prepared for each parameter are shown below in Table 2. All concentrations are expressed in terms of the molecular weight of the nutrient atom rather than the entire molecule (i.e. as NO_3-N rather than NO_3).

Table 2: Known Standard Solution Concentrations for Testing

Evaluation	Ammonia (mg/L NH_3-N)	Nitrate (mg/L as NO_3-N)	Orthophosphorus* (mg/L PO_4-P)	Turbidity (JTU / NTU)
Repeated Concentration (4 replicates per volunteer / kit)	0.6	1	0.5	20
Range of Concentrations (5 concentrations, 1 replicate per volunteer / kit)	0.1	0.2	0.05	10
	0.25	0.6	0.2	20
	0.5	1	0.5	50
	1.5	2.5	1	100
	3	5	4	200

**Total phosphorous kits were compared against ortho-phosphorous knowns since ortho-phosphorous is a subset of total phosphorous.*

In addition to these tests, each volunteer was asked to evaluate the ease of use (1-10 scale) of each kit and to provide any feedback about their experience. They also recorded their analysis time to the nearest minute during their first, middle, and last tests to provide an indication of expected times to complete the testing in the field.

2.2 Kit Descriptions and Testing Methods by Parameter

2.2.1 Ammonia

For ammonia, all three kits employed a variation of the direct nesslerization method to quantify the amount of ammonia in the sample [5] . The nessler reagent is a solution of potassium tetraiodomercurate(II), which reacts with ammonia to form a yellow tint in proportion to the amount of ammonia present. Because each method utilizes the nessler reagent, hazardous waste is generated by all methods and proper disposal must be considered. All methods are reported as ppm NH3-N. An overview of the field kit cost, size and sample aliquot requirements is provided in Table 3.

Table 3: Ammonia Field Kit Overview

Manufacturer and Model No.	Range (mg/L NH_3-N)	Initial Kit Cost	Reagent Refill Costs	# Available Tests	Cost per Test	Aliquot Volume (mL)	Size (cm)		
							L	W	H
Chemetrics Ammonia K-1510	Low Range: 0, 0.1 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 High Range: 1 – 10 (by 1)	$65.72	$29.21	30	$0.97	25	21.5	17.5	6.2
Hach Low Range Ammonia NI-8	0 - 3.0 (by 0.2)	$87.59	$87.59	100	$0.88	15	23.0	17.5	15.5
Hanna Checker Low Range Ammonia HI700	0.01 - 3.00	$49.00	$14.00	25	$0.56	10	19.3	11.9	6.4

For the Chemetrics Ammonia kit (K-1510), a stabilizing reagent is added to a sample via dropper, which is then drawn into a vacuum-sealed self-filling ampoule with precisely premeasured volumes of the nessler reagent. This ampoule is then compared visually against comparator ampoules of known concentrations.

The Hanna Checker Low Range Ammonia kit (HI700) utilizes a one frequency handheld colorimeter to quantify the color change. A stabilizing solution and nessler reagent are added via droppers and the color development is compared on a manufacturer calibrated instrument. A numerical readout of the result is recorded.

The Hach Mid-Range test kit (NI-8) utilizes a similar process of adding reagents by dropper but visually compares an untreated test tube to a treated test tube using a color disc and a color comparator box.

Laboratory results were analyzed by KGS staff according to the salicylate TNTplus Method 10205 (TNT830) [6] for determination of ammonia-nitrogen using a Hach DR3900 benchtop spectrophotometer.

2.2.2 Nitrate

For the nitrate nitrogen parameter, all three kits employed a variant of the cadmium reduction method to detect the presence of nitrate in the sample [7]. The method produces a pink-orange tint in proportion to the amount of nitrate present. All kits generate hazardous wastes methods and proper disposal must be considered. An overview of the field kit cost, size and sample aliquot requirements is provided in Table 4.

Table 4: Nitrate Field Kit Overview

Manufacturer and Model No.	Range (mg/L)	Initial Kit Cost	Reagent Refill Costs	# Available Tests	Cost per Test	Aliquot Volume (mL)	Size (cm)		
							L	W	H
Chemetrics Nitrate K-6904	0, 0.4, 0.7, 1.0, 1.4, 1.8, 2.5, 3.5, 4.5	$76.70	$76.70	30	$2.56	25	21.5	17.5	6.2
Hach Nitrate NI-14	0 – 10 (by 0.5)	$88.75	$88.75	50	$1.78	10	21.0	6.5	15.0
LaMotte Nitrate Nitrogen Low Range Comparator 3615-01	0, 0.2, 0.4, 0.6, 0.8, 1	$125.15	$125.15	40	$3.13	15	17.0	12.0	14.8

For the Chemetrics Nitrate kit (K-6904), a cadmium reagent powder packet is added to a sample to precipitate out and remove cadmium. An aliquot of the treated sample is then drawn into a vacuum-sealed self-filling ampoule with precisely premeasured reagent to produce a pink color. This ampoule is then compared visually against comparator ampoules of known concentrations.

The Hach Nitrate Low Range kit utilizes a two-step procedure which includes addition of one reagent powder packet to precipitate out and remove cadmium particles, and then a second reagent powder packet is added to develop a pink color. The color is then visually compared against an untreated, control test tube using a color disc and a color comparator box to determine the nitrate-nitrogen concentration.

The LaMotte Nitrate Nitrogen Low Range Comparator (3615-01) kit utilizes a mixed acid reagent solution followed by a nitrate-reducing reagent powder packet to generate color. A sliding color range comparator is utilized to visually compare the treated sample to an untreated one.

Laboratory results were analyzed by KGS staff according to ion chromatography by EPA method 300.0 [8] using a Dionex ICS3000 ion chromatograph system.

2.2.3 Phosphorus

One kit measuring total phosphorus was evaluated during this study using an adaptation of the heteropoly molybdenum blue method [9]. All other kits measured orthophosphate with most utilizing an adaptation of the ascorbic acid method [10] to quantify the amount of orthophosphate in the sample. One kit utilized the stannous chloride method [11]. The information provided with the LaMotte test strip kit did not indicate the chemical method utilized to generate the results. In all cases, the reagents react with the orthophosphate to produce a blue color in proportion to the amount of ammonia present. Except for the LaMotte test strips, all methods generate hazardous wastes. An overview of the field kit

cost, size and sample aliquot requirements is provided in Table 5. Most orthophosphate kits generated results reported as mg/L PO_4^{-3}. These results were all converted to mg/L PO_4-P to provide some comparability between total phosphorus and orthophosphate methods.

Table 5: Phosphorus and Orthophosphate Field Kit Overview

Manufacturer and Model No.	Range (mg/L PO_4)*	Initial Kit Cost	Reagent Refill Costs	# Available Tests	Cost per Test	Aliquot Volume (mL)	Size (cm)		
							L	W	H
Chemetrics Phosphate K-8510	LR: 0, 0.1 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 HR: 0 – 10 (by 1)	$64.27	$26.18	30	$0.87	25	19.0	14.6	6.3
Hach Orthophosphate PO-23	0 – 4.0 (by 0.2)	$142.00	$142.00	100	$1.42	10	36.0	19.5	18.0
Hanna Phosphate Low Range HI713	0.01 - 2.50	$49.00	$8.00	25	$0.32	10	19.3	11.9	6.4
Hanna Phosphorus High Range HI706	0.0 - 15.0 (mg/L PO_4-P)*	$49.00	$8.00	40	$0.20	10	19.3	11.9	6.4
LaMotte Low Range Phosphate 3121-02	0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2.0	$91.25	$91.25	50	$1.83	10	16.7	11.0	14.7
LaMotte Phosphate Test Strips, Low Range 3021-G-ENV	0, 0.1, 0.2, 0.3, 0.5, 1, 2.5	$31.62	$31.62	25	$1.26	10	21.3	10.9	4.8

**All kits with the exception of the Hanna Phosphorus High Range measure orthophosphate as PO_4^{-3}. Total phosphorous kits were compared against ortho-phosphorous knowns since ortho-phosphorous is a subset of total phosphorous.*

For the Chemetrics Phosphate Chemets Kit (K-8510), an activator solution is added to the sample to acidify the sample via dropper. An aliquot of the treated sample is then drawn into a vacuum-sealed self-filling ampoule where orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid, which is then reduced by

stannous chloride to the intensely colored molybdenum blue. The results are compared visually against either low range or high range color comparator standards.

The Hach Orthophosphate Kit (PO-23) contains the reagents and supplies to measure orthophosphate as well as metaphosphate. The kit uses the ascorbic acid method via the addition of one reagent powder packet to generate color. The color is then visually compared against an untreated control test tube using a color disc and a color comparator box to determine the orthophosphate concentration. Results must be divided by ten to get results in mg/L PO_4^{-3}.

The Hanna Checker Phosphate Low Range kit (HI713) utilizes a one frequency handheld colorimeter to quantify the color change. A reagent powder packet is added, and the color development is measured on a manufacturer-calibrated instrument. A numerical readout of the result is recorded.

The Hanna Checker Phosphorus High Range kit (HI706) uses an adaptation of the heteropoly molybdenum blue method [9] using a one frequency handheld colorimeter to quantify the color change. One reagent containing sulfuric acid is added by dropper followed by a reagent powder packet. Color development is measured on a manufacturer-calibrated instrument with results in mg/L P. A numerical readout of the result is recorded.

The LaMotte Orthophosphate Kit (3121-02) uses the ascorbic acid method through the addition of a phosphate acid reagent solution followed by a measured spoonful of phosphate reducing reagent to generate color in a sample. A sliding color range comparator is utilized to visually compare the treated sample to an untreated one.

The LaMotte Insta-Test Analytic Low Range Phosphate Test Strips – Environmental (3021-G-ENV) is a recently developed product by LaMotte and was provided for free evaluation upon the purchase of the orthophosphate kit. Each test strip contains reagent pads, which are immersed in a test tube filled with sample and then visually compared to a color chart on the side of the test strip container. Results are reported as ppb PO_4^{-3}.

Laboratory results were analyzed by KGS staff according to the ascorbic acid TNTplus Method 10209/10210 (TNT843) for determination of total and reactive phosphorus (orthophosphate) [12] using a Hach DR3900 benchtop spectrophotometer. Results are reported as mg/L P.

2.2.4 Turbidity

One turbidity test kit, the LaMotte Turbidity kit (7519-01), was evaluated during this study. This kit compares the turbidity of the sample with the same volume of turbidity-free sample containing a measured amount of standardized turbidity reagent. The readings are made by looking down through the column of liquid at a black dot. Drops of the

turbidity standard are added until a similar amount of “cloudiness” is produced between the two samples. A conversion table is used to convert the amount of standard added to a result in Jackson Turbidity Units (JTU). This method generates a hazardous waste so proper disposal methods must be considered. An overview of the field kit cost, size and sample aliquot requirements is provided in Table 5.

Laboratory results were analyzed by KGS staff according to the Hach method 8195 [13], Determination of Turbidity by Nephelometry, using a Hach 2100N Turbidimeter. Results are reported in Nephelometric Turbidity Units (NTU).

Table 6: Turbidity Field Kit Overview

Manufacturer and Model No.	Range (JTU)	Initial Kit Cost	Reagent Refill Costs	# Available Tests	Cost per Test	Aliquot Volume (mL)	Size (cm)		
							L	W	H
LaMotte Turbidity 7519-01	0- 50 (by 5s), 60, 70, 75, 80, 90, 100, 150, 200	$80.15	$13.50	35	$0.39	50	16.7	11.0	14.7

3.0 Results

The testing for this study took place on nine (9) days between October and December 2018. Eighteen (18) volunteers performed tests, and a total of 634 tests were completed. Volunteers were grouped according to experience with field kit use. The “experienced” group included seven volunteers that had between four and 33 years of experience with field-testing. The “moderate” group included five volunteers with either one to two years’ experience with field sampling or graduate students with theses including field sampling. The “new” group included five undergraduate and graduate students from Civil Engineering who had not previously utilized field sampling kits before.

Results were compiled into a database and analyzed for percent recovery, percent difference, and linear regression best fits by parameter.

3.1 Ammonia

Four volunteers tested water samples for ammonia using the Chemetrics (K-1510), Hach (NI-8), and Hanna (HI700) kits. A total of 158 tests were run for ammonia, 54 for the Hach and Hanna kits and 50 for the Hanna kit due to the volunteer’s time constraints shortening available time. The KGS laboratory analyzed 16 samples for comparison.

Figure 1 depicts the percent recovery for each kit for all the samples analyzed (replicate analysis and linear regression). Because the Chemetrics and Hach kits utilize discrete (non-continuous) concentration values, standard deviations are of limited value for these kits because the value is largely dependent upon how close the concentration value was to the discrete intervals within the kit’s range.

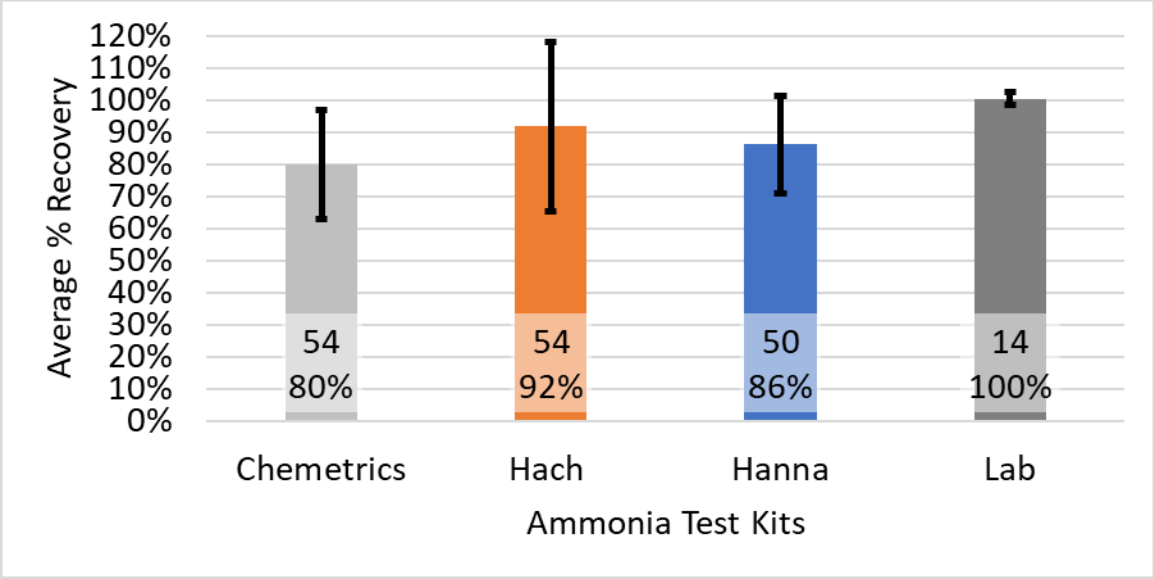


Figure 1: Average Percent Recovery for Each Kit of the Ammonia Parameter
Error bars are present to represent standard deviation. Labels indicate the count and percent recovery.

While the Hach unit has the best overall accuracy, it is the least precise unit. The Chemetrics has the lowest recovery, however one experienced volunteer did not make use of the second higher range (1-10 mg/L) color comparator for the Chemetrics unit. This oversight could also be expected to occur with field volunteers, so these results were retained. However, excluding these results from the dataset does not increase its percent recovery above the Hach or Hanna kits. The Hanna kit had the lowest standard deviation of the field kits (15%) and offers continuous, rather than discrete, results over the range.

Figure 2 depicts the linear regression data (30 analyses per kit) gathered by volunteers for each of the kits as compared to the laboratory results. Table 7 depicts the R^2 values and the slope and intercepts for each trend line depicted in Figure 2. Results indicate that the Hanna kit had a slight negative bias but the best overall correlation to the known concentration (R^2 = 0.9996). The Hach kit did not show bias (slope = 1.00) but had a slightly lower correlation. The Chemetrics kit did not perform as well as the other two units, even when removing the results effected by not utilizing the secondary color comparator.

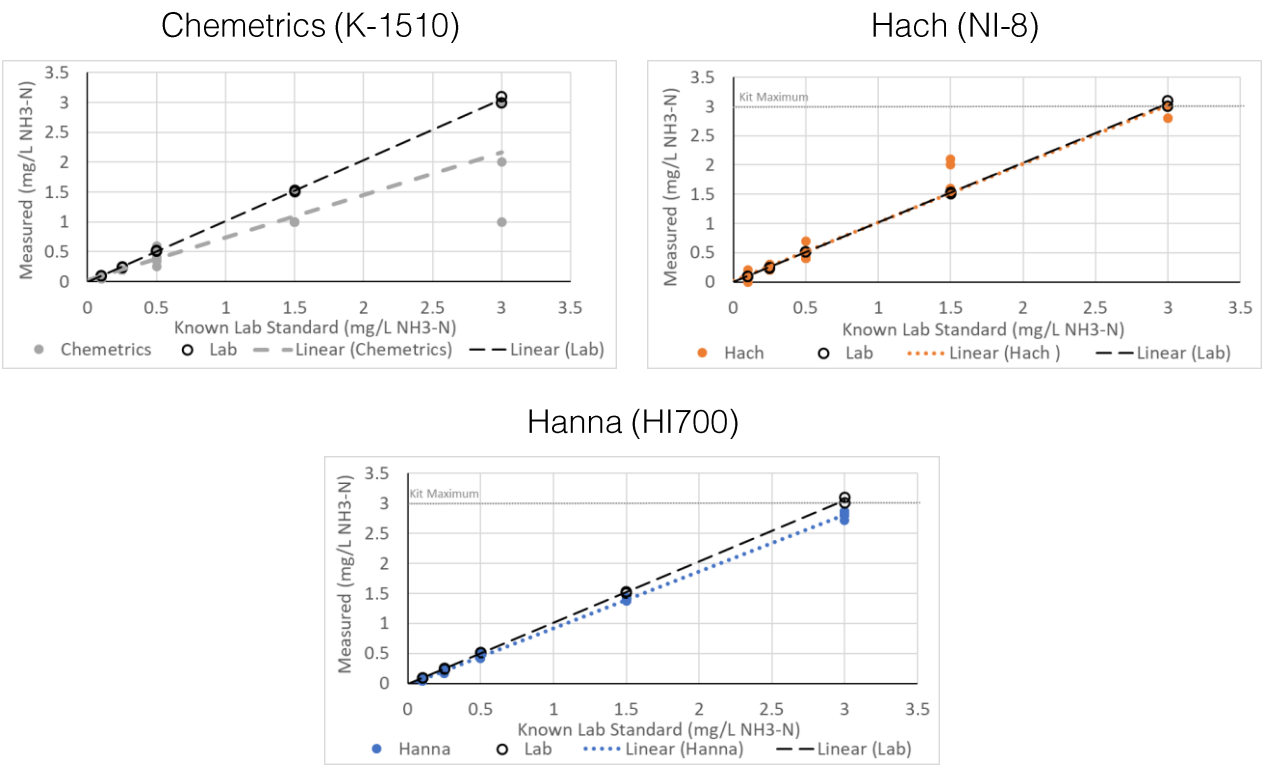


Figure 2: Linear Regression Data for Ammonia Kits

Table 7: Line of Best Fit and R^2 Values for the Ammonia Kits and Laboratory

Kit	n	Volunteers	Slope (Intercept)	R^2
Chemetrics	30	3	0.71 (0.02)*	0.7765
Hach	30	3	1.00 (0.02)	0.9772
Hanna	30	3	0.94 (-0.02)	0.9987
Lab	12	-	1.02 (0.00)	0.9996

**Exclusion of four data points in which higher comparator was not used results in slope of 0.90, intercept of -0.05 and R^2 of 0.9479.*

Table 8 depicts the average percent difference between the results obtained for each volunteer and the known value. These results show that each volunteer operated the kits with similar levels of proficiency.

Table 8: Percent Difference for Ammonia Parameter by Volunteer and Kit

Volunteer	N	Chemetrics	Hach	Hanna	Overall % Per Volunteer
Experienced #1	50	16% (14)	14% (18)	12% (18)	14%
Experienced #2	54	37% (18)	21% (18)	19% (18)	25%
Experienced #3	24	19% (8)	25% (8)	13% (8)	19%
Moderate #4	30	25% (10)	31% (10)	25% (10)	27%
Overall % Per Kit		25%	21%	17%	21%

Table 9 summarizes the average time for the volunteers to complete the testing and their evaluation of the ease of use on a scale from 1-10 with one being "hard" and ten being "easy." The Chemetrics and Hach kits both took about four minutes to run, almost half the time of the Hanna kit, but the Hanna kit was rated as the easiest to use. For the Chemetric kit, one volunteer recorded that the ampoules had a "different hue than the comparators," and another that the "tips did not always break evenly."

Table 9: Ammonia Kit Analysis Time and Ease of Use Rating

	Chemetrics	Hach	Hanna Checker
Average Time (minutes)	4	4	7
Ease of Use Rating	8	8	10
Volunteers (n)	4	4	4

Note: Ease of use rated from 1 as hard and 10 as easy.

It should also be noted that reagents within the self-filling ampoules of the Chemetrics kit arrived with an expiration date of less than six months as compared to expiration dates of 4-5 years later for the kits.

3.2 Nitrate

Due to the average analysis time for these kits, ten volunteers evaluated nitrate using the Chemetrics (K-6904), Hach (NI-14), and LaMotte (3615-01) kits over multiple days. A total of 139 tests were run; 55 for Chemetrics, 45 for Hach, and 39 for LaMotte. The KGS laboratory analyzed 16 samples for comparison.

Despite the number of volunteers participating, the length of time required to complete the analyses did not allow for all the tests to be performed before the known concentrations prepared by the laboratory expired. At least 4 replicates were performed

for each kit for each concentration in the linear analysis, and the known concentration was replicated at least 16 times for each kit. Results identified by volunteers as greater than the maximum testing limit were excluded from the data analysis.

Figure 3 depicts the percent recovery for each kit for all the samples analyzed (replicate analysis and linear regression). Because each of the kits utilize discrete (non-continuous) concentration values, standard deviations are of limited value for these kits because the value is largely dependent upon how close the concentration value was to the discrete intervals within the kit's range. The Chemetrics kit had recovery above 100%, indicating a high bias as well as the largest standard deviation. The LaMotte kit had a slightly higher recovery but over a smaller range (1.0 mg/L NO_3-N maximum) as compared to the Hach kit, while the standard deviations were equivalent.

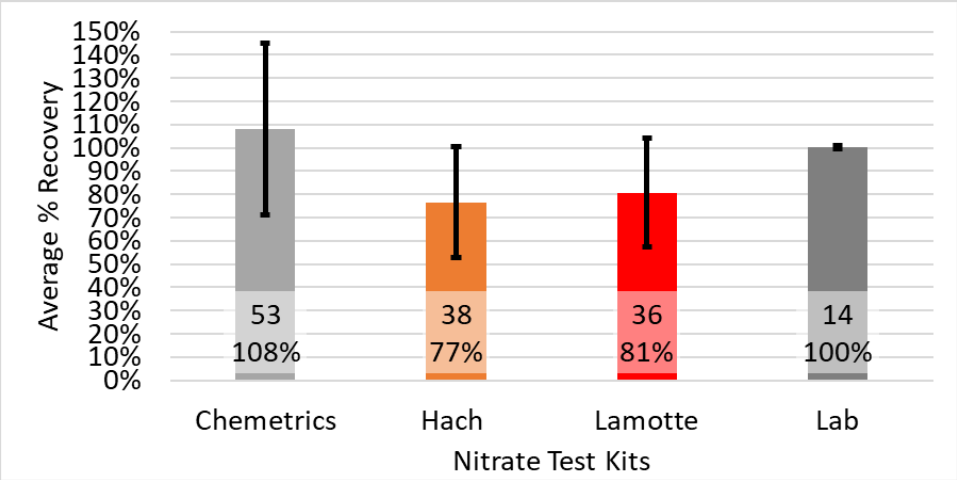


Figure 3: Average Percent Recovery for Each Kit of the Nitrate Parameter
Error bars are present to represent standard deviation. Labels indicate the count and percent recovery.

Figure 4 depicts the linear regression data gathered by the volunteers for the Chemetrics (31 analyses), Hach (25 analyses), and LaMotte (23 analyses) kits, respectively. Results greater than the kit maximum collected by the volunteers were excluded from the analysis. Table 10 depicts the R^2 values and the slope and intercepts for each trend line depicted in Figure 4.

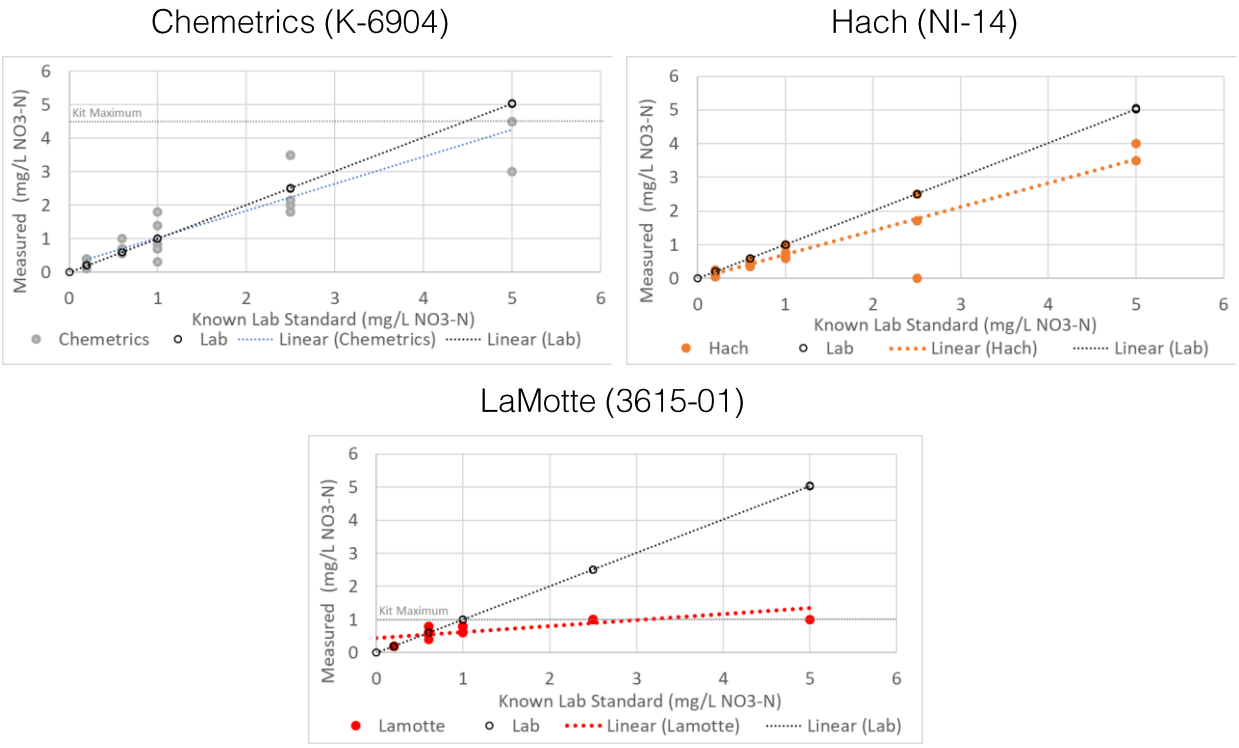


Figure 4: Linear Regression Data for Nitrate Kits

Table 10: Lines of Best Fit and R^2 Values for the Nitrate Kits and Laboratory

Kit	n	Volunteers	Slope (Intercept)	R^2
Chemetrics	29	5	0.81 (0.22)	0.8687
Hach	20	4	0.71 (0.00)	0.8322
LaMotte*	20	5	0.18 (0.45)	0.5351
Lab	12	-	1.01 (0.00)	1.0000

*Exclusion of all results from known concentrations above 1.0 mg/L maximum results in slope of 0.68, intercept of 0.13 and R^2 of 0.7474.

Despite a low bias for higher concentrations and a high bias for lower concentrations (slope 0.81, intercept 0.22), the Chemetrics kit had the best overall correlation to the known concentration (R^2 = 0.8687). The Hach kit had a lower bias on high samples (slope 0.71) but was more accurate at low ranges. Although the Hach unit allows for a maximum testing limit of 10 mg/L NO_3-N with dilution, five of the analyses were reported at >1 mg/L NO_3-N because analysts did not perform a reanalysis of the sample using a dilution due to the time required. These results were excluded from data analysis.

The LaMotte unit has a maximum of 1.0 mg/L NO_3-N. However concentrations above 1.0 mg/L NO_3-N were not found by volunteers to be greater than the test maximum. All volunteers indicated that the 2.5 mg/L NO_3-N known was 1.0 mg/L NO_3-N, and one of

the five replicates of the 5.00 mg/L NO_3-N concentration was measured at 1.0 mg/L NO_3-N. Therefore the results show no correlation with the known values. When excluding all results above 1.0 mg/L NO_3-N, the kit still performs with less precision and accuracy than the other kits.

Table 11 depicts the average percent difference between the results obtained for each volunteer and kit. There is no trend between the volunteer experience and precision of the results.

Table 11: Percent Differences for Nitrate Parameter Divided by Volunteer and Kit

Volunteer	N*	Chemetrics	Hach	LaMotte	Overall % Per Volunteer
Experienced #1	3			15% (3)	15%
Experienced #3	18		51% (7*)	40% (9)	45%
Experienced #12	12	4% (4)		21% (8)	12%
Experienced #13	8	0% (4)	49% (4)		24%
Moderate #15	10	20% (5)	67% (4*)		43%
Moderate #17	15	18% (5)	20% (3*)	27% (4*)	22%
New #11	27	23% (8)	38% (18)		30%
New #14	16	9% (8)	12% (4)	17% (4)	12%
New #16	12	40% (8*)		44% (4*)	42%
New #18	15	36% (10)		34% (4*)	35%
Overall % Per Kit		22%	31%	29%	27%

*Total count includes greater than values while counts by test method exclude greater than values.

Table 12 summarizes the average time for the volunteers to complete the testing and their evaluation of the ease of use on a scale from 1-10 with one being “hard” and ten being “easy.” All kits took 18-19 minutes to analyze, which is a considerable amount of time for a volunteer to spend on one parameter.

Table 12: Nitrate Kit Analysis Time and Ease of Use Rating

	Chemetrics	Hach	LaMotte
Average Time (minutes)	18	19	18
Ease of Use Rating	8	6	7
Volunteers (n)	8	6	7

Note: Ease of use rated from 1 as hard and 10 as easy.

The Chemetrics kit was rated the easiest to use, with the Hach kit the hardest. Yet based on volunteer feedback, easiest was evaluated by some in terms of simplicity and not in terms of time requirements or desirability. Despite being rated as the easiest kit, one volunteer noted of the Chemetrics kit, "it is difficult to shake for three minutes. Time consuming test for volunteers." Another volunteer noted that the Chemetrics ampoule broke unevenly causing the ampoule to only half fill. A third volunteer said of the Chemetrics kit, "I like this kit - easy to use." Thus, the views are mixed. For the Hach unit, one volunteer commented that "the hue of red is not the same hue" as the comparator and that there was "too much shaking." Similarly, the LaMotte kit was thought to be "too hard to differentiate colors" by one volunteer. These statements represent are all the comments provided on these kits. Thus, technical limitations (color differentiation, ampoule breaking) and time and labor requirements for the nitrate kits evaluated may hinder their use in some volunteer contexts.

3.3 Phosphorous

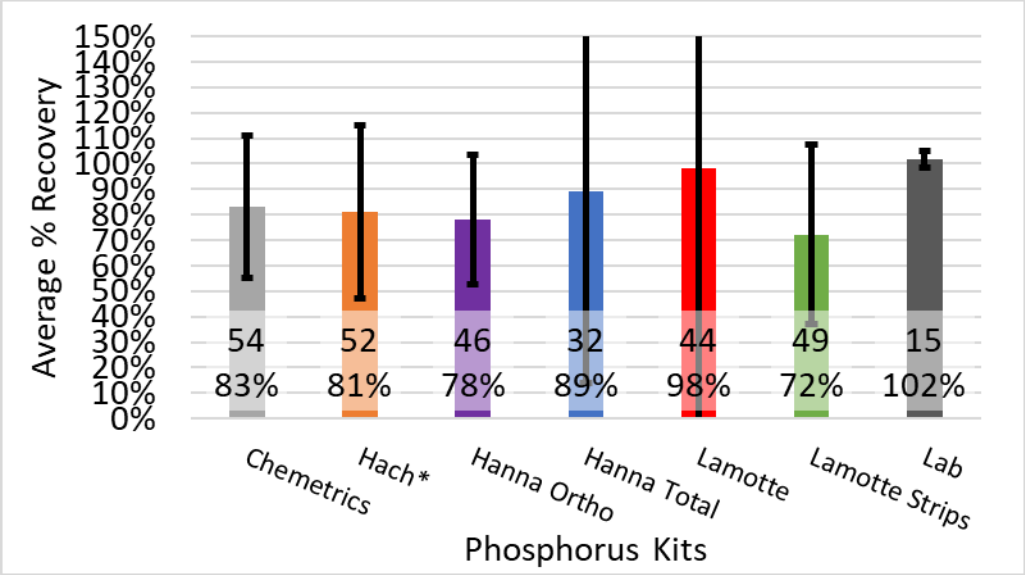
Eleven volunteers tested water samples for phosphorus over multiple days using the Chemetrics (K-8510), Hach (PO-23), Hanna phosphate (ortho) (HI713), Hanna phosphorus (total) (HI706), LaMotte (3121-02), and LaMotte strips (3021-G-ENV) kits. To distinguish between the two Hanna kits, the HI713 will be called Hanna ortho and the HI706 called Hanna total. While all kits except the Hanna total unit generate measurements in mg/L PO_4 (orthophosphate), all results were converted to mg/L PO_4-P for comparability.

A total of 293 tests were analyzed: 54 for Hach, 54 for Chemetrics, 53 for Hanna ortho, 39 for Hanna total, 44 for LaMotte, and 49 for LaMotte Strips. The KGS laboratory analyzed 29 samples for comparison. For the Hanna total kit, the number of analyses was hindered by frequent retests required due to kit error readings. For the LaMotte kits, the number of tests performed was limited by the volume of reagent powder in the kits. Delays due to shipment of extra reagents for the Hanna ortho kits caused these kits to be analyzed against a separate set of prepared standards, which were also tested separately by the KGS laboratory to confirm their concentrations.

For data analysis purposes, results greater than the kits' maximum range were excluded from the analysis. For the Hach kit, one experienced volunteer was observed to have skipped a step in the instructions to divide the results by 10, affecting 12 results. While this error could be expected to be replicated by other field samplers, these results were corrected for data analysis.

Figure 3 depicts the percent recovery for each kit for all the samples analyzed (replicate analysis and linear regression). Because each of the kits utilize discrete (non-continuous) concentration values, standard deviations are of limited value for these kits because the value is largely dependent upon how close the concentration value was to

the discrete intervals within the kit's range. Only the two Hanna kits provided continuous results.



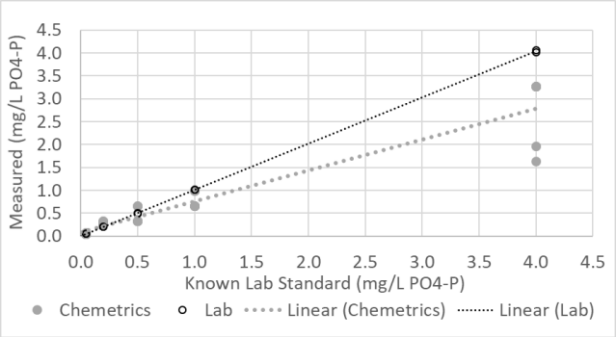
**Hach kit recoveries are corrected values based on observation that one volunteer did not divide the results by ten. Uncorrected recovery is 266% +/- 380%.*

Figure 5: Average Percent Recovery for Each Kit of the Phosphorus Parameter
Error bars are present to represent standard deviation. Labels indicate the count and percent recovery.

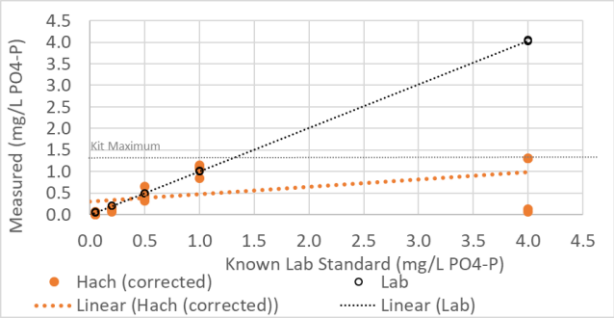
Based on percent recovery, the Chemetrics and Hanna ortho kits had the best overall performance with 83% +/- 28% and 78% +/- 26% respectively. It should be noted that both kits do not perform over the entire testing range for phosphorus results in Kentucky, with Chemetrics at a maximum of 3.26 mg/L PO_4-P and Hanna ortho at 0.82 mg/L PO_4-P, after conversion of units. When correcting for the volunteer calculation error, the Hach kits performed with similar recovery (80%) but slightly greater standard deviation (34%). Because the Hanna total kit was only sensitive to 0.1 mg/L PO_4-P, the standard deviation on the results was high (75%) despite the good average recovery (96%). As will be better demonstrated in the linear regressions below, both LaMotte kits were unreliable.

Figure 6 depicts the linear regression data for each kit. Table 13 depicts the R^2 values and the slope and intercepts for each trend line depicted in Figure 6. As previously mentioned, the Hanna kits frequently returned error messages indicating inverted sample vials or under range results. Seven of the recorded results for the Hanna total unit indicated "errors" with some re-analyses of these errors generating results and were thus not recorded. Because of the frequent kit errors, the number of reagents packets was only enough to perform two to three analyses per known concentration for the linear regression analysis. Depletion of the LaMotte kit reagent powder only allowed for four replicates to be performed for each concentration.

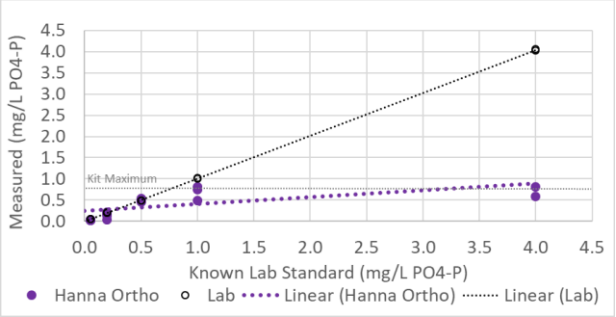
Chemetrics (K-8510)



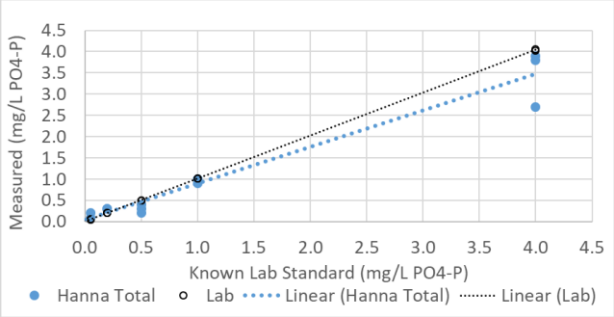
Hach* (PO-23)



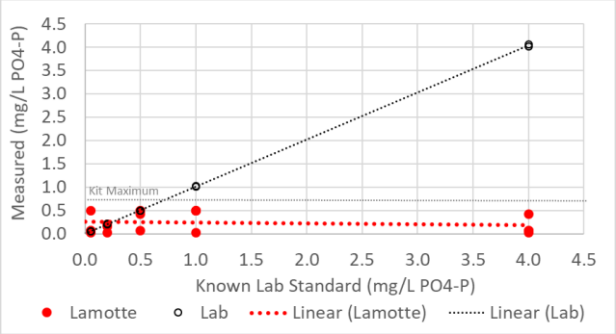
Hanna Ortho (HI713)



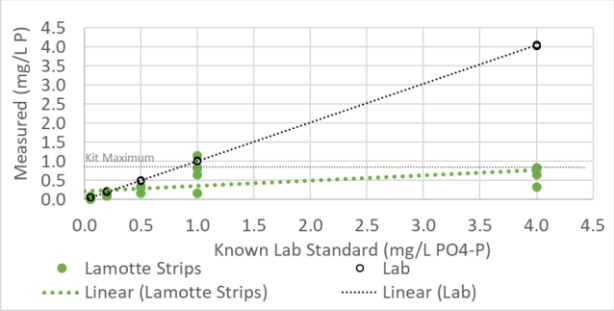
Hanna Total (HI706)



LaMotte (3121-02)



LaMotte Strips (3021-G-ENV)



**Hach kit recoveries are corrected values based on observation that one volunteer did not divide the results by ten.*

Figure 6: Linear Regression Data for Phosphorus Kits

Table 13: Lines of Best Fit and R^2 Values for Phosphorus Kits and Laboratory

Kit	n	Volunteers	Slope (Intercept)	R^2	Kit Maximum (mg/L PO_4-P)
Chemetrics	30	3	0.67 (0.08)	0.8990	3.26
Hach*	27	4	0.17 (0.31)*	0.2499*	1.3
Hanna ortho	23	5	0.16 (0.24)	0.3901	0.82
Hanna total	13	2	0.86 (0.04)	0.9541	15.0
LaMotte	20	3	-0.02 (0.26)	0.0184	0.65
LaMotte Strips	25	3	0.14 (0.21)	0.3864	0.82
Lab	10	-	1.01 (0.00)	0.9990	-

**Hach kit recoveries are corrected values based on observation that one volunteer did not divide the results by 10. Note that results above 1 mg/L PO_4-P did not perform well. If all results from the 4.0 mg/L concentration are excluded, the slope is 1.01, intercept -0.02, and R^2 0.9997.*

Only the Hanna total (R^2 = 0.9541) and Chemetrics (R^2 = 0.8990) had good correlation with the known concentrations, though the Chemetrics unit had a lower bias at the upper ranges (0.67 slope) than the Hanna total kit (0.86 slope). The corrected Hach data showed an almost perfect correlation at concentrations below 1.0 mg/L (slope is 1.01, intercept -0.02, and R^2 0.9997). However, results in the high range were often reported as greater than the kit's range and therefore the kits overall performance is poor (R^2 = 0.2499) when considering these results. Similarly, because high concentrations were not seen as above the Hanna ortho kit's testing range, the correlation is skewed for this kit at high levels, although it has better correlation at concentrations less than 1.0 mg/L. For the LaMotte kit, the high concentrations often appeared to have a lighter hue and were therefore mistaken as having a lower concentration. The LaMotte strips were extremely variable in performance.

Table 14 depicts the average percent difference between the results obtained for each volunteer and kit. No trends are evident between volunteer experience and improved performance with kit usage.

Table 14: Percent Differences for Phosphorus by Volunteer and Kit

Volunteer	n	Chemetrics	Hach	Hanna ortho	Hanna total	LaMotte	LaMotte Strips	Overall % Per Volunteer
Experienced #2	16	-	-	54% (16)	-	-	-	54%
Experienced #3	53	48% (12)	39% (9)	79% (12)	23% (4)	35% (12)	28% (4)	47%
Experienced #8	28	24% (10)	56% (10)	-	-	-	65% (8)	48%
Experienced #10	12	-	24% (4)	-	155% (4)	-	29% (4)	69%
Experienced #12	10	-	99% (10)	-	-	-	-	99%
Moderate #4	39	89% (8)	165% (8)	-	14% (7)	100% (8)	69% (8)	89%
Moderate #5	30	50% (10)	80% (5)	-	42% (5)	59% (5)	95% (5)	64%
Moderate #7	30	-	-	-	36% (10)	114% (10)	68% (10)	73%
Moderate #9	7	-	-	50% (7)	-	-	-	50%
New #6	14	-	-	68% (14)	-	-	-	68%
New #11	38	42% (8)	6% (8)	-	189% (6)	6% (8)	27% (8)	47%
Overall % Per Kit		33%	74%	63%	67%	47%	55%	63%

Table 15 summarizes the average time for the volunteers to complete the testing and their evaluation of the ease of use on a scale from 1-10 with one being "hard" and ten being "easy." The Chemetrics and Hach kits both took about four minutes to run, almost half the time of the Hanna kit, but the Hanna kit was rated as the easiest to use. For the Chemetric kit, one volunteer recorded that the ampoules had a "different hue than the comparators," and another that the "tips did not always break evenly."

Table 15: Phosphorus Kit Analysis Time and Ease of Use Rating

	Chemetrics	Hach	Hanna ortho	Hanna total	LaMotte	LaMotte Strips
Average Time (minutes)	5	4	8	9	9	3
Ease of Use Rating	8	7	9	6	7	8
Volunteers (n)	5	7	4	6	5	7

Note: Ease of use rated from 1 as hard and 10 as easy.

The ease of use ratings ranged from the Hanna ortho as easiest (9) to the Hanna total as hardest (6). Volunteer comments help to elaborate on these ratings. For the Chemetrics kit, one volunteer thought there was an "odd color match", another thought the color was "clear", and a third volunteer had some trouble "making sure all the ampoule tips are cleaned out of the sample cup." For the Hach kits, comments included "fast but color hard to distinguish – bulky box with unnecessary reagents" and "kit is

easy to use but the color variation is too tight." One volunteer found "the reagent packets are a little difficult to pour into the vial" for the Hanna total unit, and another thought the Hanna ortho kit was "very sensitive to glassware cleanliness." The LaMotte kit received two comments to the effect that the higher concentrations were hard to match because the vial appears dark, but the color best matched low concentrations. For the LaMotte strips comments included "I thought the readings were somewhat subjective" and "hard to know where to compare because the outside looks different than the bottom center."

3.4 Turbidity

Three volunteers tested 40 water samples for turbidity using the LaMotte kit (7519-01). The number of tests that could be analyzed was limited by the volume of reagents provided. The KGS laboratory analyzed 14 samples for comparison.

The average recovery for the kit was 124% +/- 47% as compared to 106%+/-2% for the laboratory. Figure 7 depicts the linear regression data from the 24 samples analyzed with the LaMotte kit for this purpose while

Table 7 16 shows the R^2 values and the slope and intercepts for each trend line. The results show a wide range of results obtained at each of the concentrations tested with a low bias with higher turbidity.

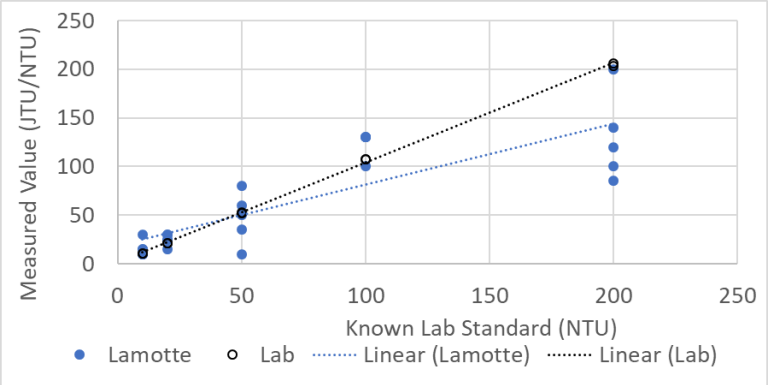


Figure 7: Linear Regression Data for LaMotte Turbidity Kit

Table 16: Lines of Best Fit and R2 Values for the Turbidity Kit and Laboratory

Kit	n	Volunteers	Slope (Intercept)	R^2
LaMotte	24	3	0.62 (18.8)	0.6960
Lab	10	-	1.02 (1.46)	0.9993

Table 17 depicts the average percent difference for each volunteer for the turbidity kit. Also, in this table is an overall percent difference for the turbidity kit. As with other kits, there is no correlation between the volunteer experience and the percent difference in the results.

Table 17: Percent Differences for Turbidity Parameter by Volunteer

Volunteer	n	LaMotte
Experienced #2	14	38%
Moderate #9	8	29%
New #6	18	52%
Overall % Diff Per Kit		35%

Time averages and ease of use rating were also gathered for the LaMotte turbidity kit. The average time per test was three minutes and the average ease of use rating out of ten was seven.

4.0 Discussion

As a laboratory study of field sampling kits, this provides a preliminary evaluation of kit performance which should be verified by field trials to confirm performance with unknown samples of variable ranges.

The goal was to evaluate the performance of the kits over a range representative of concentrations found in the WWKY database for each parameter. Many kits were tested against concentrations above its stated range. The decision was made to include results for concentrations known to be above the kit range because this would be expected in field deployment. However, under this study design it was shown that for many kits, it was often difficult to distinguish when results were above the maximum testing range. Further, for some kits very high concentrations were visually interpreted as low concentrations, which calls into question all results.

Because this study utilized kits with discrete interval ranges and the varied maximums, the statistical value of the results is limited. Additional studies utilizing different or additional known concentrations would be beneficial for evaluation purposes.

Some of the errors made in the volunteer measurements, such as forgetting to use a higher comparator, forgetting to divide results by ten, or difficulty in evaluating the color hue, can be expected to be repeated by other volunteers in the future, but not necessarily with the same frequency that occurred in this study. Volunteers were provided limited training on the kits simply because volunteers can often go several months before repeating a measurement and often must rely upon the printed instructions. It is unknown how extensive training on the kit use might have affected results. In many cases, the printed instructions could be improved for better clarity.

Because of the large amount of time required to accomplish the analyses for this study and a reliance on unpaid student volunteers and citizen scientists to perform the tests, involvement of many different volunteers was necessary to accomplish the study. However, using more volunteers decreases the comparability between datasets

because not all volunteers performed the same set of evaluations. With the broadness of the scope of the evaluation, this was a necessary factor, but future focused studies should consider more consistency in volunteer evaluations across kits.

It is unclear what effect performing multiple kits at the same time on the same concentrations might have had on the studies. Volunteers may have developed a "feel" for what the result might have been and were possibly biased by those expectations.

Results show that none of the kits provide an adequate substitute for laboratory results when making decisions where data quality is essential. However, for several parameters the quality of the data is sufficient to provide an initial cost-effective screening of results which might be followed by the more expensive but higher-quality laboratory results.

5.0 Conclusions

This study evaluated the cost, size, quality, time, and ease of use of field kits for ammonia, nitrate, phosphorus, and turbidity. Based upon the results, some recommendations can be made regarding which field-testing kits are the most appropriate for the WWKY program and other similar citizen science programs. Table 18 summarizes the recommendations for each parameter with additional explanation in the sections that follow.

Table 18: Summary of Field Kit Recommendations by Parameter

Parameter	Manufacturer and Model No.	Maximum	Cost per Test	Analysis Time (minutes)	Comments
Ammonia	Hanna Checker Low Range (HI700)	3.00 mg/L NH_3-N	$0.56	7	Strong data quality and highest rated by volunteers.
Nitrate	Chemetrics (K-6904)	4.5 mg/L NO_3-N	$2.56	18	No kit had great data quality, and the analysis time is long. Recommended only when costs prohibit lab analysis.
Phosphorus	Chemetrics (K-8510)	10 mg/L PO_4 (3.26 mg/L PO_4-P)	$0.87	5	Best option due to performance over a larger range than most other kits. Higher results may bias low.
Phosphorus	Hach (PO-23)	4.0 mg/L PO_4 (1.3 mg/L PO_4-P)	$1.42	4	If results are expected to be less than 1 mg/L PO_4-P, kit is an alternative option but requires more training.
Turbidity	Not Recommended				

5.1 Ammonia

The ammonia parameter provided some of the best overall value of the kits evaluated with all ammonia kits performing relatively well. However, the Hanna Checker Low Range Ammonia (HI 700) kit is recommended as the best field- testing kit option. It is the cheapest kit in terms of initial cost ($49) and cost per analysis ($0.56). It also had the strongest linear correlation to laboratory data as well as the lowest percent difference in readings. Combining this with the small physical size and high score of 10 in the ease of use category, the Hanna Checker is a cost effective, accurate, and easy-to-use field-testing kit. Although the kit took slightly longer to analyze (seven minutes) than the other units, much of this time does not require direct observation due to a count down timer of the unit. Therefore, Hanna kit is recommended as the best field-testing kit for ammonia.

5.2 Nitrate

For nitrate, none of the kits provide a particularly attractive option for field sampling by citizen scientists. Each kit took 18-19 minutes and volunteers provided feedback that they believed it was too long for volunteers. Of the three kits evaluated, the Chemetrics Nitrate kit (K-6904) is the best overall option. The cost per test is $2.56, which is the middle price point of the three options, but the kit covers the range of results expected in Kentucky, unlike the LaMotte kit, and has the best linear correlations and lowest percent difference. It was also rated as the easiest to use by the volunteers.

5.3 Phosphorus

It is more difficult to make a recommendation for the phosphorous and orthophosphate parameters. None of the kits performed particularly well due in part to mistakes made by volunteers or limitations in the kit's range or resolution. The errors made by volunteers cannot be neglected when recommending kits for field-testing as mistakes made in a laboratory setting are still very likely to be made in the field.

The Hanna Phosphorus High Range (HI706) kit was the only kit that measured total phosphorus and was the lowest priced unit at $0.20 per analysis. However, the kit often had “error” readings requiring re-testing. While the linear regression data and percent recoveries were well correlated to known results, the percent difference for the unit was amongst the highest. The high variability is due to a resolution of only 0.1 mg/L PO_4-P when concentrations from 0.1 to 0.5 have been proposed as TMDL target in different areas of the state. It was also rated as the hardest to use.

The Chemetrics Phosphate kit (K-8510) is recommended as the best option overall despite limitations. It was one of the lower cost options ($0.87 per analysis), was relatively fast (five minutes) and was among the kits rated easiest to use. Although the linear correlation was biased low, the kit provides one of the largest ranges with one of the best percent recoveries and lowest percent difference amongst volunteers.

The Hach Orthophosphate kit (PO-23) may be a potential option for results less than 1.0 mg/L PO_4-P. The kit provided almost perfect correlation with the known concentrations in

this range, so training could be provided to reject all results equal to or greater than the maximum level. It was one of the fastest kits (four minutes), was rated moderately easy to use, and was a medium cost per analysis ($1.42). However, the kit had multiple drawbacks. It comes in an overly large box which allows for testing of metaphosphate, which is unnecessary for screening purposes. Further, although it has instructions to report the results as phosphorus rather than phosphate, none of the volunteers utilized this option and one volunteer skipped the instruction to divide the result by ten. Therefore, careful training of field samplers would be recommended with this kit.

5.4 Turbidity

The only kit tested for turbidity was the LaMotte kit. However, it is not recommended for use as it failed to correlate with the laboratory results and had an extremely high percent difference in readings. Unless more accurate turbidity testing field kits can be located and evaluated, turbidity testing should be performed by the laboratory to ensure accurate results.

5.5 Follow Up Studies

It is recommended that a future study is conducted to evaluate performance outside of a laboratory setting. This study did not evaluate any of the effects of interfering chemicals that may be encountered in field environments. Further, evaluation of conditions such as uncleanliness of glassware, performance over the reagent lifespan, differing light conditions, and other such conditions would be recommended prior to long-term investment and utilization of the field kits.

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