Standard Operating Procedures for Non-tidal Monitoring

Integration of Citizen-based and Nontraditional Monitoring into the Chesapeake Bay Program Partnership

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Acknowledged Works

Much of the information in this manual has been adapted from the following methods manuals:

Alliance for the Chesapeake Bay. 2012. Citizen Monitoring Program Manual

EcoCheck. (2013). Sampling and data analysis protocols for Mid-Atlantic non-tidal stream indicators. Wicks EC, Fries AS, Kelsey RH, (eds). IAN Press, Cambridge, Maryland, USA.

Alliance for Aquatic Resource Monitoring. 2013. Antietam Watershed Association Water Quality Monitoring Manual

Virginia Citizen Water Quality Monitoring Program. 2007. Virginia Citizen Water Quality Monitoring Program Methods Manual

Center for Marine Conservation & U. S. EPA. Volunteer Estuary Monitoring: A Methods Manual, Second Edition.

U.S. EPA. 1997. Volunteer Stream Monitoring: A Methods Manual. EPA 841-B-97-003.

U.S. EPA. 1996. Recommended Guidelines for Sampling and Analyses in the Chesapeake Bay Monitoring Program. EPA 903-R-96-006.

Table of Contents

1 Before You Begin

1.1 Safety, Equipment List, and Volunteer Responsibilities

1.1.1 Safety – General Precautions

a) Always perform water-monitoring activities under the guidance of an adult.

b) Read all instructions to familiarize yourself with the test procedure before you begin. Note any precautions in the instructions.

c) Keep all equipment and chemicals out of the reach of young children and pets.

d) Avoid contact between chemicals and skin, eyes, nose and mouth.

e) Read the label on each reagent container prior to use. Some containers include precautionary notices or antidote information on the back of the container.

f) In the event of an accident or suspected poisoning, immediately call the Poison Control Center phone number in the front of your local telephone directory or call your physician. Be prepared to give the name of the reagent in question and its code number. Most kit reagents are registered with POISINDEX, a computerized poison control information system available to all local poison control centers.

1.1.2 Protect Yourself & Your Equipment: Use Proper Technique

a) Wear safety goggles or glasses when handling reagent chemicals.

b) Use the test tube caps or stoppers, not your fingers, to cover test tubes during shaking or mixing.

c) When dispensing a reagent from a plastic squeeze bottle, hold the bottle vertically upside-down (not at an angle) and gently squeeze it (if a gentle squeeze does not suffice, the dispensing cap or plug may be clogged).

d) Wipe up any reagent spills, liquid or powder, as soon as they occur. Rinse area with a wet sponge, and then dry.

e) Thoroughly rinse test tubes before and after each test. Dry your hands and the outside of the tubes.

f) Tightly close all reagent containers immediately after use. Do not interchange caps from different containers.

g) Avoid prolonged exposure of equipment and reagents to direct sunlight. Protect them from extremely high temperatures. Protect them from freezing.

1.2 Monitor Responsibilities

Choose a regular sampling day: Choose a convenient day of the week for sampling. Samples should be taken at regular weekly or monthly intervals. If it is not possible to sample on the same day each week, try to sample within 2 days (either side) of your regular day spacing the sampling dates, 5 to 9 days apart. Sample at the same time of day each week; if you are sampling multiple locations, be sure to always sample your sites in the same order each monitoring run to achieve similar sample timing.

Record your test results: Record data on a data collection form provided. Always record the test results as you go along. Keep a copy of the data collected for your records and to provide a backup copy should the original be lost.

Provide comments as necessary: The "Comments" section can be used to record general observations about the site especially changes due to erosion, recent notable weather, and any problems you had with the sampling procedures.

Submit data to database: If you have access to the internet, submit your data to the project's online database.

Send datasheets once every three months. Mail the data sheets to the Alliance or your Watershed Coordinator every three months so that we can maintain a current database.

Stay certified: Attend a recertification session every other year to maintain your skills and learn new information and techniques. You can also attend any training session to refresh yourself of the concepts and procedures between re-certifications.

2 QA/QC Procedures

2.1 Certification and Re-certification

2.1.1 Certification

All monitors that wish to submit Tier II data must gain monitor certification. Monitors can become certified at their initial training session by demonstrating a mastery of the sampling procedures and complete understanding of the quality assurance protocols used during data collection to be assessed by a Project Team member or Certified Trainer. Monitors must also pass a test that assesses the monitor's understanding of QA/QC procedures outlined in this SOP and the project QAPP with a 90% score.

Monitors that attend an initial training and are unable to pass the requirements to become certified at the end of the training will be encouraged to continue practicing their monitoring procedures. Un-certified monitors are encouraged assist a certified monitors in the field until they have become comfortable with the procedures and QA/QC protocols. Un-certified monitors are allowed to retake the certification test, and demonstrate proper sampling and analysis technique up to three times in order to become a certified monitor.

When a monitor achieves certification, they may be assigned a site and begin to collect Tier II data and submit it to the project database.

2.1.2 Re-certification

The Project Team and Certified Monitors will host recertification sessions biennially for monitors that have passed the initial training and wish to maintain their certification. Recertification sessions are conducted in a fashion that is similar to a lab practical. Monitors are checked to assure that: they remain proficient in methodology and understanding of basic water quality parameters; their equipment is operational and properly calibrated / verified; and they have an adequate supply of viable chemicals, procedures, equipment verification/check, and updated information about monitoring.

The recertification session is set up with a "station" for each water quality parameter. Monitors perform the test and compare their results to a known or controlled result. Project staff observe the monitors' methods and ensure that monitors correctly perform the tests and accurately record the data. After completing and "passing" one parameter, the monitor moves through each of the other stations while completing a datasheet that serves as documentation of re-certification. Replacement equipment, datasheets, information, and chemicals are given if needed. Alliance for the Chesapeake Bay retains documentation of recertification sessions.

2.1.3 Field Audits

Project Team members, the QC manager, or Certified Trainers may accompany monitors in the field and observe field collection procedures as part of the recertification process for monitors. Monitors will demonstrate proper sample collection, analysis, labelling, and preservation in accordance with this SOP.

2.2 Pre-monitoring checks

2.2.1 Equipment Check

Prior to going out into the field, monitors should check their equipment for cleanliness, breakage, probe function and battery life, and chemical expiration dates. If a monitor finds that their equipment is damaged and will affect the quality of the data they collect they will not collect data that day and mark the reason on their data sheet. The monitor should contact their Project Team member to get the equipment repaired or replaced prior to the next scheduled sample.

Monitors measuring dissolved oxygen using the Winkler titration will check the viability of their sodium thiosulfate solution prior to each monitoring event and record the results on their field datasheet. Sodium thiosulfate is used for monitoring dissolved oxygen. By using a standard solution of iodate-iodide, with 10 mg/L dissolved oxygen value, the monitor must record a value of 9.4 – 10 mg/L with their sodium thiosulfate measurement.

If results of the first check are above or below these intended values, a second check is performed. If the second check yields unacceptable values or if the two checks are greater than 0.4 mg/L apart from each other, the monitor is instructed to abandon the dissolved oxygen test because the sodium thiosulfate is no longer viable. The monitor must replace all expired chemicals prior to sampling again.

2.2.2 Calibration

Monitors will calibrate any equipment that requires calibration prior to being used (within 24 hours of use), using standard solutions and following the manufacturer's instructions. Monitors will note on their data sheet that they calibrated their equipment.

After sampling, it is recommended that monitors check their probes against the standard solutions used for calibration to identify instrument drift. If pH is outside of +/- 0.20 units, DO is +/- 0.3 mg/L, or specific conductance is +/- 5% of verification standards, the data must be flagged and the probe must be assessed and fixed or replaced if needed.

Monitors record these calibration and verification values on their datasheet and values are entered into the online database.

Thermometers that are verified should be re-verified every year. Thermometers must be verified against the Alliance master precision thermometer that is annually verified against an NIST-traceable thermometer to 0.2° C.

2.3 Field QC

2.3.1 Duplicates

If monitors are using the Winkler titration method for measuring DO they will perform the dissolved oxygen test on the actual water sample in duplicate. Monitors are instructed to do a third titration if their two initial titrations differ by more than 0.6mg/L. The two closest values are recorded on the datasheet.

Monitors collecting samples for Tier II laboratory analysis will perform duplicate samples at least 10% of the time. Duplicates consist of either collecting a larger sample for mixing and splitting it between two containers or immersing sample containers side by side in the water at the same time.

2.3.2 Replicates

Monitors will perform replicate samples of all other parameters (DO using Winkler titration method must be done in duplicate each sample) 10% of the time. The quality control samples are prepared and analyzed for all parameters of interest. The field replicate data are used to determine the overall precision of the field and laboratory procedures.

2.3.3 Field Blanks

Monitors will perform blank samples 10% of the time for samples to be sent to a lab for analysis. Monitors will perform all field procedures including preserving the samples as required and taking to the lab for analysis using deionized water provided by the laboratory. Results from field blanks will be recorded and appropriately marked during database entry.

3 Field Monitoring Procedures

3.1 Field Sampling Procedures

3.1.1 Best Practices

a) Use of protective gloves. Gloves serve a dual purpose: 1) protecting the sample collector from potential exposure to sample constituents and 2) minimizing accidental contamination of samples by the collector. Wearing protective gloves at all times while sampling is recommended. Latex or nitrile gloves may be used for common sampling conditions.

b) Safety always comes first. All sampling should be conducted with the proper equipment and least amount of danger to field personnel.

c) Permission must be obtained from landowners before entering private property.

d) Care should be taken not to disturb the bottom when sampling. When entering a stream, always walk in an upstream direction.

e) Surface water should always be collected facing upstream and in the center of main area of flow. Therefore, unless safety is an issue, samples should be obtained from a bridge or instream.

f) Samples should be collected in the main flow representative of the stream you are monitoring (for small streams, this is usually mid-channel) just below the water surface, about 0.3 meters (1 foot) deep.

g) Whenever possible, collect field measurements directly from the sample site, not from bucket. If the field parameters need to be measured in the bucket, collect water quality samples (nutrients, etc.) first before placing the multi probe instrument in the bucket.

h) When there are obvious standing pools of water during low or no flow conditions, do not collect samples or field measurements. Make a note of this on the data sheet.

i) When collecting bacterial samples:

   i. DO NOT rinse the bacteria sample bottle before collecting the sample.
   ii. If sample bottles contain a dechlorinating tablet (usually small white tablet) and you are collecting an unchlorinated sample, dump out the tablet before collecting the sample.
   iii. Be careful not to insert fingers into the mouth of the container or on the interior of the cap.

3.1.2 Streambank and Instream Sampling

If possible, wade into the stream to collect the sample. If wading to the sample site, always proceed upstream to allow the flow of the water to push any disturbed sediment downstream of where you will be collecting the sample.

When sampling from the streambank, care should be taken to sample from an area that will most closely represent the entire stream. Typically, this will be the area of the greatest flow in the stream and away from stagnant pools or eddies.

Step	Bacteria Samples	Nutrient and Chlorophyll Samples
1.	Walk upstream to the sample location. Be sure any sediment or debris disturbed from your movement in the streambed is not present where you will collect the sample.	Walk upstream to the sample location. Be sure any sediment or debris disturbed from your movement in the streambed is not present where you will collect the sample.
2.	Submerge the container; neck first into the water. The mouth of the bottle should be completely below the water surface approximately 3-6 inches.	Lower the sample bottle so that one edge of the opening is just below the water.
3.	Invert the bottle so the neck is upright and pointing into the water flow.	Allow the bottle to fill to the neck of the bottle.
4.	Move the bottle forward away from the body for at least six inches.	Lift the filled container. Do not pour out any excess water.
5.	Return the filled container quickly to the surface. Pour any excess water and cap.	

3.1.3 Dock or Bridge Sampling

1. Sample in the center of main flow from or as close as you can get on the dock or bridge. If sampling from a bridge sample from the safest side of the bridge and where contamination is least likely to occur. Typically, sampling on the upstream side of the bridge or dock is less likely to be contaminated.

2. During rainy periods, avoid sampling where storm water runoff from the bridge can affect sample.

3. Obtain field parameters (DO, pH, temperature) first before lowering a sample bucket.

4. When lowering the sample bucket, allow it to fill ¼ the way full and retrieve. Swirl the contents and dump the rinse away from the sample location to avoid kicking up sediment.

5. Repeat step 4 two more times and on the final time fill ½ to ¾ the way full.

6. Retrieve the bucket and collect the samples in the following order.

1. Bacteria
    - Open the bottle without touching the inner wall of the bottle or lid.
    - Invert the bottle by holding to the main body of the bottle and lower into the bucket 3-6 inches.
    - Fill the bottle in a 'U' from the side of the bucket closest to you to the opposite end.
    - At the end, bottle opening should be facing up and remove from the bucket.
    - Pour off any excess water and cap with the lid.

2. Nutrients
    - Open the bottle and tilt so that one side of the bottle will be below the waterline of the bucket.
    - Allow the bottle to fill to the neck of the bottle.
    - Remove the bottle and cap. Do not pour off any excess sample.

7. In situations where field parameters must be obtained from the bucket, all water samples must be collected prior to inserting the probe in the bucket.

3.2 Air Temperature Measurement

Equipment: armored, digital thermistor, or probe

Temperature is reported in degrees Celsius (ºC). Always measure air temperature before water temperature.

Method:

1. Locate a place near your site and hang the thermometer out of the direct sun.

2. Wait 3-5 minutes to allow the thermometer to equilibrate.

3. Record air temperature to the nearest 0.5 ºC for the armored thermometer or to the nearest tenth of a degree for the digital thermistor or probe on Page 2 of the datasheet.

3.3 Recording General Observations

Record weather and general observations on the datasheet.

3.4 Water Clarity & Turbidity Measurement

3.4.1 Secchi Disk

Equipment: 8" Secchi disk with attached line

Method:

1. Remove sunglasses if you are wearing them and stand with the sun to your back. Try to lower the disk into a shaded area.

2. Lower the disk into the water until the disk barely disappears from sight. Note the depth reading, in meters, based on the length of line submerged. Each mark is one-tenth (or 0.1) meter.

3. Slowly raise the disk and record the depth at which it reappears (i.e. is barely perceptible).

4. Average the two depth readings obtained above. The average of the two readings is considered to be the limit of visibility, or index of transparency. Record this average to the nearest tenth of a meter on your data form.

3.4.2 Transparency Tube

Transparency tubes are a type of equipment used for measuring transparency of water in streams and rivers. They are helpful for measuring transparency in situations where the stream is too shallow for the Secchi disk to be practical and for running waters where flow is too fast that the Secchi disk cannot remain vertical. Sample water collected either directly from the stream or from the sampling bucket is analyzed.

Equipment: Transparency tube

Method:

1. Close the drain tube by squeezing the crimp.

2. Fill the transparency tube with your sample water. Water may be collected directly from the stream in the vicinity of the sampling location if the stream is too small to fill the bucket, or sample water collected in the sampling bucket may be used (See 5.4, "Collecting the Water Sample"). To collect water directly from the stream, point the top of the tube in the upstream direction and collect surface water, being careful not to disturb the stream bed. To analyze water collected in the bucket, pour sample water from the bucket water directly into the transparency tube.

3. While looking down through the opening of the tube, partially open drain crimp, slowly draw off sample (Control flow by squeezing the crimp).

4. When the black and white pattern begins to appear, immediately tighten the crimp.

5. Record the level of water remaining via the centimeter ruler on the side of tube.

3.4.3 Turbidity Kit

This test is performed by comparing the turbidity of a measured amount of the sample with an identical amount of turbidity-free water containing a measured amount of standardized turbidity reagent. The readings are made by looking down through the column of liquid at a black dot. If turbidity is present, it will interfere with the passage of light through the column of liquid. Small amounts of turbidity will cause a "blurring" of the black dot in the bottom of the tube. Large amounts of turbidity may provide sufficient "cloudiness" so that it is not possible to see the black dot when looking down through the column. Any color that may be present in the sample should be disregarded. This determination is concerned only with the haziness or cloudy nature of the sample.

Equipment: Turbidity kit – LaMotte 7519-01

Method:

1. Fill one Turbidity Column to the 50 mL line with the sample water. If the black dot on the bottom of the tube is not visible when looking down through the column of liquid, pour out a sufficient amount of the test sample so that the tube is filled to the 25 mL line.

2. Fill the second Turbidity Column with an amount of turbidity-free water that is equal to the amount of sample being measured. Distilled water is preferred; however, clear tap water may be used. This is the "clear water" tube.

3. Place the two tubes side by side and note the difference in clarity. If the black dot is equally clear in both tubes, the turbidity is zero. If the black dot in the sample tube is less clear, proceed to Step 4.

4. Shake the Standard Turbidity Reagent vigorously. Add 0.5 mL to the "clear water" tube. Use the stirring rod to stir contents of both tubes to equally distribute turbid particles. Check for amount of turbidity by looking down through the solution at the black dot. If the turbidity of the sample water is greater than that of the "clear water", continue to add Standard Turbidity Reagent in 0.5 mL increments to the "clear water" tube, mixing after each addition until the turbidity equals that of the sample. Record total amount of Standard Turbidity Reagent added.

5. Each 0.5 mL addition to the 50 mL size sample is equal to 5 Jackson Turbidity Units (JTUs). If a 25 mL sample size is used, each 0.5 mL addition of the Standard Turbidity Reagent is equal to 10 Jackson Turbidity Units (JTUs). See Table 3.4-1 below. Rinse both tubes carefully after each determination.

Table 3.4-1-1. Turbidity Test Results – from LaMotte 7519-01 instructions

TURBITITY TEST RESULTS

Number of Measured Additions	Amount in mL	50 mL Graduation	25 mL Graduation
1	0.5	5 JTU	10 JTU
2	1.0	10 JTU	20 JTU
3	1.5	15 JTU	30 JTU
4	2.0	20 JTU	40 JTU
5	2.5	25 JTU	50 JTU
6	3.0	30 JTU	60 JTU
7	3.5	35 JTU	70 JTU
8	4.0	40 JTU	80 JTU
9	4.5	45 JTU	90 JTU
10	5.0	50 JTU	100 JTU
15	7.5	75 JTU	150 JTU
20	10.0	100 JTU	200 JTU

3.5 Water Temperature Measurement

Equipment: armored, digital thermistor, or probe

Method:

Surface Sampling:

1. Place your probe or thermometer 0.3 m beneath the surface of the water
2. Wait for the probe or thermometer to stabilize
3. Record your reading

Sample with bucket:

1. Hang thermometer in the bucket
2. Wait for the probe or thermometer to stabilize
3. Record your reading

3.6 Water Depth Measurement

Equipment: Secchi disk (for <3 m deep), or measuring tape with weighted end

Method:

1. At your sampling site, lower the measuring device into the water until it is resting on the bottom and the line is slack.

2. Record the depth reading, to the nearest tenth, based on the length of line submerged.

3.7 Dissolved Oxygen

3.7.1 **Winkler Titration Method**

Equipment: LaMotte Dissolved Oxygen Test Kit

Sodium Thiosulfate Check:
Prior to each sampling event (either the night before or the day of), you must run a test to make sure your Sodium Thiosulfate is still fresh and functional. Sodium Thiosulfate is fairly unstable and can degrade very suddenly, making it necessary to check it before each DO sampling. Perform this check at home before you go out. Here is how you do the check…

1. Rinse the titrating tube (small glass vial with plastic lid with hole in it) with a small amount of Iodate-Iodide Standard Solution (in large amber bottle).

2. Pour into waste container.

3. Repeat step 1 and 2 two more times

4. Pour 20 ml of the Iodate-Iodide Standard Solution into the rinsed titrating tube.

5. Add 8 drops of Sulfuric Acid (hold the bottle vertical to ensure equal drop size) to the 20 ml of solution and mix by swirling. Then place plastic cap (with hole in it) onto titrating tube.

6. Fill titrating syringe to the "0" mark with Sodium Thiosulfate.

7. Titrate using the Sodium Thiosulfate.

8. When solution turns a pale yellow color, but not clear:
    a) Remove cap, leaving syringe in cap.
    b) Add 8 drops Starch Solution (white bottle). Swirl titration sample gently to mix to a uniform blue color. Recap glass tube and continue titration process.

9. Continue adding Sodium Thiosulfate until solution turns from blue to clear.

10. Read results on syringe - Record your results under the Dissolved Oxygen portion on your field datasheet.

11. If results are less than 9.4 mg/l or greater than 10.0 mg/L, perform a 2nd test and record in the space on datasheet marked "2nd check".

12. Dispose of solution in titrating tube and syringe by pouring down sink and flushing with additional tap water.

13. Keep the amber bottle solution at home- you don't need to take into the field.

DO Sampling Method:

NOTE: Duplicate tests are run simultaneously on each sample to guard against error. If the amount of DO in the second test is more than 0.6 ppm different than the first test, you should do a third test. Record the average of the two closest results.

Since you will be doing two tests at the same time, thoroughly rinse both water sampling bottles with the sample water, filling and dumping the waste water downstream three times before collecting your sample.

1. Using the first sample bottle, submerge about 1/2 of the bottle opening allowing the water to gently flow into the bottle. Try to fill the bottle without causing a lot of bubbles. Submerge the filled bottle.

2. Turn the submerged bottle upright and tap the sides of the bottle to dislodge any air bubbles clinging to the inside of the bottle. Cap the bottle while it is still submerged.

3. Retrieve the bottle and turn it upside down to make sure that no air bubbles are trapped inside. If any air bubbles are present, empty the sample bottle downstream and refill. Fill the second sample bottle. Once two satisfactory samples have been collected, proceed immediately with Steps 4 & 5.

4. Place both sample bottles on a flat surface and uncap. While holding the bottle vertical, add 8 drops of Manganese Sulfate Solution followed by 8 drops of Alkaline Potassium Iodide Solution to each sample bottle. Always add the Manganese Sulfate first. Cap each sample bottle and mix by inverting gently several times. A precipitate will form. Allow the precipitate to settle to the shoulder of the bottle. Mix both bottles again and allow the precipitate to settle to the shoulder again.

5. Add 8 drops of the Sulfuric Acid both sample bottles. Cap the bottles and gently shake to mix, until both the reagent and the precipitate have dissolved. A clear-yellow to brown-orange color will develop. If brown flecks are present, keep mixing the samples until the flecks will not dissolve any further.

NOTE: Following the completion of Step 5, the samples have been "fixed," which means that dissolved oxygen cannot be added to the sample bottles. The titration procedure described in Steps 6-13 may be performed at a later time (but must be performed within 8 hours of sample collection). This means that several samples can be collected and "fixed" in the field and then carried back to a testing station for the remaining steps.

6. Pour 20 ml of the solution from one of the sample bottles into one of the glass tubes with a hole in its cap. Fill to white line so that the bottom of the meniscus (the curved surface of the liquid in the tube) rests on the top of the white line. The amount is critical so be sure to use the glass dropper to add or remove the sample solution from the tube. Place

cap on the tube.

7. Fill syringe (titrator) to the 0 mark with Sodium Thiosulfate solution. Be sure that there are no air bubbles in the syringe. Refer to kit manual for instructions on how to properly fill syringe.

8. To titrate the solution in the tube, insert the syringe into the cap of tube. Add 1 drop of Sodium Thiosulfate to test tube and gently swirl the glass tube to mix. Add another drop of the Sodium Thiosulfate and swirl the tube. Continue this process one drop at a time until the yellow-brown solution in the glass tube turns a pale yellow (lighter than the original yellow-brown solution but not clear). Once you reach this point, take the cap off while leaving the syringe in the cap.

9. Add 8 drops of Starch Solution to the glass tube. Swirl the tube gently to mix. The solution should turn from light yellow to dark blue.

10. Recap the glass tube and continue the titration process with the Sodium Thiosulfate remaining in the syringe (adding one drop at a time and swirling as described in Step 9), until the test tube solution turns from blue to clear. This is the endpoint. If the solution turns blue again, ignore it. Do not add any more Sodium Thiosulfate than is necessary to produce this first color change. Be sure to gently swirl the test tube after each drop.

NOTE: When the dissolved oxygen level is above 10 ppm, the solution in the tube will still be blue when the plunger tip of the titrator reaches 10 units. If it reaches this 10 unit line, do not go beyond that line. Usually, this will only happen when the water temperature is cold. In this case, refill the syringe to the 0 line from the Sodium Thiosulfate bottle and continue adding a drop at a time and swirling until reaching the endpoint.

11. Using the scale on the side of the syringe, read the total number of units of Sodium Thiosulfate used. Each line is 0.2 units. This number equals the number of parts per million (ppm) or milligrams per liter (mg/l) of dissolved oxygen in the water sample.

12. Carry out Steps 7-12 on second sample bottle and second glass tube.

13. Record the results of the two tests on the data sheet. If the difference between Test 1 and Test 2 is more than 0.6 ppm, you should do a third test and record the two results which are within 0.6 ppm.

NOTE: If using transparency tube to measure turbidity, perform this measurement now.

3.7.2 Electronic Probe Method

Equipment: Various models of dissolved oxygen probes and meters

Calibrating Dissolved Oxygen Probes and Meters

With practice and proper care for the DO probe, users can complete the entire DO probe calibration process within 5-10 minutes.

NOTE: Some probes may differ in displaying values. For DO probes, parts per million (ppm), and milligrams per liter (mg/L) are the same value. In addition, barometric pressure may be displayed in millibars (mBar) or in millimeters of mercury (mmHg).

Method:

1. Record the date of calibration. Calibration must be done each day you collect DO samples

2. Record the temperature of the probe just before you calibrate the probe

3. Set the barometric pressure (BP) mmHg or mBar- Most probes allow the user to adjust the barometric pressure readout of the probe for calibrating DO. The standard unit for barometric pressure is millimeters of mercury (mmHg) or millibars (mBar). You can get local barometric pressure readings from www.weatherunderground.com or www.noaa.gov. If using weather station data, it is important to adjust the reading by the altitude of the weather station. Appendix B explains how to calculate the correct reading.

4. Calculate the Theoretical DO Value mg/L- Prior to calibrating your probe, you should determine the theoretical DO value to confirm your probes readout. To determine the theoretical value, please follow the instructions found in Appendix B.

5. Record the mg/L reading of the calibrated DO level. If everything is working properly, the probe should display the correct DO level based on the altitude and temperature that you are calibrating at. The theoretical DO value and the probes calibrated readout should be within 0.2 mg/L. If not, try to recalibrate the probe or perform maintenance on the probe based on manufacturer instructions.

6. Turn off the probe if the manufacturer says so. If not, keep the probe on at all times while you are taking it out to the field and performing your field samples.

Measure DO

1. Place your probe 0.3 m beneath the surface of the water

2. Wait for the probe to stabilize, and then record your reading

Post Sampling Calibration Check
After the sample run is complete, return the probe to the calibration station to perform a quick post check. The post check consists of placing the probe in the DO calibration chamber and letting it equalize. This may take between 2 to 10 minutes depending on the condition of the probe.

1. Measure and record the temperature. If you did the morning calibration indoors, the probe temperature should be roughly close to the same as the morning calibration. If you are calibrating the probe outside, the temperature may be different from the earlier reading. This should not affect the post check.

2. Record the barometric pressure reading of the probe. This may have changed from the morning reading due to weather changes. You can get current local barometric pressure readings from the Internet. Remember to adjust any weather station data based on the instructions found in Appendix B.

3. As in the morning calibration, use Appendix B to determine your theoretical DO level.

4. Record the DO reading of the probe (ppm or mg/L). DO NOT recalibrate the probe. The purpose of this check is to see if the probe has drifted out of acceptable limits during the day.

5. Calculate the difference between the probe reported value and the theoretical DO value. If the probe is functioning properly there should be a difference of less than 0.50 mg/L from the afternoon theoretical DO level and the probe readout. If the calibration difference is greater than 0.50 mg/L the probe needs service and you must flag the data because the probe did not hold onto the calibration. If the calibration difference is 0.16 to 0.50 mg/L. The calibration of the probe is approaching the limits of accuracy and preventative maintenance may be required. It may be wise to clean the probe or replace the probe membrane when this occurs.

3.8 pH

3.8.1 Electronic probe method

Equipment: Various models of pH probes and meters

Calibration

The pH probe calibration procedure a similar protocol used in calibrating the DO probe. Most meters allow calibrating the pH probe using two different buffers. In most cases the use the 7.00 and 4.00 pH buffer solutions is suitable. If you are experiencing pH values above 7.00, calibrate using 7.00 and 10.00 buffer.

Use fresh buffer solution when you calibrate the probe and check the readings at the end of the day. If the probe is capable in doing so, please record the probe readings to the nearest hundredth unit place (Ex. 7.01) when performing the calibration.

1. Record the date of calibration. Calibration must be done each day you perform samples.

2. Record the temperature of the probe during calibration.

3. Record the probe reading as you place the probe in the 7.00 buffer solution. Gently swirl the buffer or the probe to obtain an accurate reading.

4. Calibrate the probe, the probe should now read a value close to 7.00 pH units. Most manufacturers of buffers provide a table showing the pH result that probes should display based on temperature. Check against this value displayed on the probe is close to this value.

5. Clean the probe with distilled or deionized water and blot dry

6. Immerse the probe in the 4.00 (or 10.00) buffer solution, record the stabilized value.

7. Calibrate the probe and it should now read a value close to 4 (or 10) pH units. Again, consult the buffer solution table to ensure accuracy.

After calibration, you may turn off the probe if the manufacturer says so. If not, the probe should be kept on at all times while going out into the field and prior to the post check. Follow manufacturer instructions regarding transporting of the probe into the field to prevent damage and drying out of the pH probe.

Field Sampling

IMPORTANT NOTE- When traveling to a sample station, keep the probe tip stored in the protective cap. This will keep the glass sensor hydrated.

1. Turn the probe on.

2. Dip the electrode about 2 to 3 cm either directly into the water or in your sampling bucket. Let the reading stabilize. This may take about 2 to 3 minutes.

3. Once the reading has stabilized record the reading on your datasheet.

4. Turn off the probe and replace the protective cap.

End of Day Calibration Check

To ensure the probe has maintained proper calibration, it is important to verify no significant probe drift has occurred. The procedures listed below will verify the probe did not drift outside QA/QC specifications. DO NOT CALIBRATE the probe during this check. Doing so will invalidate the data collected during the sample run.

1. Rinse off the probe and probe tip with distilled water and wipe dry using a soft cloth. Washing the probe will remove any material that may reduce probe life.

2. Place the probe into a container of pH 7.00 buffer. You may use the same buffer used during the morning calibration as long as the buffer was covered and appears clean.

3. Allow the probe to stabilize and record the temperature and pH reading in the "End of Day Temp C" and the "End of Day pH 7 Check" columns on the "pH Probe Calibration Form."

4. Rinse the probe and repeat the end of day check process using the 4.00 or 10.00 buffer.

If both buffer checks are within 0.20 units from the calibration values, the probe is within specifications. If the readings are greater than 0.20 units, flag all pH data collected during the sample run by typing "pH probe flag" in the "Additional comments" section when entering data into the online database. Also note "pH probe flag" at the top of the hard copy datasheet. This is because sometime during the sample run, the probe exceeded QA/QC specifications.

3.8.2 Colorimetric Kit

Equipment: LaMotte or Hach pH kits

Method:
Look on the front of black box to determine whether you have a wide range pH kit or a narrow range pH kit (i.e. cresol red, phenol red, bromthymol blue, thymol blue).

1. Rinse one sample test tube and cap twice with water from the stream or bucket

2. Fill the sample test tube to the black line with water from the stream or bucket. The bottom of the meniscus should be even with the line. Use plastic dropper to add or remove water from test tube.

3. For wide range pH kit, add ten drops of the wide range indicator while holding the reagent bottle completely upside down. For narrow range kits, add 8 drops of the indicator while holding the reagent bottle completely upside down.

4. Cap the test tube and mix the sample thoroughly.

5. Slide the tube in the comparator slot, hold it up to the sunlight, and record the pH value from the color in the comparator that most closely matches the sample tube color. When the color observed is between 2 colors on the comparator, the value is reported to the nearest 0.5 unit (for wide range kit) or 0.1 unit for other pH kits.

3.9 Salinity, Conductivity, and Total Dissolved Solids

Equipment: Various models of conductivity probes and meters

Calibration
Most probes that test for conductivity and TDS use a pre-made calibration solution with a specific conductivity value. The probe is immersed in the solution and calibrated to the value of the solution. It is good to use a calibration solution concentration similar to what you may find in the field to ensure accuracy.

1. Record the date of calibration. Calibration must be done each day you perform samples.

2. Record the temperature of the probe while you are calibrating the probe.

3. Write down the conductivity listed on the probe when you immerse the probe into the conductivity solution and record the value prior to calibration.

4. Record the conductivity solution that you will use to calibrate the probe. The standard unit for these solutions is in microsiemens per centimeter (mS/cm) but probes may use different units.

5. Write down the conductivity reading after you have calibrated the probe in the solution. The probe should be very close to the calibrated buffer solution but may be off by a couple of units.

Measure salinity, conductivity & TDS

1. Prior to sampling, rinse the probe with deionized or distilled water.

2. Select the appropriate mode and range on the meter, beginning with the highest range and working down. Some probes will auto select the correct range.

3. Place the probe into the sample water, and read the salinity, conductivity or TDS of the water sample on the meter's scale.

NOTE: If your probe does not automatically select the appropriate measurement range, and the reading is in the lower 10 percent of the range that you selected, switch to the next lower range. If the reading is above 10 percent on the scale, then record this number on your data sheet.

4. Rinse the probe with distilled or deionized water between each sample and before post sampling calibration check. Replace the cap for storage and transport.

Post sampling calibration check

1. Record the temperature of the probe at the end of the day when you are performing the calibration check.

2. Record the temperature of the probe at the end of the day when you are performing the calibration check.

3. Write down the conductivity listed on the probe when you immerse the probe into the conductivity solution and record the value.

4. Calculate the difference between the pre and post sampling calibration values.

5. Standard rule of thumb is if the probe difference is less than 10.00%, you should be confident of the probe values. To calculate the relative percent difference use the formula:

$$RPD\% = \frac{AbsoluteValue(Sample1 - Sample2)}{Average(Sample1 + Sample2)} \times 100\%$$

6. Initial the person calibrating and using the probe for your records. This is good to know in case something happens to the probe that you may not be aware of due to someone else is using it.

3.10 Nitrate – Nitrogen and Orthophosphate Kits

Equipment:

- Nitrate – Nitrogen kit w/ all chemicals and clean glassware (Hach NI-14 14161000, LaMotte, 3110, LaMotte 3354)
- Orthophosphate kit w/ all chemicals and clean glassware (Hach PO-19 224800, Hanna HI 38061, Hanna HI 713)
- Clean polypropylene sample bottle or scintillation vial (60 ml)

Method:

1. Rinse the sample bottle with sample water and dispose of downstream

2. Repeat step 1 three times.

3. Fill the bottle with sample water and cap. Process the sample as soon as possible.

4. Make sure the sample is well mixed prior to analysis by shaking the sample bottle.

5. Follow the protocol for each nutrient type as outlined in the instructions accompanying the kit. Reagents should be maintained at about 20° C to yield best results.

6. Record your results on the data sheet.

3.11 Alkalinity

Equipment: LaMotte 4491-DR-01, LaMotte 3467-01, or LaMotte 4533-DR-01

Method:

1. Rinse the sample bottle with sample water and dispose of downstream
2. Repeat step 1 three times.
3. Fill the bottle with sample water to the 5mL line.
4. Add an indicator tablet.
5. Cap and swirl the vial to mix until the tablet dissolves.
6. Fill the titration syringe with the titration reagent.
7. Insert the titrator syringe into the center hole of the test tube cap.
8. While gently swirling the tube, slowly press the plunger to titrate until the solution color changes from blue-green to purple. Consult the alkalinity endpoint color chart.
9. Read the test result directly from the scale where the large ring on the titrator meets the titrator barrel. Record as ppm on your data sheet.
10. Make sure the sample is well mixed prior to analysis by shaking the sample bottle.
11. Follow the protocol for each nutrient type as outlined in the instructions accompanying the kit. Reagents should be maintained at about 20° C to yield best results.
12. Record your results on the data sheet.

Equipment: Hanna HI 775 Digital Checker

Pre Sample Check:

1. Turn the meter on by pressing the button, all segments will be displayed. When the display shows ‘’Add’’, ‘’C.1’’ with ‘’Press’’ blinking, the meter is ready.
2. Fill the cuvette to the 10 mL line on the cuvette with unreacted sample and replace the cap. Place the cuvette into the meter and close the meter’s cap.
3. Press the button. When the display shows “Add”, “C.2” with “Press” blinking the meter is zeroed.

4. Wipe the standardized cuvette clean with a Kimwipe.

5. Place the standardized cuvette into the meter and close the meter's cap.

6. Press and hold the button until the timer is displayed on the LCD (the display will show the countdown prior to the measurement) or, alternatively, wait for 3 minutes and press the button.

7. Record your standard reading on your data sheet.

Method:

1. Turn the meter on by pressing the button, all segments will be displayed. When the display shows ''Add'', ''C.1'' with ''Press'' blinking, the meter is ready.

2. Fill the cuvette to the 10 mL line on the cuvette with unreacted sample and replace the cap. Place the cuvette into the meter and close the meter's cap.

3. Press the button. When the display shows "Add", "C.2" with "Press" blinking the meter is zeroed. Note: Any chlorine present in the sample will interfere with the reading. To remove the chlorine interference add one drop of HI 93755-53 Chlorine Remover to the unreacted sample.

4. Remove the cuvette, open it and using a 1 mL syringe carefully add exactly 1.00 mL of Alkalinity Reagent to the sample. Replace the cap and gently invert 5 times. Place the cuvette back into the meter. Note: Pay attention not to spill reagent otherwise full color development may be inhibited.

5. Press the button. The instrument directly displays the concentration of alkalinity in ppm of CaCO3. Alkalinity conversion: 1 ppm CaCO3 = 0.02 meq/L = 0.056 dkH The meter automatically turns off after 10 minutes.

3.12 Phosphate

Equipment:

- Hanna HI 713 Phosphate Low Range Checker
- Clean polypropylene sample bottle or scintillation vial (60 ml)

Pre Sample Check:

1. Turn the meter on by pressing the button, all segments will be displayed. When the display shows ‘’Add’’, ‘’C.1’’ with ‘’Press’’ blinking, the meter is ready.

2. Fill the cuvette to the 10 mL line on the cuvette with unreacted sample and replace the cap. Place the cuvette into the meter and close the meter’s cap.

3. Press the button. When the display shows “Add”, “C.2” with “Press” blinking the meter is zeroed.

4. Wipe the standardized cuvette clean with a Kimwipe.

5. Place the standardized cuvette into the meter and close the meter’s cap.

6. Press and hold the button until the timer is displayed on the LCD (the display will show the countdown prior to the measurement) or, alternatively, wait for 3 minutes and press the button.

7. Record your standard reading on your data sheet.

NOTE: If your standard value is outside ±5 mg/L of the expected value, acquire a second standard to check the Digital Checker again. If the second standard is outside ±5 mg/L of the expected value, replace the Digital Checker immediately and do not use for sample analysis.

Method:

1. Rinse the sample bottle with sample water and dispose of downstream three times.

2. Fill the bottle with sample water and cap. Process the sample as soon as possible.

3. Make sure the sample is well mixed prior to analysis by shaking the sample bottle.

4. Turn the meter on by pressing the button. All segments will be displayed. When the display shows “Add”, “C.1” with “Press” blinking, the meter is ready.

5. Fill the cuvette with 10 mL of unreacted sample and replace the cap. Place the cuvette into the meter and close the meter’s cap.

6. Press the button. When the display shows “Add”, “C.2” with “Press” blinking the meter is zeroed.

7. Remove the cuvette from the meter and unscrew the cap. Add the content of one packet of HI 713-25 reagent. Replace the cap and shake gently for 2 minutes until the powder is completely dissolved. Place the cuvette back into the meter.

8. Press and hold the button until the timer is displayed on the LCD (the display will show the countdown prior to the measurement) or, alternatively, wait for 3 minutes and press the button.

9. The instrument directly displays the concentration of phosphate in ppm. The meter automatically turns off after 2 minutes.

10. Record your results on your datasheet.

3.13 Bacteria

Equipment: Coliscan Easygel Kit

Sample collection:

Note the amount of rainfall within 48 hours prior to sampling and record in the bacteria section of the datasheet.

Collecting by wading:

4. Wade into the main flow of the stream

5. Take a few steps upstream with minimal disturbance;

6. Un-cap the sterile and pre-labeled bottle without touching the inside of the lid

7. Using a U motion dip the bottle into the water down and away from yourself allowing the bottle to fill ¾ full.

8. Cap the bottle and place sample on ice in cooler immediately (cooler temperature should be 1°C to 4°C. NOTE: Do not freeze your sample.

Collecting using a bucket:

1. Make sure not to touch inside of bucket with your hands.

2. If sampling from a dock or pier, go as far as possible to the end of the pier to collect your sample.

3. Throw the bucket out as far as possible in the main channel, and try not to disturb the bottom.

4. Rinse the bucket three times with stream water collected downstream of your sampling location.

5. Fill the bucket with the sample water to 1/3 full.

6. Un-cap the sterile and pre-labeled bottle without touching the inside of the lid

7. Using a U motion dip the bottle into the water down and away from yourself allowing the bottle to fill ¾ full.

8. Cap the bottle and place sample on ice in cooler immediately (cooler temperature should be 1°C to 4°C. NOTE: Do not freeze your sample.

Collecting using a sampling pole (from bridge or dock):

If sampling from a boat make sure that the boat motor has not stirred up the water. If the water is shallow, sampling should be done through wading.

1. Un-cap your sterile and pre-labeled bottle and secure it to the end of the pole.

2. Extend the pole outward and dip at approximately 0.3 m below the surface.

3. Cap the bottle and place sample on ice in cooler immediately (cooler temperature should be 1°C to 4°C. NOTE: Do not freeze your sample.

After sampling bacteria wash your skin that came in contact with the water with disinfectant or soap to reduce your chances of becoming sick.

Bacteria Sample Plating

Write the site designation, sample #, date, and time on the bottom of the Petri dish lid with a permanent marker. It is best to use small lettering on the outer rim of the dish.

1. Use proper technique to keep pipette sterile: open pipette packet bulb-side first so that you do not contaminate the tip.

2. Gently mix the water sample in the bottle. Pipette the desired volume (1.0 – 5.0 milliliters) of sample water directly into Coliscan media bottle. It is best to dispense 2-ml in two separate allotments for a total of 4 ml while using a 3 ml disposable pipette. Be careful not to let the bottle lid touch anything to prevent sample contamination.

3. Record the expiration date of the media bottle on your datasheet.

4. Gently mix (do not shake) bottle of Coliscan media containing the sample water, and then pour the entire contents into a Petri dish. Only open the Petri dish long enough to pour in the sample.

5. Gently swirl Petri dish so the Coliscan media covers the entire bottom. For safety purposes, tape the Petri dish shut at this point.

6. Allow the media to solidify for approximately 60 minutes prior to incubation. (Amount of time will vary based on room temperature.)

7. Put plates in incubator and try to maintain at 37^{o}C (= 98.6^{o}F) for 24 hours. If no incubator is available, place the dish in a safe warm place out of direct sunlight, such as on top of a fridge or a water heater. Depending on temperature, the plates may need to be incubated for

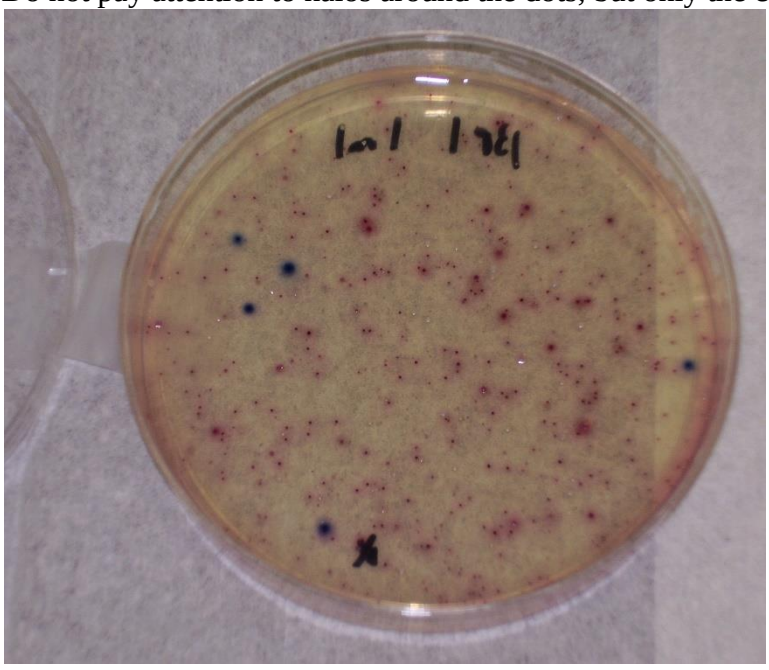
48 to 72 hours.

8. Record the average incubator temperature on the datasheet as well as the # of hours that the plates were in the incubator.

NOTE: As soon as plates are removed from incubator, they must be scored.

Bacteria Scoring

1. Place the Petri dishes on a white background or in natural sunlight. Count the number of dark blue (NOT TEAL) to purple (NOT PINK) colored colonies larger than pinprick size on each plate. Do not pay attention to halos around the dots, but only the center color.



2. Record this number in the column labeled "Total # of purple or dark blue colonies on plate" on the data form. Repeat for replicate #2.

3. Calculate the number of E. coli per 100 milliliters of water by following the instructions on the datasheet and record.

4. Calculate the average number of E. coli per plate and record on the datasheet. This is the value you will report in the online database.

Bacteria Monitoring Cleanup and Disposal

1. Throw used pipettes in the trash.

2. Rinse empty Coliscan bottles 2-3 times with tap water and dispose of in the trash can. (If media bottles are not rinsed, pathogens could grow in the remaining media.)

3. Add bleach or rubbing alcohol to each Petri dish to completely cover the solid media. Allow dishes to stand for at least 10 minutes to ensure all bacteria have been killed.

4. Place the plates in a zip-lock bag and dispose of in the trash.

4 Lab sample collection preparation and handling

4.1 Nutrient and Grab Samples

Collecting from a boat:

1. Water samples should be taken one meter from the surface and one meter from the bottom of the water column at sites with depths greater than four meters. Because it is below the layer of mixing caused by wind, boating, and other activities, sampling one meter below the surface gives a better representation of the surface water column.

2. At sites with depths less than four meters, water samples should be taken one meter from the surface.

3. Facing upstream, extend the pole and bottle, rinse the bottle out three times, and take the sample the fourth time.

4. After samples are taken, immediately place the sample on ice up to the shoulders of the bottle. The lid should not be immersed under the ice, in case ice water leaks into the sample bottle, diluting the concentration of the sample.

5. On the field data sheet, record the time, date, and any other information about the water sampling event.

Collecting by wading:

1. Wade into the main flow of the stream

2. Take a few steps upstream with care not to disturb the sediment;

3. Un-cap the pre-labeled bottle

4. Using a U motion dip the bottle into the water down and away from yourself allowing the bottle to fill to the shoulder

5. After samples are taken, immediately place the sample on ice up to the shoulders of the bottle. The lid should not be immersed under the ice, in case ice water leaks into the sample bottle, diluting the concentration of the sample.

Collecting with a sampling pole:

1. Attach the sample bottle to the sampling pole, making sure that the clamp is tight.

2. The sampling point in the stream or river should have a low to medium flow and not be in eddies or stagnant water.

3. Facing upstream, extend the pole and bottle, rinse the bottle out three times, and take the sample the fourth time.

4. Fill the bottle up to the shoulders and immediately cap and place on ice. The lid should not be immersed under the ice, in case ice water leaks into the sample bottle, diluting the concentration of the sample.

4.2 Chemical preservatives and reagents

The nutrient sample bottles contain a small amount of sulfuric acid as a preservative. When sampling it is important to fill the bottle to the needed level and not pour out the preservative or excess sample from the bottle.

The bacteria sample bottle contains a dechlorinating tablet. When collecting non-chlorinated water, discard the tablet. Samplers should discard the tablet just prior to collecting a bacteria sample at the site. Discard the tablet by dumping out of the bottle without touching the lip or inner wall of the sample bottle. The tablets are harmless to the environment and may be left at the site.

4.3 Sample container handling and preservation

Proper sample containers and sample preservation are essential to sample integrity. Samples not preserved properly may be rejected by the laboratory.

a) Sample containers should be inspected and any torn, punctured or cracked sample containers discarded.

b) After collecting the sample, make sure the lids are secured tightly to prevent contamination from water seepage in or out of the container.

c) Sample containers and coolers should be stored with the tops securely fastened. Containers with loose fasteners should be replaced or taped to prevent loss of sample containers during transport.

d) In the field, unless specified otherwise, all samples should be placed in an ice filled cooler immediately after collection. To ensure samples do not exceed the 4°C holding temperature, sample containers shall be placed upright and if possible, covered with ice in such a manner that the container openings are above the level of ice. Bacteria sample bottles should be stored in bags, placed in coolers and surrounded with wet ice.

e) Glass sample containers should be packed in bubble wrap or other waterproof protective materials to minimize accidental breakage.

f) The laboratory will provide temperature bottles that they use to determine sample temperature upon arrival at the lab. Make sure that every cooler used to ship samples to the lab contains one of these bottles.

4.4 Sample Bottle Identification

Each sample container must include a label with the following information.

a) Station ID or description

b) Date and time of sample collection

c) Collector's initials

d) Sample depth in meters (surface samples are reported as 0.3)

e) Parameter name and/or group code,

f) Container number

g) Preservative used and volume filtered, if applicable.

Samples will not be analyzed if this information is missing. If more than one container is needed for a parameter (such as a duplicate sample), each container collected for that parameter must have a label with identical information in addition to an indication of 1 of 3, 2 of 3, 3 of 3, etc., as required. Split samples should be designated as S1 and S2.

Please remember to fill out the labels on the bottle with a waterproof pen before taking the samples.

It is essential that the actual sampling site match the labeling information. Always check the labeling information against the actual site. Samples not labeled properly may be rejected by the laboratory.

4.5 Transport of Samples

After collecting the samples at the site:

1. Place the bottles in the cooler filled with ice. Coolers should have enough ice to come up to the necks of the sample bottles.

2. Place any chain of custody forms in the Ziploc bag taped to the inner lid of the cooler.

3. Transport the cooler with samples to the designated drop off point or laboratory by the specified time.

5 Lab Procedures

Lab work will be performed by a NELAP, federal, or state approved lab. The following are the approved methods and their corresponding SOPs for reference for laboratories. It is expected that laboratories will be in compliance with these methods and will already be in possession of the procedural documentation for these methods.

Parameter	Method	Appendix
Silicate	US EPA method 366.0	Appendix C
Nitrate - Nitrogen	USEPA Method 352.1	Appendix D
Nitrite - Nitrate	USEPA Method 353.3	Appendix E
Ammonia - Nitrogen	USEPA Method 350.1	Appendix F
Total Nitrogen	USEPA Method 351.2	Appendix G
Total Phosphorus	USEPA Method 365.4	Appendix H

Laboratories will perform QA/QC measures including: method blanks, matrix spikes, replicates, check standard.

6 Cleanup and Storage of Water Monitoring Equipment

a) Rinse the thermometer in tap water and store upright.

b) Pour contents of DO sampling bottles and chemical kits into the sink. Rinse all the bottles and containers thoroughly with tap water. Put all equipment away until next sampling time.

c) Store all chemical reagents in a dark, cool place and out of the reach of children and pets!

d) Save expired chemicals and give them to your monitoring coordinator or trainer at the next recertification event for proper disposal.

NOTE: If you conduct the sampling procedures away from home or on a boat, you need a special container for safe disposal of the test samples. A plastic milk jug or jar works well and is easy to obtain. Fill this container about ½ to ¾ full with kitty litter to absorb the moisture. When the litter is saturated, place the closed jar in double plastic garbage bags and dispose of in the trash.

6.1 Maintenance for pH meter

Follow maintenance and care guidelines as specified by the manufacturer manual. Below are some general day to day care tips.

1. Ensure the probe is cleaned and well maintained. After each sample run, rinse off the probe with distilled water. Use a soft cloth and gently dry the probe and glass sensor.

2. Store the probe tip in the cap provided by the manufacturer. Inside this cap, place a small cotton ball or piece of paper towel soaked with pH 4.00 buffer (or probe storage solution). This will keep the probe in working condition until the next field sampling event.

3. If you see any biological growth (mold, algae, etc.), use mild soap or warm (~30o C) pH 4.00 buffer to clean. Rinse with distilled water and dry.

4. If the calibration or end of day check indicates there is a problem with the probe, and standard cleaning does not produce acceptable results, replacement of the sensor cap may be necessary. Contact a Project Team Member to get a replacement sensor cap.

Appendix A

Field Data Sheet

Chesapeake Monitoring Cooperative

Non-tidal Field Data Sheet

Site Name & # _____ Stream Name _____

Date _____/_____/_____ Time (military time) _____ Rainfall (mm last 48 hrs) _____

Monitors: _____

Parameter	Method Used (Circle Applicable)	Calibration Pre / Post Sampling		Measurement 1st / 2nd / 3rd Replicate or Circle observation		
Weather Conditions (cloud cover)				Clear / Partly Cloudy Cloudy / Fog or Haze		
Stream Flow				Low / Med / High / NA		
Water Color				Clear / Milky / Muddy Oil slick / Other		
Air Temperature (°C)	Armored Classic / Digital / Probe	Verified? Y / N				
Water Temperature (°C)	Armored Classic / Digital / Probe	Verified? Y / N				
Dissolved Oxygen (mg/L)	Winkler Titration / Probe					
pH	Kit / Probe / ColorpHast Strips					
Conductivity (µS/cm)	Probe					
TDS (mg/L)	Probe					
Turbidity (JTU)	LaMotte 7519					
Water Clarity (cm)	Secchi Disk / Turbidity Tube					
Alkalinity (mg/L)	Hanna Digital Checker	Pre only:				
Alkalinity (mg/L)	LaMotte 4491-DR-01 / LaMotte 3467-01 LaMotte 4533-DR-01					
Phosphate (mg/L)	Hanna Digital Checker	Pre only:				
Orthophosphate (mg/L)	Hach PO-19 224800 Hanna HI 38061					
Nitrate (mg/L)	Hach NI-14 1416100 / LaMotte 3110 LaMotte 3354					

Comments:_____

_____

_____

Use this chart to determine if your two replicates are within range of each other. If not, perform a third test.

Parameter	Acceptable Range
Temperature	Armored (+/- 1° C) / Digital (+/- 0.5° C)
Dissolved Oxygen Sodium Thiosulfate Check	Only perform 1 test. If <9.4 or >10 mg/L, do a second test. If both tests are not within 0.4 mg/L of each other, do not measure DO.
Dissolved Oxygen	+/- 0.6 mg/L
pH	+/- 1 pH unit
TDS / Conductivity	± 2% FS
Nitrate	Low range (0–1 mg/L) = +/- 0.1 mg/L Mid range (1–10 mg/L) = +/- 1 mg/L
Phosphate	+/- 0.04 mg/L
Alkalinity	< 100 mg/L = +/- 10 mg/L > 100 mg/L = +/- 20 mg/L
Turbidity	+/- 5 JTU

E. coli Bacteria Measurement (using Coliscan Easygel plates)
Incubation time: _____ hours (to nearest hour)

Incubation temp: _____.____ o C (to nearest half degree)

Media expiration date: _____ Plate expiration date: _____

Amount of water sample added to media bottle (max 5 ml per Rep):

Rep1:_____(A1)Rep2:_____(A2)

Total # of purple or dark blue colonies on plate: Rep1:_____(B1) Rep2:_____(B2)

Note: disregard any pink, blue-green or white colonies- these are not E. coli bacteria

To calculate the Total Colonies of E. coli bacteria per 100 ml of water:

1. Divide 100 by the ml of water used. 2. Multiply this quotient by the number of purple colonies counted

Rep1: ([100 ÷ A1] * B1) = _____(C1) **Rep2: ([100 ÷ A2] * B2) = _____ (C2)**

Total Time Spent Monitoring:(Includes travel to and from monitoring site; equipment preparation; sample collection; water's edge time; and time spent filling out data sheets):

Name: _____ Hours: _____ (Round to nearest 15 min.)

Name: _____ Hours: _____ (Round to nearest 15 min.)

Name: _____ Hours: _____ (Round to nearest 15 min.)

Lead Monitor Signature: _____ **Date:** _____

Once datasheets have been entered in the database, send original forms to your coordinator or:

Alliance for the Chesapeake Bay
Attn: Chesapeake Monitoring Coop
612 Hull St. Suite 101C
Richmond, VA 23225

Or

ALLARM
Dickinson College-Environmental Studies Dept.
P.O. Box 1773-College & Louther Streets
Carlisle , PA 17013

Appendix B

Theoretical DO Calculation

How to Calculate Theoretical Dissolved Oxygen Values

From: Virginia Citizen Water Quality Monitoring Program Methods Manual - October 2007

Proper calibration of Dissolved Oxygen (DO) probes is important to collect accurate data. An easy way to see if a probe is calibrated correctly is to compare the probe's results against a theoretical DO value. This value is what the DO level should be based on temperature and barometric pressure.

DO Level based on temperature

The top table on the attached chart allows users to find the DO level based on temperature. The top and side axis of the table corresponds to the temperature that the probe is reporting. The intersection of the two axes displays the DO reading. Write this number down to start calculating the theoretical DO level.

Correction factor for barometric pressure

Barometric pressure is a way to tell how much atmosphere is pressing down on a surface. Weather systems and elevation above (or below) sea level can change this value. The bottom table of the attached chart will help compensate for these changes in pressure. Dissolved oxygen probes normally show pressure in millimeters of mercury (**mmHg**) or millibars (**mBar**).

Having a barometer on hand is a good way to get pressure data. A weather station can also provide pressure data. Websites such as www.weatherunderground.com are useful to find local weather stations. Please note that most barometers and weather stations report pressure in inches of mercury (**inHg**).

Note about using weather station pressure readings

Weather stations compensate pressure readings to make it appear as if the station is at sea level. To account for this, subtract the barometric pressure by 1.01 inHg per 1,000 feet in elevation of the weather station. This final value is known as **absolute barometric pressure**.

Example: Find the absolute barometric pressure of a station located 222 feet above sea level that reported 30.12 inHg.

$$30.12 \text{ inHg} - \frac{1.01 \text{ inHg}}{1000/\ 222 \text{ feet}} \rightarrow 30.12 - \frac{1.01}{4.50} \rightarrow 30.12 - 0.22 == 29.90 \text{ inHg absolute barometric pressure}$$

Once finding the absolute pressure, use the bottom table found on the attached chart to find the proper correction factor to use. The formulas at the bottom of the chart will help in converting inHg barometric pressure readings into **millibars** (mBar) or **millimeters of mercury** (mmHg) that are commonly used to calibrate a dissolved oxygen probe. Use this value to find the correction factor to use in the final calculation.

Example: A barometric pressure of 970 millibars you would use a correction factor of 0.96 (second column, bottom row).

Theoretical DO Calculation

To find the theoretical DO value, use the following formula.

Theoretical DO = (DO level based on temperature) x (barometric pressure correction factor)

Example: If a probe had a temperature of 18.4 C and the barometric pressure was 970 mBar, the theoretical DO value would be 9.00 mg/L (9.37mg/L x 0.96 correction factor).

Dissolved Oxygen Saturation

Directions- To determine theoretical DO saturation, multiply the O2 concentration value (found in the top chart) by the barometric pressure correction factor (bottom chart).

Example: Find the DO saturation for at a temperature of **18.4 C** at **730 mmHg** pressure: 9.37 x 0.96= **9.00 mg/L**

Temp in ^{O}C	**O_2 concentrations in mg/l**									
	0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
5	12.75	12.71	12.68	12.65	12.61	12.58	12.55	12.52	12.48	12.45
6	12.42	12.39	12.36	12.32	12.29	12.26	12.23	12.2	12.17	12.14
7	12.11	12.08	12.05	12.02	11.99	11.96	11.93	11.9	11.87	11.84
8	11.81	11.78	11.758	11.72	11.69	11.67	11.64	11.61	11.58	11.55
9	11.53	11.5	11.47	11.44	11.42	11.39	11.36	11.33	11.31	11.28
10	11.25	11.23	11.2	11.18	11.15	11.12	11.1	11.07	11.05	11.02
11	10.99	10.97	10.94	10.92	10.89	10.87	10.84	10.82	10.79	10.77
12	10.75	10.72	10.7	10.67	10.65	10.63	10.6	10.58	10.55	10.53
13	10.51	10.48	10.46	10.44	10.41	10.39	10.37	10.35	10.32	10.3
14	10.28	10.26	10.23	10.21	10.19	10.17	10.15	10.12	10.1	10.08
15	10.06	10.04	10.02	9.99	9.97	9.95	9.93	9.91	9.89	9.87
16	9.85	9.83	9.81	9.79	9.76	9.74	9.72	9.7	9.68	9.66
17	9.64	9.62	9.6	9.58	9.56	9.54	9.53	9.51	9.49	9.47
18	9.45	9.43	9.41	9.39	**9.37**	9.35	9.33	9.31	9.3	9.28
19	9.26	9.24	9.22	9.2	9.19	9.17	9.15	9.13	9.11	9.09
20	9.08	9.06	9.04	9.02	9.01	8.99	8.97	8.95	8.94	8.92
21	8.9	8.88	8.87	8.85	8.83	8.82	8.8	8.78	8.76	8.75
22	8.73	8.71	8.7	8.68	8.66	8.65	8.63	8.62	8.6	8.58
23	8.57	8.55	8.53	8.52	8.5	8.49	8.47	8.46	8.44	8.42
24	8.41	8.39	8.38	8.36	8.35	8.33	8.32	8.3	8.28	8.27
25	8.25	8.24	8.22	8.21	8.19	8.18	8.16	8.15	8.14	8.12
26	8.11	8.09	8.08	8.06	8.05	8.03	8.02	8	7.99	7.98
27	7.96	7.95	7.93	7.92	7.9	7.89	7.88	7.86	7.85	7.83
28	7.82	7.81	7.79	7.78	7.77	7.75	7.74	7.73	7.71	7.7
29	7.69	7.67	7.66	7.65	7.63	7.62	7.61	7.59	7.58	7.57
30	7.55	7.54	7.53	7.51	7.5	7.49	7.48	7.46	7.45	7.44

Barometric Pressure Correction factor:

mmHg (mBar)	**Corr. Factor**	**mmHg (mBar)**	**Corr. Factor**	**mmHg (mBar)**	**Corr. Factor**	**mmHg (mBar)**	**Corr. Factor**
775-771 (1033-1028)	**1.02**	**750-746 (1000-995)**	**0.987**	**725-721 (967-961)**	**0.953**	**700-696 (934-928)**	**0.92**
770-766 (1027-1021)	**1.014**	**745-741 (994-988)**	**0.98**	**720-716 (960-955)**	**0.947**	**695-691 (927-921)**	**0.914**
765-761 (1020-1014)	**1.007**	**740-736 (987-981)**	**0.973**	**715-711 (954-948)**	**0.94**	**690-686 (920-915)**	**0.907**
760-756 (1013-1008)	**1**	**735-731 (980-975)**	**0.967**	**710-706 (947-941)**	**0.934**	**685-681 (914-908)**	**0.9**
755-751 (1007-1001)	**0.993**	**730-726 (974-968)**	**0.96**	**705-701 (940-935)**	**0.927**	**680-676 (907-901)**	**0.893**

Appendix C
Laboratory Method – Silicate – US EPA 366.0

Method 366.0

Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

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National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Method 366.0

Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

1.0 Scope and Application

1.1 This method provides a procedure for the determination of dissolved silicate concentration in estuarine and coastal waters. The dissolved silicate is mainly in the form of silicic acid, H_4SiO_4, in estuarine and coastal waters. All soluble silicate, including colloidal silicic acid, can be determined by this method. Long chain polymers containing three or more silicic acid units do not react at any appreciable rate[1], but no significant amount of these large polymers exists in estuarine and coastal waters.[2,3] This method is based upon the method of Koroleff,[4] adapted to automated gas segmented continuous flow analysis.[5-7]

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Silicate	12627-13-3

1.2 A statistically determined method detection limit (MDL) of 0.0012 mg Si/L has been determined by one laboratory in seawaters of three different salinities.[8] The method is linear to 6.0 mg Si/L using a Flow Solution System (Perstorp Analytical Inc., Silver Spring, MD).

1.3 Approximately 60 samples per hour can be analyzed.

1.4 This method should be used by analysts experienced in the use of automated gas segmented continuous flow colorimetric analyses, and familiar with matrix interferences and procedures for their correction. A minimum of 6-months experience under supervision is recommended.

2.0 Summary of Method

2.1 An automated gas segmented continuous flow colorimetric method for the analysis of dissolved silicate concentration is described. In the method, β-molybdosilicic acid is formed by reaction of the silicate contained in the sample with molybdate in acidic solution. The β-molybdosilicic acid is then reduced by ascorbic acid to form molybdenum blue. The absorbance of the molybdenum blue, measured at 660 nm, is linearly proportional to the concentration of silicate in the sample. A small positive error caused by differences in the refractive index of seawater and reagent water, and negative error caused by the effect of salt on the color formation, are corrected prior to data reporting.

3.0 Definitions

3.1 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2 Dissolved Analyte (DA) -- The concentration of analyte in an aqueous sample that will pass through a 0.45 μm membrane filter assembly prior to sample acidification or other processing.

3.3 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether method performance is within acceptable control limits, and whether the laboratory is capable of making accurate and precise measurements.

This is basically a standard prepared in reagent water that is analyzed as a sample.

3.4 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all labware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or apparatus.

3.6 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.7 Method Detection Limit (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[8]

3.8 Reagent Water (RW) -- Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing and Materials (ASTM). Reverse osmosis systems or distilling units followed by Super-Q Plus Water System that produce water with 18 megohm resistance are examples of acceptable water sources.

3.9 Refractive Index (RI) -- The ratio of velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as estuarine or sea water versus reagent water. The correction for this difference is referred to as refractive index correction in this method.

3.10 Stock Standard Solution (SSS) -- A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

3.11 Quality Control Sample (QCS) - A solution of method analyte of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.12 SYNC Peak Solution - A colored solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by most data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

4.0 Interferences

4.1 Interferences caused by hydrogen sulfide, such as occur in samples taken from deep anoxic basins can be eliminated by oxidation with bromine or stripping with nitrogen gas after acidification. Interferences of phosphate at concentrations larger than 0.15 mg P/L is eliminated by the use of oxalic acid in the color development step of this method. Interferences of fluoride at concentrations greater than 50 mg F/L can be reduced by complexing the fluoride with boric acid.[4]

4.2 Glassware made of borosilicate glass should be avoided for use in silicate analysis. Plastic labware such as polyethylene volumetric flasks and plastic sample vials, should be used.

4.3 Sample turbidity and particles are removed by filtration through a 0.45 µm non-glass membrane filters after sample collection.

4.4 This method corrects for refractive index and salt error interferences which occur if sampler wash solution and calibration standards are not matched with samples in salinity.

4.5 Frozen samples should be filled about 3/4 full in the sample bottles. The expansion of water on freezing will squeeze some of the brine out of the bottle if the bottle was overfilled. The overfill of the sample bottle during freezing will drastically alter the nutrient concentrations in the sample that remains.

5.0 Safety

5.1 Water samples collected from the estuarine and coastal environment are generally not hazardous. However, the individual who collects samples should use proper technique.

5.2 Good laboratory technique should be used when preparing reagents. A lab coat, safety goggles, and gloves should be worn when preparing the sulfuric acid reagent.

6.0 Equipment and Supplies

6.1 Gas Segmented Continuous Flow Autoanalyzer Consisting of:

6.1.1 Autosampler.

6.1.2 Analytical cartridge with reaction coils for silicate analysis.

6.1.3 Proportioning pump.

6.1.4 Monochromator or spectrophotometer equipped with a tungsten lamp (380-800 nm) and a low refractive index flowcell.

6.1.5 Strip chart recorder or computer based data acquisition system.

6.2 Glassware and Supplies

6.2.1 All labware used in the analysis must be low in residual silicate to avoid sample or reagent contamination. Soaking with lab grade detergent, rinsing with tap water, followed by rinsing with 10% HCl (v/v) and thoroughly rinsing with reagent water was found to be effective.

6.2.2 Glassware made of borosilicate glass should be avoided for storage of solutions for silicate analysis. Plastic containers are preferable for silicate analysis.

6.2.3 Non-glass membrane filters with 0.45 µm nominal pore size. Plastic syringes with syringe filters, pipets, 60 mL polyethylene bottles, and polyethylene volumetric flasks, plastic sample vials.

6.2.4 Drying oven, desiccator and analytical balance.

7.0 Reagents and Standards

7.1 Stock Reagent Solutions

7.1.1 Sulfuric Acid Solution (0.05 M) - Cautiously add 2.8 mL of concentrated Analytical Reagent Grade sulfuric acid (H_2SO_4) to approximately 800 mL of reagent water, mix then bring up to 1 L with reagent water.

7.1.2 Ammonium Molybdate Solution (10 g/L) - Dissolve 10 g of ammonium molybdate (VI) tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in approximately 800 mL of 0.05 M sulfuric acid solution and dilute to 1000 mL with 0.05 M sulfuric acid solution. Store in an amber plastic bottle. This solution is stable for one month. Inspect the solution before use. If a white precipitation forms on the wall of container, discard the solution and make a fresh one.

7.1.3 Stock Silicate Solution (100 mg Si/L) - Quantitatively transfer 0.6696 g of pre-dried (105°C for 2 hours) sodium hexafluorosilicate (Na_2SiF_6) to a 1000 mL polypropylene flask containing approximate 800 mL of reagent water, cover with plastic film and dissolve on a stir plate using a Teflon-coated stirring bar. Complete dissolution usually takes 2-24 hours. Dilute the solution to 1000 mL in polyethylene volumetric flask with reagent water. Store the stock solution in a plastic bottle. This solution is stable for one year if care is taken to prevent contamination and evaporation.

7.1.4 Low Nutrient Sea Water (LNSW) - Obtain natural low nutrient seawater from surface seawater in the Gulf Stream or Sargasso Sea (salinity 36 ‰, < 0.03 mg Si/L) and filter through 0.45 µm pore size non-glass membrane filters. In addition, commercially available low nutrient sea water (< 0.03 mg Si/L) with salinity of 35 ‰ (Ocean Scientific International, Wormley, U.K.) can be used.

7.2 Working Reagents

7.2.1 Dowfax Start-up Solution - Add 2 mL of Dowfax 2A1 surfactant (Dow Chemical Company) to 1000 mL reagent water and mix gently.

Note: Dowfax 2A1 contains (w/w) 47% benzene, 1,1-oxybis, tetrapropylene derivatives, sulfonate, sodium salt, 1% sodium sulfate, 3% sodium chloride and 49% water.

7.2.2 Working Molybdate Reagent - Add 0.5 mL Dowfax 2A1 to 250 mL of ammonium molybdate solution, mix gently. Prepare this solution daily. This volume of solution is sufficient for an 8-hour run.

7.2.3 Ascorbic Acid Solution - Dissolve 4.4 g of ascorbic acid ($C_6H_8O_6$) in 200 mL of reagent water and 12.5 mL of acetone(C_3H_6O), dilute to 250 mL with reagent water. Store in a plastic container. This solution is stable for one week if stored at 4°C. Discard the solution if it turns brown.

7.2.4 Oxalic Acid Solution - Dissolve 50 g of oxalic acid ($C_2H_2O_4$) in approximately 800 mL of reagent water and dilute to 1000 mL with reagent water. Store in a plastic container. This solution is stable for approximately 3-months.

7.2.5 Refractive Index Matrix Solution - Add 0.5 mL Dowfax 2A1 to 250 mL of 0.05 M sulfuric acid solution and mix gently.

7.2.6 Colored SYNC Peak Solution - Add 50 µL of blue food coloring solution to 1000 mL reagent water and mix thoroughly. The solution should give a peak of between 25 to 100 percent full scale, otherwise the volume of food coloring added needs to be adjusted.

7.2.7 Calibration Standards - Prepare a series of calibration standards (CAL) by diluting suitable volumes of Stock Silicate Solution (Section 7.1.3) to 100 mL with

reagent water or low nutrient seawater. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards with equal increments in concentration should be used to construct the calibration curve.

When working with samples of a narrow range of salinities (± 2 ‰), it is recommended that the CAL solutions be prepared in Low Nutrient Seawater (Section 7.1.4) diluted to the salinity of samples, and the Sampler Wash Solution also be Low Nutrient Seawater (Section 7.1.4) diluted to that salinity. If this procedure is performed, it is not necessary to perform the salt error and refractive index corrections outlined in Sections 12.2 and 12.3.

When analyzing samples of varying salinities, it is recommended that the calibration standard solutions and sampler wash solution be prepared in reagent water and corrections for salt error and refractive index be made to the sample concentrations (Section 12.2 and 12.3).

7.2.8 Saline Silicate Standards - If CAL solutions will not be prepared to match sample salinity, then saline silicate standards must be prepared in a series of salinities in order to quantify the salt error, the change in the colorimetric response of silicate due to the change in the ionic strength of the solution. The following dilutions prepared in 100 mL volumetric flasks, diluted to volume with reagent water, are suggested.

Salinity (‰)	Volume of LNSW(mL)	Volume(mL) Si stock std	Conc. mg Si/L
0	0	1.5	1.5
9	25	1.5	1.5
18	50	1.5	1.5
27	75	1.5	1.5
35	98	1.5	1.5

8.0 Sample Collection, Preservation and Storage

8.1 Sample Collection - Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods: hydrocast or submersible pump systems.

8.1.1 A hydrocast uses a series of sampling bottles (Niskin, Go-Flo or equivalent) that are attached at fixed intervals to a hydro wire. These bottles are sent through the water column open and are closed either electronically or via a mechanical messenger when the bottles have reached the desired depth.

8.1.2 In a submersible pump system, a weighted hose is sent to the desired depth in the water column and water is pumped from that depth to the deck of the ship for sample processing.

8.1.3 For collecting surface samples, an acid - cleaned plastic bucket or a large plastic bottle can be used as convenient samplers. Wash the sampler three times with sample water before collecting samples.

8.1.4 Samples must be filtered through a 0.45 µm non-glass membrane filters as soon as possible after collection.

8.1.5 60-mL high density polyethylene bottles are used for sample storage. Sample bottles should be rinsed 3 times with about 20 mL of sample, shaking with the cap in place after each rinse. Pour the rinse water into the cap to dissolve and rinse away salt crusts trapped in the threads of the cap. Finally, fill the sample bottle about 3/4 full, and screw the cap on firmly. The expansion of water on freezing will squeeze some of the brine out of the bottle if the bottle was overfilled.

8.2 Sample Preservation - After collection and filtration, samples should be analyzed as soon as possible. If samples will be analyzed within 24 hours then keep refrigerated in tightly sealed, high density polyethylene bottles in the dark at 4°C until analysis can be performed.

8.3 Sample Storage - If samples are to be frozen for long-term storage ensure that none of the sample bottles are filled more than 3/4 full and the cap is firmly screwed on. Place the bottles upright on a rack and store in the freezer (-20°C).

Before analysis, frozen samples must be taken out of the freezer and allowed to thaw in a refrigerator at 4°C in the dark. Thawing times depend upon sample salinities. It was found that the frozen low salinity estuarine water took 4 days to thaw. After completely thawing, take samples out of the refrigerator and mix thoroughly. Keep samples in the dark at room temperature overnight before analysis.

Effects of thawing conditions on the recoveries of frozen samples are more pronounced in low salinity estuarine

waters than high salinity coastal waters as shown in following results:

Day	Recovery (%)			Remark
	S=35.85	S=18.07	S=2.86	
0	100.00	100.00	100.00	
7	102.44	102.65	89.37	a
14	98.59	101.06	86.49	a
21	99.51	99.30	83.49	a
27	98.86			a
		98.86	91.43	b
35	98.70			b
		98.66	92.98	b
42	100.87			b
49		102.44	79.12	c
		103.92	79.10	d
		99.92	89.68	e
56	103.47			c
	104.12			d
	99.35			e
84		100.80	91.71	f
		99.90	93.81	g
91	100.65			f
	100.22			g

S = Salinity
a, overnight thawing at room temperature
b, 20 hours thawing at room temperature
c, 24 hours thawing at room temperature
d, 8 hours thawing at room temperature then heating at 80°C for 16 hours
e, 24 hours thawing at room temperature in the dark
f, 4 days thawing at room temperature in the dark
g, 4 days thawing at 4°C in a refrigerator in the dark

To ensure a slow process of depolymerization of polysilicate to occur, thawing the frozen samples in the dark at 4°C for 4 days is critical condition for obtaining high recoveries of silicate in frozen samples. A maximum holding time for frozen estuarine and coastal waters is two months.[9-11]

9.0 Quality Control

9.1 Each laboratory using this method is required to implement a formal quality control(QC) program. The minimum requirements of this program consists of an initial demonstration of performance, continued analysis of Laboratory Reagent Blanks (LRB), laboratory duplicates and Laboratory Fortified Blanks (LFB) with each set of samples as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The Initial demonstration of performance is used to characterize instrument performance by determining the MDL and LDR and laboratory performance by analyzing quality control samples prior to analysis of samples using this method.

9.2.2 Method Detection Limits (MDLs) should be established using a low level seawater sample containing, or fortified at, approximately 5 times the estimated detection limit. To determine MDL values, analyze at least seven replicate aliquots of water which have been processed through the entire analytical method. Perform all calculations defined in the method and report concentration in appropriate units. Calculate the MDL as follows:

$$MDL = (t)(S)$$

where, S = the standard deviation of the replicate analyses

t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; t = 3.143 for six degrees of freedom.

MDLs should be determined every 6-months or whenever a significant change in background or instrument response occurs or a new matrix is encountered.

9.2.3 The LDR should be determined by analyzing a minimum of eight calibration standards ranging from 0.03 to 5.00 mg Si/L across all sensitivity settings (Absorbance Units Full Scale) of the detector. Standards and sampler wash solutions should be prepared in low nutrient seawater with salinities similar to that of samples, therefore a correction factor for salt error, or refractive index, will not be necessary. Normalize responses by multiplying the response by the Absorbance Units Full Scale output range setting. Perform the linear regression of normalized response vs. concentration and obtain the constants m and b, where m is the slope and b is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration C_C, that is within 100 ± 10% of the known concentration, C, where $C_C = (R-b)/m$. That concentration

defines the upper limit of the LDR for the instrument. Should samples be encountered that have a concentration that is 90% of the upper limit of LDR, then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) - A laboratory should analyze at least one LRB with each set of samples. LRB data are used to assess contamination from the laboratory environment. Should an analyte value in the LRB exceed the MDL, then laboratory or reagent contamination should be suspected. When the LRB value constitutes 10% or more of the analyte concentration determined for a sample, duplicates of the sample must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank (LFB) - A laboratory should analyze at least one LFB with each set of samples. The LFB must be at a concentration that is within the daily calibration range. The LFB data are used to calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90 -110%, the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90 -110%. When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

Upper Control Limit = x + 3S
Lower Control Limit = x - 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix (LFM)

9.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the total number of samples or one sample per sample set, whichever is greater. The analyte added should be 2-4 times the ambient concentration and should be at least four times greater than the MDL.

9.4.2 Calculate percent recovery of analyte, corrected for background concentration measured in a separate unfortified sample. These values should be compared with the values obtained from the LFBs. Percent recoveries may be calculated using the following equation:

$$R = \frac{(C_S - C)}{S} \times 100$$

where, R = percent recovery

C_S = measured fortified sample concentration (background + addition in mg Si/L)

C = sample background concentration (mg Si/L)

S = concentration in mg Si/L added to the environmental sample.

9.4.3 If the recovery of the analyte falls outside the required control limits of 90-110%, but the laboratory performance for that analyte is within the control limits, the fortified sample should be prepared again and analyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related and the sample data should be flagged.

10.0 Calibration and Standardization

10.1 At least five calibration standards should be prepared daily for system calibration.

10.2 A calibration curve should be constructed for each run by analyzing a set of calibration standard solutions. A run should contain no more than 60 samples.

It is suggested that a large set of samples be analyzed in several sets with individual calibration curves.

10.3 Place the calibration standards before samples for each run. All the calibration solutions should be analyzed in duplicate.

10.4 The calibration curve containing five data points or more should have a correlation coefficient 0.995.

10.5 Place a high standard solution cup and follow by two blank cups to quantify the **carry-over** of the system. The difference in peak heights between two blank cups is due to the carry over from the high standard cup. The carry-over coefficient, k, is calculated as follows:

$$k = \frac{P_{b1} - P_{b2}}{P_{high}} X\ 100$$

where, P_{high} = the peak height of the high silicate standard

P_{b1} = the peak height of the first blank sample

P_{b2} = the peak height of the second blank sample.

The carry over coefficient, k, for a system should be measured in seven replicates in order to obtain a statistically significant number. The k should be remeasured when a change in the plumbing of the manifold or replacement of pump tube occur.

The carry over correction (CO) on a given peak, i, is proportional to the peak height of the preceding sample, P_{i-1}.

$$CO = kxP_{i-1}$$

To correct a given peak height reading, P_i, one subtracts the carry over correction.[12,13]

$$P_{i,c} = P_i - CO$$

where $P_{i,c}$ is corrected peak height. The correction for carry over should be applied to all the peak heights throughout a run. The carry over should be less than 2%.

10.6 Place a high standard solution at the end of a run to check sensitivity drift. The sensitivity drift should be ± 5% during the run.

11.0 Procedure

11.1 If samples are frozen, thaw the sample at 4°C in the dark as outlined in Section 8.3. Mix samples thoroughly prior to analyses.

11.2 Turn on the continuous flow analyzer and PC components and warm up at least 30 minutes.

11.3 Set up the cartridge and pump tubes as shown in Figure 1.

Note: Fluctuation of ambient temperature can cause erratic results due to the effect of temperature on kinetics of color development. The laboratory temperature should be maintained as close to a constant temperature as possible. The cartridge should be away from the direct path of air flow from a heater or air conditioner. In cases such as on a ship where the fluctuation of temperature can be extreme, the temperature effect can be minimized by increasing the length of mixing coil 1 (Figure 1) to bring the formation of silicomolybdic acid reaction to completion.

11.4 Set the wavelength at 660 nm on the spectrometer/monochrometer.

Note: The absorption spectra of silicomolybdeum blue complex has two maxima at 820 nm and 660 nm with 820 nm higher than 660 nm. This method measures absorbance at 660 nm because the detector works in the range of 380 to 800 nm. The sensitivity of the method is satisfactory at 660 nm. The sensitivity, however, can be improved by using 820 nm if this wavelength is available on the detector.

11.5 On the monochromator, set the Absorbance Unit Full Scale at an appropriate setting according to the highest concentration of silicate in the samples. The highest setting used in this method was 0.2 for 6 mg Si/L.

11.6 Prepare all reagents and standards.

11.7 Begin pumping the Dowfax start-up solution (Section 7.2.1) through the system and obtain a steady baseline. Place the reagents on-line. The reagent baseline will be higher than the start-up solution baseline. After the reagent baseline has stabilized, reset the baseline.

NOTE: To minimize the noise in the reagent baseline, clean the flow system by sequentially pumping the

sample line with reagent water, 1 N HCl solution, reagent water, 1N NaOH solution for a few minutes each at the end of the daily analysis. Make sure to rinse the system well with reagent water after pumping NaOH solution to prevent precipitation of $Mg(OH)_2$ when seawater is introduced into the system. Keep the reagents and samples free of particulate. Filter the reagents and samples if necessary.

If the baseline drifts upward, pinch the waste line for a few seconds to increase back pressure. If absorbance drops down rapidly when back pressure increases, this indicates that there are air bubbles trapped in the flow cell. Attach a syringe at the waste outlet of the flowcell. Air bubbles in the flowcell can often be eliminated by simply attaching a syringe for a few minutes or, if not, dislodged by pumping the syringe pistion. Alternatively, flushing the flowcell with alcohol was found to be effective in removing air bubbles from the flowcell.

For analysis of samples with a narrow range of salinities (± 2 ‰), it is recommended that the wash water in the sampler be prepared in Low Nutrient Seawater diluted to the salinity of samples in place of reagent water. For samples with varying salinities, it is suggested that reagent waters and procedures in Sections 12.2 and 12.3 be employed.

11.8 A good sampling rate is approximately 60 samples per hour with 40 seconds of sample time and 20 seconds of wash time.

11.9 Use 10% HCl followed by reagent water to rinse sample cups. Place CAL solutions and saline standards (optional) in sampler. Complete filling the sampler tray with samples, laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Place a blank every ten samples and between samples of high and low concentrations.

11.10 Commence analysis.

11.11 If the reagent water is used as wash solution instead of Low Nutrient Seawater and an operator wants to quantify the refractive index correction due to the difference in salinities between sample and wash solution, the following procedures are used. Replace ammonium molybdate solution (Section 7.1.2) with refractive index matrix solution (Section 7.2.5). All other reagents remain the same. Replace the synchronization cup with the colored SYNC peak solution (Section 7.2.6). Commence analysis and obtain a second set of peak heights for all standards and samples. The peak heights obtained from these measurements must be subtracted from the peak heights of samples analyzed with color developing reagent pumping through the system. If a low refractive index flowcell is used, the correction for refractive index is negligible. This procedure is optional.

12.0 Data Analysis and Calculations

12.1 Concentrations of silicate are calculated from the linear regression, obtained from the standard curve in which the concentrations of the calibration standards are entered as the independent variable, and their corresponding peak heights are the dependent variable.

12.2 Refractive Index Correction for Estuarine and Coastal Samples (optional)

12.2.1 Obtain a second set of peak heights for all standards and samples with refractive index matrix solution being pumped through the system in place of color reagent (ammonium molybdate solution). All other reagents remain the same. The peak heights for the refractive index correction must be obtained at the same Absorbance Unit Full Scale range setting and on the same monochromator as the corresponding samples and standards.

12.2.2 Subtract the refractive index peak heights from the peak heights obtained from the silicate determination.

12.2.3 An alternative approach is to measure the relationship between the sample salinity and refractive index on a particular detector.

First analyze a set of silicate standards in reagent water with color reagent and obtain a linear regression from the standard curve.

Prepare a set of different salinity samples with LNSW. Analyze these samples with refractive index matrix solution being pumped through the system in place of color reagent (ammonium molybdate solution). All other reagents remain the same. The peak heights for the refractive index correction must be obtained at the same Absorbance Unit Full Scale setting and on the same monochromator as the corresponding standards.

For each sample, the apparent silicate concentration due to refractive index is then calculated from its peak height obtained with refractive index reagent and the regression of silicate standards obtained with color reagent pumping

through the system. Salinity is entered as the independent variable and the apparent silicate concentration due to refractive index in that detector is entered as the dependent variable. The resulting regression allows the operator to calculate apparent silicate concentration due to refractive index when the salinity is known. Thus, the operator would not be required to obtain refractive index peak heights for all samples.

12.2.4 Refractive index correction can be minimized by using a low refractive index flowcell. An example of typical results using a low refractive index flowcell follows:

Salinity (‰)	Apparent silicate conc. due to refractive index (mg Si/L)
4.5	0.0003
9.0	0.0005
18.0	0.0016
27.0	0.0027
36.0	0.0042

12.2.5 An example of a typical equation is:

Apparent silicate (mg Si/L) = $0.00001953*S^{1.5}$

where S is sample salinity. The form of fitted equation might vary as the design of flowcell, so the operators are advised to obtain the appropriate equation which has the best fit of their own data with the least fitting coefficients.

12.3 Correction for Salt Error in Estuarine and Coastal Samples

12.3.1 When calculating concentrations of samples of varying salinities from standards and wash solution prepared in reagent water, it is usual to first correct for refractive index errors, then correct for the change in color development due to the differences in ionic strength between samples and standards (salt error). The refractive index correction is negligible, so is optional, but correction for salt error is necessary.

12.3.2 Plot the salinity of the saline standards (Section 7.2.8) as the independent variable, and the apparent concentration of silicate (mg Si/L) from the peak height (corrected for refractive index) calculated from the regression of standards in reagent water, as the dependent variable for all 1.50 mg Si/L standards. The resulting regression equation allows the operator to correct the concentrations of samples of known salinity for the color suppression due to salinity effect, e.g., salt error. An example of typical results follows:

Salinty (‰)	Peak height of 1.50 mg Si/L	Uncorrected Si conc.calculated from standards in reagent water
0	2503	1.50
9	2376	1.32
18	2282	1.27
27	2250	1.25
36	2202	1.23

12.3.3 An example of a typical equation to correct for salt error is:

$$\text{Corrected mg Si/L} = \frac{\text{Uncorrected mg Si/L}}{1 - 0.02186 \times \sqrt{S}}$$

where S is salinity.

12.3.4 Results of sample analyses should be reported in mg Si/L or in µg Si/L.

mg Si/L = ppm (parts per million)
µg Si/L = ppb (part per billion)

13.0 Method Performance

13.1 Single Laboratory Validation

13.1.1 Method Detection Limit - A method detection limit (MDL) of 0.0012 mg Si/L has been determined by one laboratory in seawaters of three different salinities.

Salinity (‰)	SD (µg/L)	Recovery (%)	MDL (µg/L)
36	0.3924	105	1.233
36	0.4980	107	1.565
27	0.2649	104	0.832
27	0.3362	104	1.056
27	0.4671	100	1.468
18	0.3441	101	1.081
18	0.2809	105	0.883
18	0.2432	104	0.764
3	0.3441	101	1.081
3	0.2331	102	0.733
3	0.1963	98	0.617
3	0.2809	99	0.883

13.1.2 Single Analyst Precision - A single laboratory analyzed three samples collected from the Miami River and Biscayne Bay areas of Florida. Seven replicates of

each sample were processed and analyzed with salinities ranging from 2.86 to 35.85. The results were as follows:

Sample	Salinity (‰)	Concentration (mg Si/L)	RSD (%)
1	35.85	0.097	1.2
2	18.07	1.725	1.4
3	2.86	3.322	0.9

13.1.3 Laboratory Fortified Sample Matrix - Laboratory fortified sample matrixes were processed in three different salinities ranging from 2.86 to 35.85 and ambient concentrations from 0.095 to 3.322 mg Si/L with three fortified levels at each salinity. Seven replicates of each sample were analyzed and the results were as follows:

Salinity (‰)	Concentration (mg Si/L)		RSD (%)	Recovery (%)
	Ambient	Fortified		
35.85	0.095	0.1647	0.82	99.37
35.85	0.095	0.2196	1.34	100.61
35.85	0.095	0.2747	1.74	99.62
18.07	1.725	0.5517	1.11	107.18
18.07	1.725	1.1008	0.77	104.69
18.07	1.725	1.6508	0.98	103.62
2.86	3.322	0.5421	0.99	101.03
2.86	3.322	1.0801	1.26	103.22
2.86	3.322	1.6188	0.98	100.59

13.2 Multi-Laboratory Validation

Multi-laboratory validation has not been conducted for this method and, therefore, multi-laboratory data is currently unavailable.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

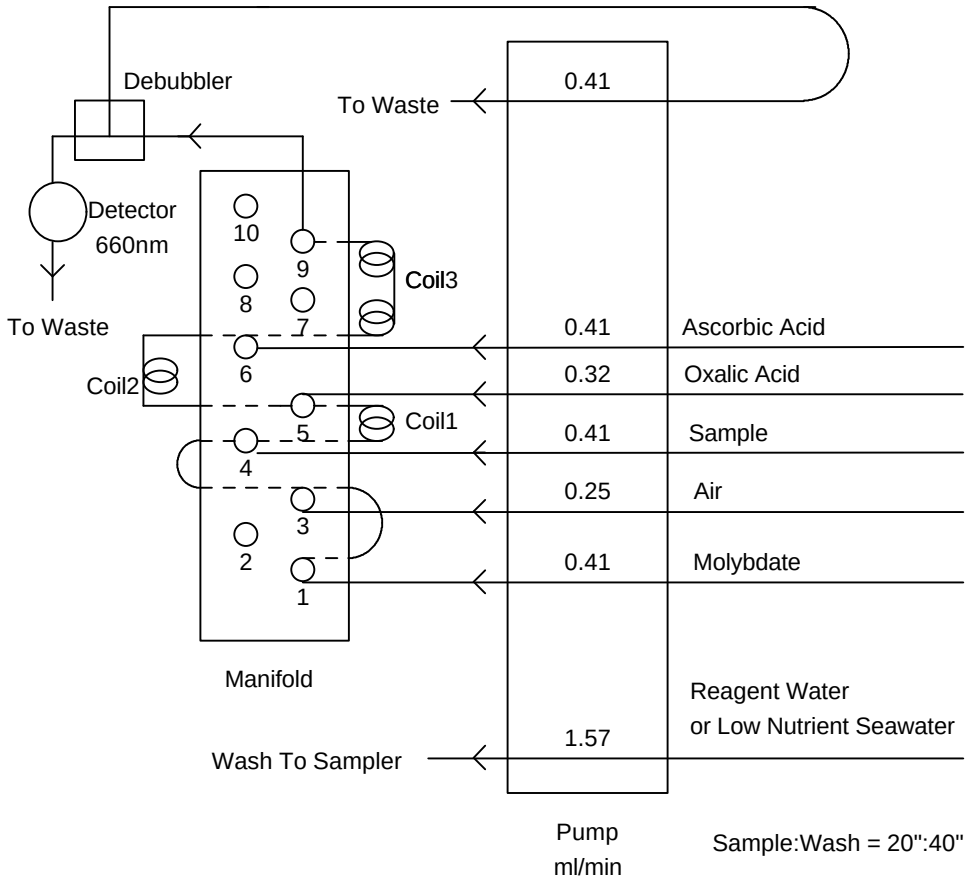

Figure 1. Manifold Configuration for Silicate Analysis.

Appendix D
Laboratory Method – Nitrate – Nitrogen – US EPA 352.1

METHOD #: 352.1	Approved for NPDES and SDWA (Issued 1971)
TITLE:	Nitrogen, Nitrate (Colorimetric, Brucine)
ANALYTE:	CAS # N Nitrogen 7727-37-9 NO3 Nitrate
INSTRUMENTATION:	Spectrophotometer
STORET No.	Total 00620

1.0 Scope and Application

1.1 This method is applicable to the analysis of drinking, surface and saline waters, domestic and industrial wastes. Modification can be made to remove or correct for turbidity, color, salinity, or dissolved organic compounds in the sample.

1.2 The applicable range of concentrations is 0.1 to 2 mg NO_3-N/liter.

2.0 Summary of Method

2.1 This method is based upon the reaction of the nitrate ion with brucine sulfate in a 13 N H_2SO_4 solution at a temperature of 100°C. The color of the resulting complex is measured at 410 nm. Temperature control of the color reaction is extremely critical.

3.0 Sample Handling and Preservation

3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc. H_2SO_4 per liter) and refrigeration.

4.0 Interferences

4.1 Dissolved organic matter will cause an off color in 13 N H_2SO_4 and must be compensated for by additions of all reagents except the brucine-sulfanilic acid reagent. This also applies to natural color present not due to dissolved organics.

4.2 The effect of salinity is eliminated by addition of sodium chloride to the blanks, standards and samples.

4.3 All strong oxidizing or reducing agents interfere. The presence of oxidizing agents may be determined with a total residual chlorine test kit.

4.4 Residual chlorine interference is eliminated by the addition of sodium arsenite.

4.5 Ferrous and ferric iron and quadrivalent manganese give slight positive interferences, but in concentrations less than 1 mg/L these are negligible.

4.6 Uneven heating of the samples and standards during the reaction time will result in erratic values. The necessity for absolute control of temperature

during the critical color development period cannot be too strongly emphasized.

5.0 Apparatus

5.1 Spectrophotometer or filter photometer suitable for measuring absorbance at 410 nm.
5.2 Sufficient number of 40-50 mL glass sample tubes for reagent blanks, standards and samples.
5.3 Neoprene coated wire racks to hold sample tubes.
5.4 Water bath suitable for use at 100°C. This bath should contain a stirring mechanism so that all tubes are at the same temperature and should be of sufficient capacity to accept the required number of tubes without significant drop in temperature when the tubes are immersed.
5.5 Water bath suitable for use at 10-15°C.

6.0 Reagents

6.1 Distilled water free of nitrite and nitrate is to be used in preparation of all reagents and standards.
6.2 Sodium chloride solution (30%): Dissolve 300 g NaCl in distilled water and dilute to 1 liter.
6.3 Sulfuric acid solution: Carefully add 500 mL conc. H_2SO_4 to 125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.
6.4 Brucine-sulfanilic acid reagent: Dissolve 1 g brucine sulfate $[(C_{23}H_{26}N_2O_4)_2 \bullet H_2SO_4 \bullet 7H_2O]$ and 0.1 g sulfanilic acid ($NH_2C_6H_4SO_3H \bullet H_2O$) in 70 mL hot distilled water. Add 3 mL conc. HCl, cool, mix and dilute to 100 mL with distilled water. Store in a dark bottle at 5 °C. This solution is stable for several months; the pink color that develops slowly does not effect its usefulness. Mark bottle with warning: CAUTION: Brucine Sulfate is toxic; take care to avoid ingestion.
6.5 Potassium nitrate stock solution: 1.0 mL = 0.1 mg NO_3-N. Dissolve 0.7218 g anhydrous potassium nitrate (KNO_3) in distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 mL chloroform per liter. This solution is stable for at least 6 months.
6.6 Potassium nitrate standard solution: 1.0 mL = 0.001 mg NO_3-N. Dilute 10.0 mL of the stock solution (6.5) to 1 liter in a volumetric flask. This standard solution should be prepared fresh weekly.
6.7 Acetic acid (1 + 3): Dilute 1 volume glacial acetic acid (CH_3COOH) with 3 volumes of distilled water.
6.8 Sodium hydroxide (lN): Dissolve 40 g of NaOH in distilled water. Cool and dilute to 1 liter.

7.0 Procedure

7.1 Adjust the pH of the samples to approximately 7 with acetic acid (6.7) or sodium hydroxide (6.8). If necessary, filter to remove turbidity.
7.2 Set up the required number of sample tubes in the rack to handle reagent blank, standards and samples. Space tubes evenly throughout the rack to allow

for even flow of bath water between the tubes. This should assist in achieving uniform heating of all tubes.

7.3 If it is necessary to correct for color or dissolved organic matter which will cause color on heating, a set of duplicate samples must be run to which all reagents except the brucine-sulfanilic acid have been added.

7.4 Pipette 10.0 mL of standards and samples or an aliquot of the samples diluted to 10.0 mL - into the sample tubes.

7.5 If the samples are saline, add 2 mL of the 30% sodium chloride solution (6.2) to the reagent blank, standards and samples. For fresh water samples, sodium chloride solution may be omitted. Mix contents of tubes by swirling and place rack in cold water bath (0 - 10°C).

7.6 Pipette 10.0 mL of sulfuric acid solution (6.3) into each tube and mix by swirling. Allow tubes to come to thermal equilibrium in the cold bath. Be sure that temperatures have equilibrated in all tubes before continuing.

7.7 Add 0.5 mL brucine-sulfanilic acid reagent (6.4) to each tube (except the interference control tubes, 7.3) and carefully mix by swirling, then place the rack of tubes in the 100°C water bath for exactly 25 minutes.
CAUTION: Immersion of the tube rack into the bath should not decrease the temperature of the bath more than 1 to 2°C. In order to keep this temperature decrease to an absolute minimum, flow of bath water between the tubes should not be restricted by crowding too many tubes into the rack. If color development in the standards reveals discrepancies in the procedure, the operator should repeat the procedure after reviewing the temperature control steps.

7.8 Remove rack of tubes from the hot water bath and immerse in the cold water bath and allow to reach thermal equilibrium (20-25°C).

7.9 Read absorbance against the reagent blank at 410 nm using a 1 cm or longer cell.

8.0 Calculation

8.1 Obtain a standard curve by plotting the absorbance of standards run by the above procedure against mg NO_3-N/L. (The color reaction does not always follow Beer's law).

8.2 Subtract the absorbance of the sample without the brucine-sulfanilic reagent from the absorbance of the sample containing brucine-sulfanilic acid and determine mg NO_3-N/L. Multiply by an appropriate dilution factor if less than 10 mL of sample is taken.

9.0 Precision and Accuracy

9.1 Twenty-seven analysts in fifteen laboratories analyzed natural water samples containing exact increments of inorganic nitrate, with the following results:

Increment as Nitrogen, Nitrate mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Bias, mg N/liter
0.16	0.92	-6.79	-0.01
0.19	0.083	+8.30	+0.02
1.08	0.245	+4.12	+0.04
1.24	0.214	+2.82	+0.04

(FWPCA Method Study 2, Nutrient Analyses).

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Appendix E
Laboratory Method – Nitrite - Nitrate – US EPA 353.3

METHOD #: 353.3	Approved for NPDES and SDWA (Issued 1974)
TITLE:	Nitrogen, Nitrate-Nitrite (Spectrophotometric, Cadmium Reduction)
ANALYTE:	CAS # N Nitrogen 7727-37-9 NO_3 Nitrate NO_2 Nitrite
INSTRUMENTATION:	Spectrophotometer
STORET No.	Total 00630

1.0 Scope and Application

1.1 This method is applicable to the determination of nitrite singly, or nitrite and nitrate combined in drinking, surface and saline waters, domestic and industrial wastes. The applicable range of this method is 0.01 to 1.0 mg/L nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2.0 Summary of Method

2.1 A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured, spectrophotometrically. Separate, rather than combined nitrate-nitrite, values are readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction step.

3.0 Sample Handling and Preservation

3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL H_2SO_4 per liter) and refrigeration.

CAUTION: Samples for reduction column must not be preserved mercuric chloride.

4.0 Interferences

4.1 Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is found in a soluble state, the sample may be pre-filtered through a glass fiber filter or a 0.45 *u* membrane filter. Highly turbid samples may be pretreated with zinc sulfate before filtration to remove the bulk of particulate matter present in the sample.

4.2 Low results might be obtained for samples that contain high concentrations of

iron, copper or other metals. EDTA is added to the samples to eliminate this interference.

4.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

4.4 This procedure determines both nitrate and nitrite. If only nitrate is desired, a separate determination must be made for nitrite and subsequent corrections made. The nitrite may be determined by the procedure below without the reduction step.

5.0 Apparatus

5.1 Reduction column: The column in Figure 1 was constructed from a 100 mL pipet by removing the top portion. This column may also be constructed from two pieces of tubing joined end to end. A 10 mm length of 3 cm I.D. tubing is joined to a 25 cm length of 3.5 mm I.D. tubing.

5.2 Spectrophotometer for use at 540 nm, providing a light path of 1 cm or longer.

6.0 Reagents

6.1 Granulated cadmium: 40-60 mesh (MCB Reagents).

6.2 Copper-Cadmium: The cadmium granules (new or used) are cleaned with dilute HCl and copperized with 2% solution of copper sulfate in the following manner:

6.2.1 Wash the cadmium with dilute HCl (6.10) and rinse with distilled water. The color of the cadmium should be silver.

6.2.2 Swirl 25 g cadmium in 100 mL portions of a 2% solution of copper sulfate (6.11) for 5 minutes or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

6.2.3 Wash the copper-cadmium with distilled water (at least 10 times) to remove all the precipitated copper. The color of the cadmium so treated should be black.

6.3 Preparation of reaction column: Insert a glass wool plug into the bottom of the reduction column and fill with distilled water. Add sufficient copper-cadmium granules to produce a column 18.5 cm in length. Maintain a level of distilled water above the copper- cadmium granules to eliminate entrapment of air. Wash the column with 200 mL of dilute ammonium chloride solution (6.5). The column is then activated by passing through the column 100 mL of a solution composed of 25 mL of a 1.0 mg/L NO_3-N standard and 75 mL of ammonium chloride - EDTA solution (6.4). Use a flow rate between 7 and 10 mL per minute.

6.4 Ammonium chloride- EDTA solution: Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetraacetate in 900 mL of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide (6.9) and dilute to 1 liter.

6.5 Dilute ammonium chloride-EDTA solution: Dilute 300 mL of ammonium chloride- EDTA solution (6.4) to 500 mL with distilled water.

6.6 Color reagent: Dissolve 10 g sulfanilamide and 1 g N-(1-naphthyl) ethylene diamine dihydrochloride in a mixture of 100 mL conc. phosphoric acid and 800

mL of distilled water and dilute to 1 liter with distilled water.

6.7 Zinc sulfate solution: Dissolve 100 g $ZnSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.

6.8 Sodium hydroxide solution, 6N: Dissolve 240 g NaOH in 500 mL distilled water, cool and dilute to 1 liter.

6.9 Ammonium hydroxide, conc.

6.10 Dilute hydrochloric acid, 6N: Dilute 50 mL of conc. HCl to 100 mL with distilled water.

6.11 Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 mL of distilled water and dilute to 1 liter.

6.12 Stock nitrate solution: Dissolve 7.218 g KNO_3 in distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform per liter. This solution is stable for at least 6 months. 1.0 mL = 1.00 mg NO_3-N.

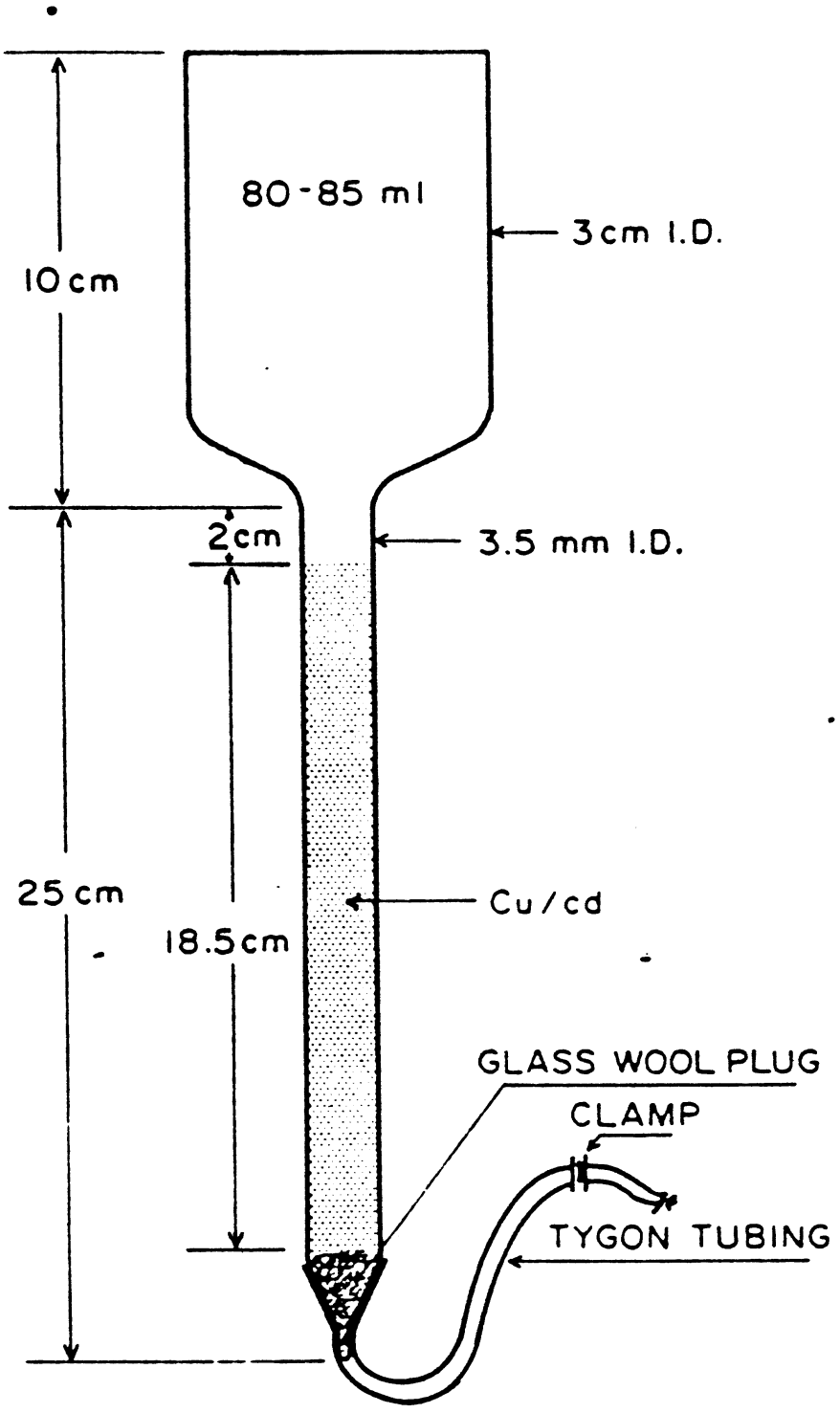

FIGURE 1. REDUCTION COLUMN

6.13 Standard nitrate solution: Dilute 10.0 mL of nitrate stock solution (6.12) to 1000 mL with distilled water. 1.0mL = 0.01 mgNO_3-N.

6.14 Stock nitrite solution: Dissolve 6.072 g KNO_2 in 500 mL of distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform and keep under refrigeration. Stable for approximately 3 months. 1.0 mL = 1.00 mg NO_2-N.

6.15 Standard nitrite solution: Dilute 10.0 mL of stock nitrite solution (6.14) to 1000 mL with distilled water. 1.0 mL = 0.01 mg NO_2-N.

6.16 Using standard nitrate solution (6.13) prepare the following standards in 100 mL volumetric flasks:

Conc., mg-NO3-N/L	mL of Standard Solution/100.0 mL
0.00	0.0
0.05	0.5
0.10	1.0
0.20	2.0
0.50	5.0
1.00	10.0

7.0 Procedure

7.1 Turbidity removal: One of the following methods may be used to remove suspended matter.

7.1.1 Filter sample through a glass fiber filter or a 0.45u membrane filter.

7.1.2 Add 1 mL zinc sulfate solution (6.7) to 100 mL of sample and mix thoroughly. Add 0.4-0.5 mL sodium hydroxide solution (6.8) to obtain a pH of 10.5 as determined with a pH meter. Let the treated sample stand a few minutes to allow the heavy flocculent precipitate to settle. Clarify by filtering through a glass fiber filter or a 0.45u membrane filter.

7.2 Oil and grease removal: Adjust the pH of 100 mL of filtered sample to 2 by addition of conc. HCl. Extract the oil and grease from the aqueous solution with two 25 mL portions of a non-polar solvent (Freon, chloroform or equivalent).

7.3 If the pH of the sample is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. NH_4OH. This is done to insure a sample pH of 8.5 after step 7.4.

7.4 To 25.0 mL of sample or an aliquot diluted to 25.0 ml, add 75 mL of ammonium chloride- EDTA solution (6.4) and mix.

7.5 Pour sample into column and collect sample at a rate of 7-10 mL per minute.

7.6 Discard the first 25 ml, collect the rest of the sample (approximately 70 ml) in the original sample flask. Reduced samples should not be allowed to stand longer than 15 minutes before addition of color reagent, step 7.7.

7.7 Add 2.0 mL of color reagent (6.6) to 50.0 mL of sample. Allow 10 minutes for color development. Within 2 hours measure the absorbance at 540 nm against a reagent blank. NOTE: If the concentration of sample exceeds 1.0 mg NO_3-N/L, the remainder of the reduced sample may be used to make an appropriate dilution before proceeding with step

7.8 Standards: Carry out the reduction of standards exactly as described for the

samples. At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration to verify the efficiency of the reduction column.

8.0 Calculation

8.1 Obtain a standard curve by plotting the absorbance of standards run by the above procedure against NO_3-N mg/L. Compute concentration of samples by comparing sample absorbance with standard curve.

8.2 If less than 25 mL of sample is used for the analysis the following equation should be used:

$$\text{mg } NO_2 + NO_3 - \text{N/L} = \frac{A \times 25}{\text{mL sample used}}$$

where:

A = Concentration of nitrate from standard curve.

9.0 Precision and Accuracy

9.1 In a single laboratory (EMSL), using sewage samples at concentrations of 0.04, 0.24, 0.55 and 1.04 mg $NO_3 + NO_2$-N/L, the standard deviations were ± 0.005, ± 0.004, ± 0.005 and ± 0.01, respectively.

9.2 In a single laboratory (EMSL), using sewage samples at concentrations of 0.24, 0.55, and 1.05 mg $NO_3 + NO_2$-N/L, the recoveries were 100%, 102% and 100%, respectively.

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Appendix F
Laboratory Method – Ammonia - Nitrogen – US EPA 350.1

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of ammonia in drinking, ground, surface, and saline waters, domestic and industrial wastes.

1.2 The applicable range is 0.01-2.0 mg/L NH_3 as N. Higher concentrations can be determined by sample dilution. Approximately 60 samples per hour can be analyzed.

1.3 This method is described for macro glassware; however, micro distillation equipment may also be used.

2.0 SUMMARY OF METHOD

2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Section 9.0, Quality Control.

3.0 DEFINITIONS

3.1 **Calibration Blank (CB)** -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 **Calibration Standard (CAL)** -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 **Instrument Performance Check Solution (IPC)** -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 **Laboratory Fortified Blank (LFB)** -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 **Laboratory Fortified Sample Matrix (LFM)** -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6 **Laboratory Reagent Blank (LRB)** -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7 **Linear Calibration Range (LCR)** -- The concentration range over which the instrument response is linear.

3.8 **Material Safety Data Sheet (MSDS)** -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 **Method Detection Limit (MDL)** -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 **Quality Control Sample (QCS)** -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11 **Stock Standard Solution (SSS)** -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 INTERFERENCES

4.1 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out.

4.2 Residual chorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid (Section 7.6)

5.3.2 Phenol (Section 7.7)

5.3.3 Sodium nitroprusside (Section 7.10)

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 An all-glass distilling apparatus with an 800-1000 mL flask.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water - Ammonia free: Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

Note: All solutions must be made with ammonia-free water.

7.2 Boric acid solution (20 g/L): Dissolve 20 g H_3BO_3 (CASRN 10043-35-3) in reagent water and dilute to 1 L.

7.3 Borate buffer: Add 88 mL of 0.1 N NaOH (CASRN 1310-73-2) solution to 500 mL of 0.025 M sodium tetraborate solution (5.0 g anhydrous $Na_2B_4O_7$ [CASRN 1330-43-4] or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ [CASRN 1303-96-4] per L) and dilute to 1 L with reagent water.

7.4 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in reagent water and dilute to 1 L.

7.5 Dechlorinating reagents: A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

7.5.1 Sodium thiosulfate: Dissolve 3.5 g $Na_2S_2O_3 \cdot 5H_2O$ (CASRN 10102-17-7) in reagent water and dilute to 1 L. One mL of this solution will remove 1 mg/L of residual chlorine in 500 mL of sample.

7.5.2 Sodium sulfite: Dissolve 0.9 g Na2SO_3 (CASRN 7757-83-7) in reagent water and dilute to 1 L. One mL removes 1 mg/L Cl per 500 mL of sample.

7.6 Sulfuric acid 5 N: Air scrubber solution. Carefully add 139 mL of conc. sulfuric acid (CASRN 7664-93-9) to approximately 500 mL of reagent water. Cool to room temperature and dilute to 1 L with reagent water.

7.7 Sodium phenolate: Using a 1-L Erlenmeyer flask, dissolve 83 g phenol (CASRN 108-95-2) in 500 mL of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 L with reagent water.

7.8 Sodium hypochlorite solution: Dilute 250 mL of a bleach solution containing 5.25% NaOCl (CASRN 7681-52-9) (such as "Clorox") to 500 mL with reagent

water. Available chlorine level should approximate 2-3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

7.9 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) (CASRN 6381-92-6) and approximately six pellets of NaOH in 1 L of reagent water.

7.10 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside (CASRN 14402-89-2) in 1 L of reagent water.

7.11 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH_4Cl (CASRN 12125-02-9), dried at 105°C, in reagent water, and dilute to 1 L. 1.0 mL = 1.0 mg NH_3-N.

7.12 Standard Solution A: Dilute 10.0 mL of stock solution (Section 7.11) to 1 L with reagent water. 1.0 mL = 0.01 mg NH_3-N.

7.13 Standard Solution B: Dilute 10.0 mL of standard solution A (Section 7.12) to 100.0 mL with reagent water. 1.0 mL = 0.001 mg NH_3-N.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH <2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ± 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[9] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]
S = standard deviation of the replicate analyses

MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3S$$
$$\text{LOWER CONTROL LIMIT} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to 10 new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to established an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift, the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where,
- R = percent recovery
- C_s = fortified sample concentration
- C = sample background concentration
- s = concentration equivalent of analyte added to sample

9.4.3 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions (Sections 7.12 and 7.13) to 100 mL with reagent water.

10.2 Process standards and blanks as described in Section 11.0, Procedure.

10.3 Set up manifold as shown in Figure 1.

10.4 Prepare flow system as described in Section 11.0, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.7 After the calibration has been established, it must be verified by the analysis of a suitable QCS. If measurements exceed ±10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Preparation of equipment: Add 500 mL of reagent water to an 800 mL Kjeldahl flask. The addition of boiling chips that have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia.

11.2 Sample preparation: Remove the residual chorine in the sample by adding dechlorinating agent (Section 7.5) equivalent to the chlorine residual. To 400 mL of sample add 1 N NaOH (Section 7.4), until the pH is 9.5, check the pH during addition with a pH meter or by use of a short range pH paper.

11.3 Distillation: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 mL Kjeldahl flask and add 25 mL of the borate buffer (Section 7.3). Distill 300 mL at the rate of 6-10 mL/min. into 50 mL of 2% boric acid (Section 7.2) contained in a 500 mL Erlenmeyer flask.

Note: The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

11.4 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples.

11.5 Allow analysis system to warm up as required. Feed wash water through sample line.

11.6 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

11.7 Switch sample line from reagent water to sampler and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3 Report results in mg NH_3-N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH_3-N/L, the standard deviation was ±0.005.

13.2 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg NH_3-N/L.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202)872-4477.

15.0 WASTE MANAGEMENT

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.3.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

Number of Values Reported	True Value (T)	Mean (X)	Residual for X	Standard Deviation (S)	Residual for S
134	0.270	0.2670	-0.0011	0.0342	0.0015
157	0.692	0.6972	0.0059	0.0476	-0.0070
136	1.20	1.2008	0.0001	0.0698	-0.0112
195	1.60	1.6095	0.0076	0.1023	0.0006
142	3.00	3.0128	0.0069	0.1677	-0.0067
159	3.50	3.4991	-0.0083	0.2168	0.0165
156	3.60	3.5955	-0.0122	0.1821	-0.0234
200	4.20	4.2271	0.0177	0.2855	0.0488
196	8.76	8.7257	-0.0568	0.4606	-0.0127
156	11.0	11.0747	0.0457	0.5401	-0.0495
142	13.0	12.9883	-0.0465	0.6961	0.0027
199	18.0	17.9727	-0.0765	1.1635	0.2106

REGRESSIONS: $X = 1.003T - 0.003$, $S = 0.052T + 0.019$

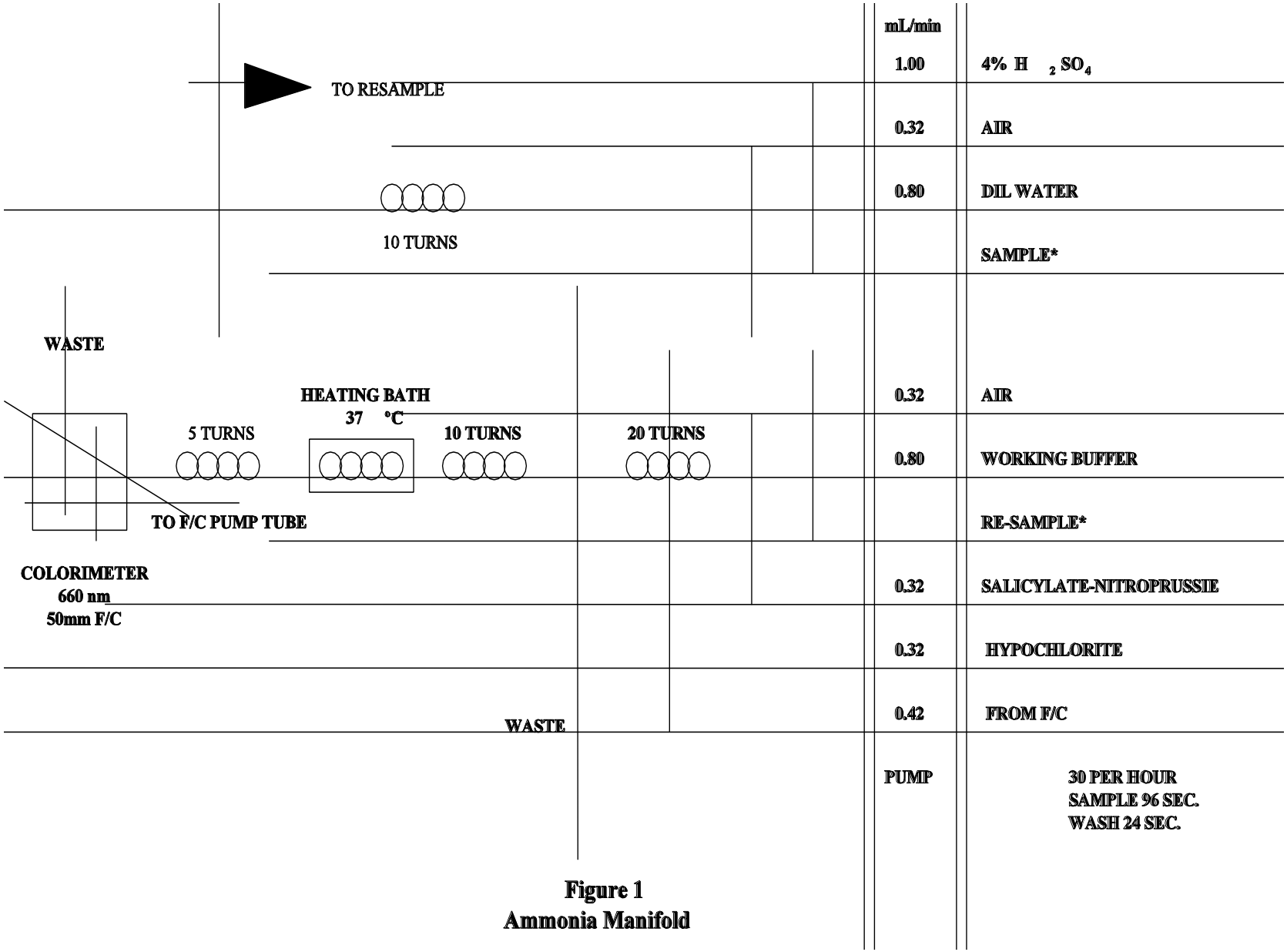

Figure 1
Ammonia Manifold

Appendix G
Laboratory Method – Total Nitrogen – US EPA 351.2

United States Environmental Protection Agency

www.epa.gov August 1993

Method 351.2, Revision 2.0: Determination of Total Kjeldahl Nitrogen by Semi-Automated Colorimetry

METHOD 351.2

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATEDCOLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 351.2

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 The applicable range is 0.1-20 mg/L TKN. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample is heated in the presence of sulfuric acid, H_2SO_4 for two and one half hours. The residue is cooled, diluted to 25 mL and analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

2.3 Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

2.4 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.5 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Section 9.0, Quality Control.

3.0 DEFINITIONS

3.1 **Calibration Blank (CB)** -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 **Calibration Standard (CAL)** -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and

surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 **Instrument Performance Check Solution (IPC)** -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 **Laboratory Fortified Blank (LFB)** -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 **Laboratory Fortified Sample Matrix (LFM)** -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6 **Laboratory Reagent Blank (LRB)** -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7 **Linear Calibration Range (LCR)** -- The concentration range over which the instrument response is linear.

3.8 **Material Safety Data Sheet (MSDS)** -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 **Method Detection Limit (MDL)** -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 **Quality Control Sample (QCS)** -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11 **Stock Standard Solution (SSS)** -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 INTERFERENCES

4.1 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Mercury (Sections 7.2 and 7.3)

5.3.2 Sulfuric acid (Sections 7.2, 7.3, and 7.4)

5.3.3 Sodium nitroprusside (Section 7.9)

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance - Analytical, capable of accurately weighing to the nearest 0.000l g.

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 Block digestor with tubes.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Ammonia free distilled or deionized water, free of the analyte of interest. ASTM Type II or equivalent.

7.2 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) (CASRN 21908-53-2) in 50 mL of 1:4 sulfuric acid (10 mL conc. H_2SO_4: [CASRN 7664-93-9] 40 mL reagent water) and dilute to 100 mL with reagent water.

7.3 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 (CASRN 7778-80-5) in 700 mL of reagent water and 200 mL of conc. H_2SO_4. Add 25 mL of mercuric sulfate solution (Section 7.1) and dilute to 1 L.

Note 1: An alternate mercury-free digestion solution can be prepared by dissolving 134 g K_2SO_4 and 7.3 g $CuSO_4$ in 800 mL reagent water and then adding 134 mL conc. H_2SO_4 and diluting to 1 L. Use 10 mL solution per 25 mL of sample.

7.4 Sulfuric Acid solution (4%): Add 40 mL of conc. sulfuric acid to 800 mL of reagent water, cool and dilute to 1 L.

Note 2: If alternate mercury-free digestion solution is used, adjust the above solution to equal the acid concentration of the digested sample (Section 11.6).

7.5 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide (CASRN 1310-73-2) in 900 mL of reagent water and dilute to 1 L.

7.6 Stock Sodium Potassium Tartrate solution (20%): Dissolve 200 g sodium potassium tartrate (CASRN 6381-59-5) in about 800 mL of reagent water and dilute to 1 L.

7.7 Stock Buffer solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) (CASRN 7558-79-4) in about 800 mL of reagent water. Add 20 g of sodium hydroxide and dilute to 1 L.

7.8 Working Buffer solution: Combine the reagents in the stated order, add 250 mL of stock sodium potassium tartrate solution (Section 7.6) to 200 mL of stock buffer solution (Section 7.7) and mix. Add xx mL sodium hydroxide solution

(Section 7.5) and dilute to 1 L. See concentration ranges, Table 2, for composition of working buffer.

7.9 Sodium Salicylate/Sodium Nitroprusside solution: Dissolve 150 g of sodium salicylate (CASRN 54-21-7) and 0.3 g of sodium nitroprusside (CASRN 13755-38-9 or 14402-89-2) in about 600 mL of reagent water and dilute to 1 L.

7.10 Sodium Hypochlorite solution: Dilute 6.0 mL sodium hypochlorite solution (CASRN 7681-52-9) (Clorox) to 100 mL with reagent water.

7.11 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl (CASRN 12125-02-9) in reagent water and bring to volume in a 1 L volumetric flask. 1 mL = 1.0 mg NH_3-N.

7.12 Teflon boiling chips.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH <2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change

in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ±10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis, or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[6] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]
S = standard deviation of the replicate analyses

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3S$$
$$\text{LOWER CONTROL LIMIT} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th sample (or more frequently, if required), and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to

be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample

9.4.3 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (Section 7.11) with reagent water.

10.2 Process standards and blanks as described in Section 11.0, Procedure.

10.3 Set up manifold as shown in Figure 1 and Table 2.

10.4 Prepare flow system as described in Section 11.0, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established

using the difference between the measured value of the calibration solution and the "true value" concentration.

10.7 After the calibration has been established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed ±10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Pipet 25.0 mL of sample, standard or blank in the digestor tube.

11.2 Add 5 mL of digestion solution (Section 7.3) and mix with a vortex mixer (See Note 1).

11.3 Add four to eight Teflon boiling chips (Section 7.12). **CAUTION:** An excess of Teflon chips may cause the sample to boil over.

11.4 Place tubes in block digestor preheated to 160°C and maintain temperature for one hour.

11.5 Reset temperature to 380°C and continue to heat for one and one half hour.

(380°C MUST BE MAINTAINED FOR 30 MINUTES)

11.6 Remove digestion tubes, cool and dilute to 25 mL with reagent water.

11.7 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the sampler and start the pump.

11.8 Flush the sampler wash receptacle with about 25 mL of 4% sulfuric acid (Section 7.4) (See Note 2).

11.9 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

11.10 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

11.11 After a stable baseline has been obtained, start the sampler and perform analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

l2.3 Report results in mg N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations of 1.2, 2.6, and 1.7 mg N/L, the precision was ±0.07, ±0.03, and ±0.15, respectively.

13.2 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations 4.7 and 8.74 mg N/L, the recoveries were 99% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg N/L.

l4.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American

Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess Reagents and samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.3.

16.0 REFERENCES

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of total Kjeldahl Nitrogen in Estuarine Water", Technicon Symposia, pp. 362-367, Vol. 1, 1967.

2. Gales, M.E. and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.

3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May 1974.

4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August 1974.

5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digestor System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600/4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 1978.

6. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

Number of Values Reported	True Value (T)	Mean (X)	Residual for X	Standard Deviation (S)	Residual for S
115	0.380	0.3891	-0.0091	0.0750	-0.0135
134	0.451	0.4807	0.0125	0.1181	0.0238
127	1.00	1.0095	-0.0000	0.1170	-0.0227
164	3.10	3.0992	0.0191	0.2821	-0.0310
138	3.50	3.4765	0.0020	0.3973	0.0512
115	5.71	5.6083	-0.0452	0.4869	-0.0417
175	7.00	6.9246	-0.0008	0.6623	0.0272
121	8.00	7.9991	0.0877	0.6283	-0.0894
120	15.0	15.0213	0.2080	1.2495	-0.0462
127	21.0	20.4355	-0.2937	1.7267	-0.0644
164	25.0	24.7157	0.0426	2.0147	-0.1067
175	26.9	26.1464	-0.4000	2.9743	0.6960

REGRESSIONS: $X = 0.986T + 0.024$, $S = 0.083T + 0.057$

TABLE 2. CONCENTRATION RANGES

Range mg/LN	Pump mL/min.		mL NaOH Buffer (Section 7.7)
	Sample	Resample	
0-1.5	0.80	0.32	250
0-5.0	0.16	0.32	120
0-10.0	0.16	0.16	80

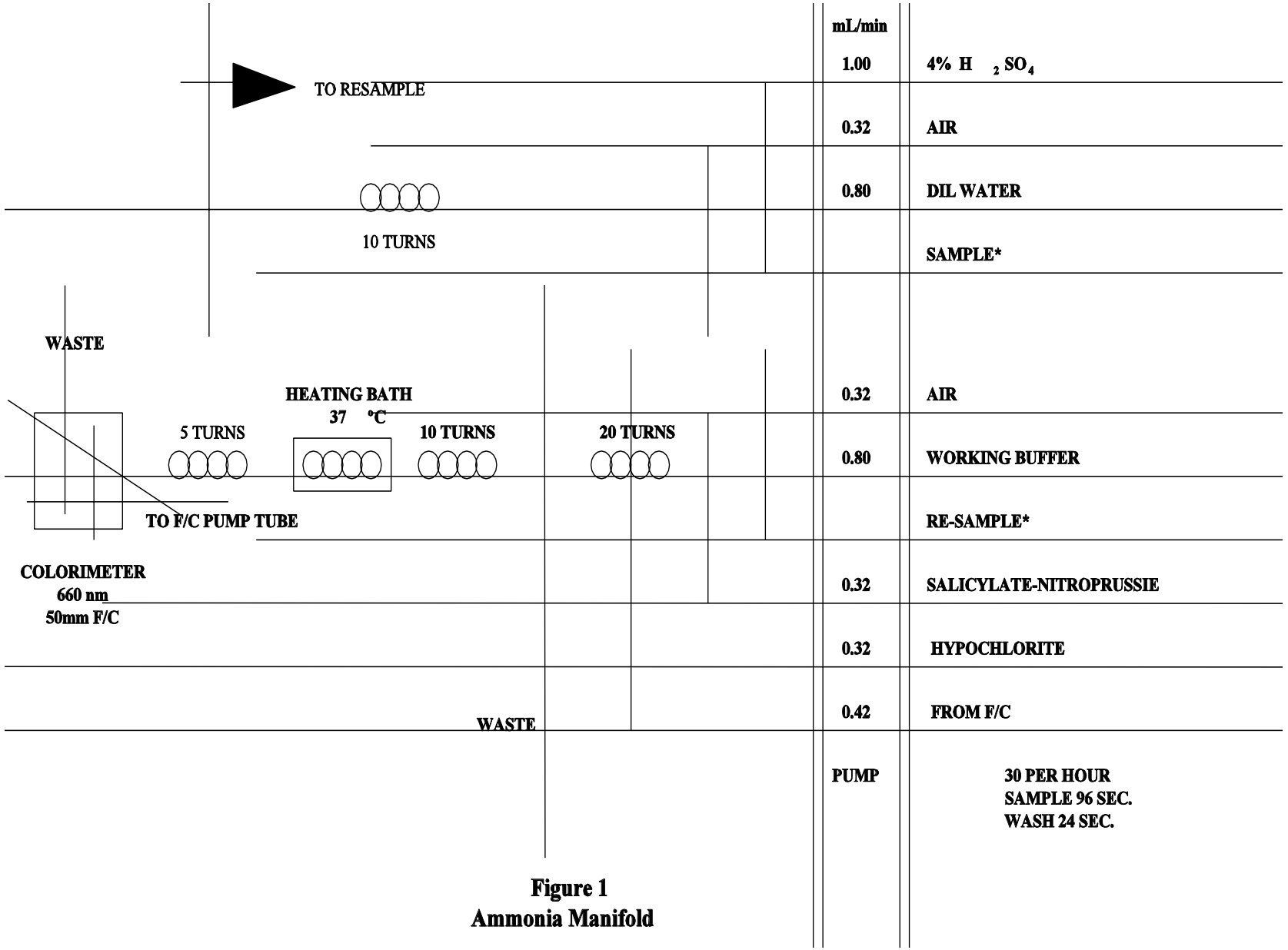

Figure 1
Ammonia Manifold

Appendix H
Laboratory Method – Total Phosphorus– US EPA 365.4

METHOD #: 365.4 Pending Approval for NPDES, CWA (Issued 1974)

TITLE: Phosphorous, Total (Colorimetric, Automated, Block Digester AA II)

ANALYTE: CAS # P Phosphorus 7723-14-0

INSTRUMENTATION: Autoanalyzer

STORET No. 00665

1.0 Scope and Application

1.1 This method covers the determination of total phosphorus in drinking water, surface water and domestic and industrial wastes. The applicable range of this method is 0.01 to 20 mg P/L.

2.0 Summary of Method

2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 mL and placed on the AutoAnalyzer for phosphorus determination.

3.0 Sample Handling and Preservation

3.1 Sample containers may be of plastic material, such as a cubitainer, or of Pyrex glass.

3.2 If the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 mL of conc. H_2SO_4 per liter and refrigeration at 4°C.

4.0 Apparatus

4.1 Block Digester BD-40

4.2 Technicon Method No. 327-74W for Phosphorus

5.0 Reagents

5.1 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 mL of 1:4 sulfuric acid (10 conc. H_2SO_4: 40 mL distilled water) and dilute to 100 mL with distilled water.

5.2 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4in 600 mL of distilled water and 200 mL of conc. H_2SO_4. Add 25 mL of mercuric sulfate solution (5.1) and dilute to 1 liter.

5.3 Sulfuric acid solution (0.72 N): Add 20 mL of conc. sulfuric acid to 800 of distilled water, mix and dilute to 1 liter.

5.4 Molybdate/antimony solution: Dissolve 8 g of ammonium molybdate and 0.2g of antimony potassium tartrate in about 800 mL of distilled water and dilute to

1 liter.

5.5 Ascorbic acid solution: Dissolve 60 g of ascorbic acid in about 600 mL of distilled water. Add 2 mL of acetone and dilute to 1 liter.

5.6 Diluent water: Dissolve 40 g of NaCl in about 600 mL of distilled water and dilute to 1 liter.

5.7 Sulfuric acid solution, 4%: Add 40 mL of conc. sulfuric acid to 800 mL of ammonia-free distilled water, cool and dilute to 1 liter.

6.0 Procedure

Digestion

6.1 To 20 or 25 mL of sample, add 5 mL of digestion solution and mix. (Use a vortex mixer).

6.2 Add 4-8 Teflon boiling chips. Too many boiling chips will cause the sample to boil over.

6.3 With Block Digester in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digester and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

6.4 Cool sample and dilute to 25 mL with distilled water. If TKN is determined the sample should be diluted with ammonia-free water.

Colorimetric Analysis

6.4.1 Check the level of all reagent containers to ensure an adequate supply.

6.4.2 Excluding the molybdate/antimony line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

6.4.3 Flush the Sampler IV wash receptacle with about 25 mL of 4% sulfuric acid (5.7).

6.4.4 When reagents have been pumping for at least five minutes, place the molybdate/antimony line in its container and allow the system to equilibrate.

6.4.5 After a stable baseline has been obtained, start the sampler.

7.0 Calculations

7.1 Prepare a standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with the standard curve.

8.0 Precision and Accuracy

8.1 In a single laboratory (EMSL) using sewage sample containing total P at levels of 0.23, 1.33, and 2.0, the precision was ± 0.01, ±0.04, and ± 0.06, respectively.

8.2 In a single laboratory (EMSL) using sewage samples of concentration 1.84 and 1.89, the recoveries were 95 and 98%, respectively.

Bibliography

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of Total Kjeldahl Nitrogen in Estuarine Water", Technicon Symposia, pp. 362-367, Vol. 1,

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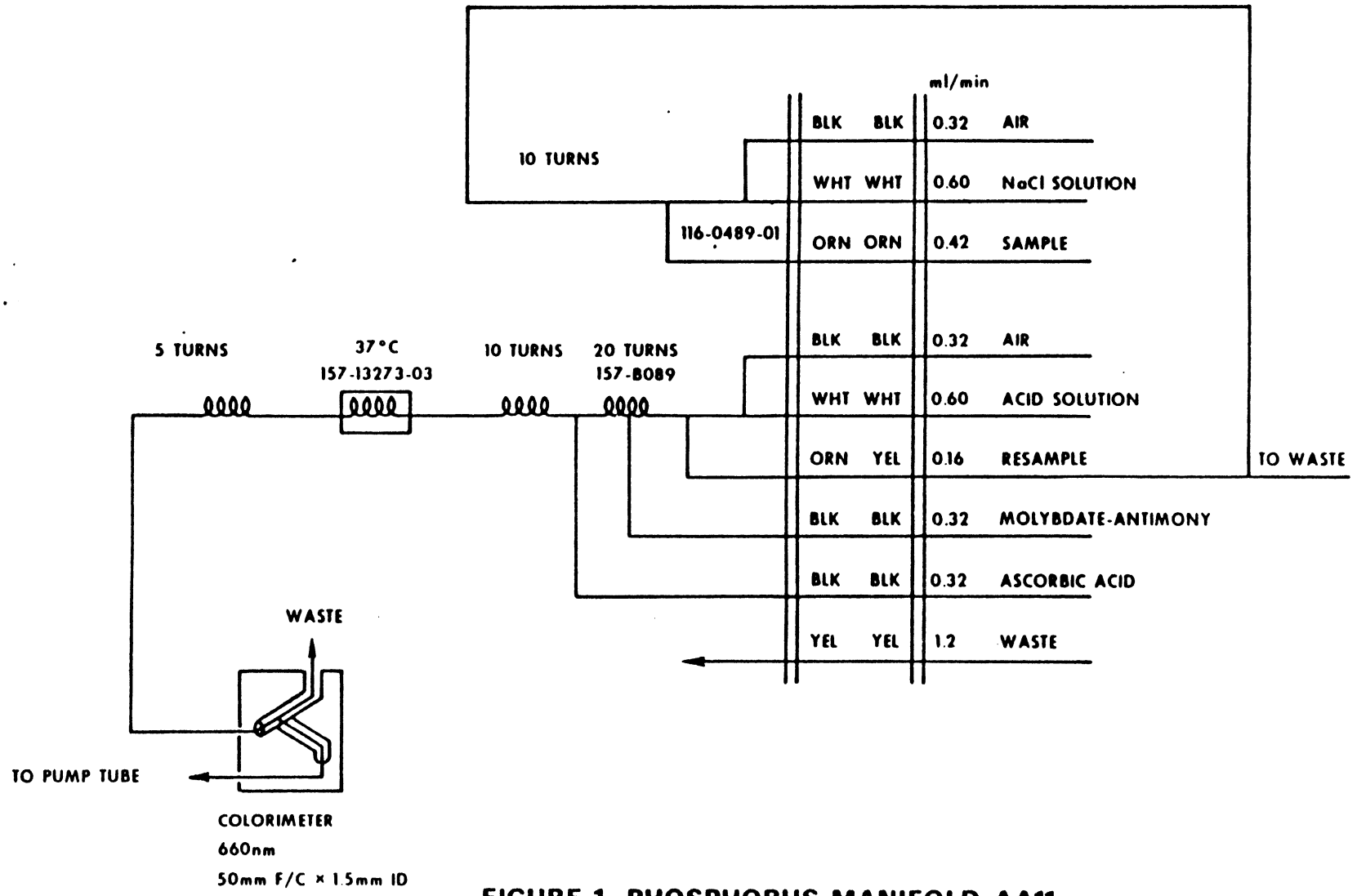

FIGURE 1. PHOSPHORUS MANIFOLD AA11

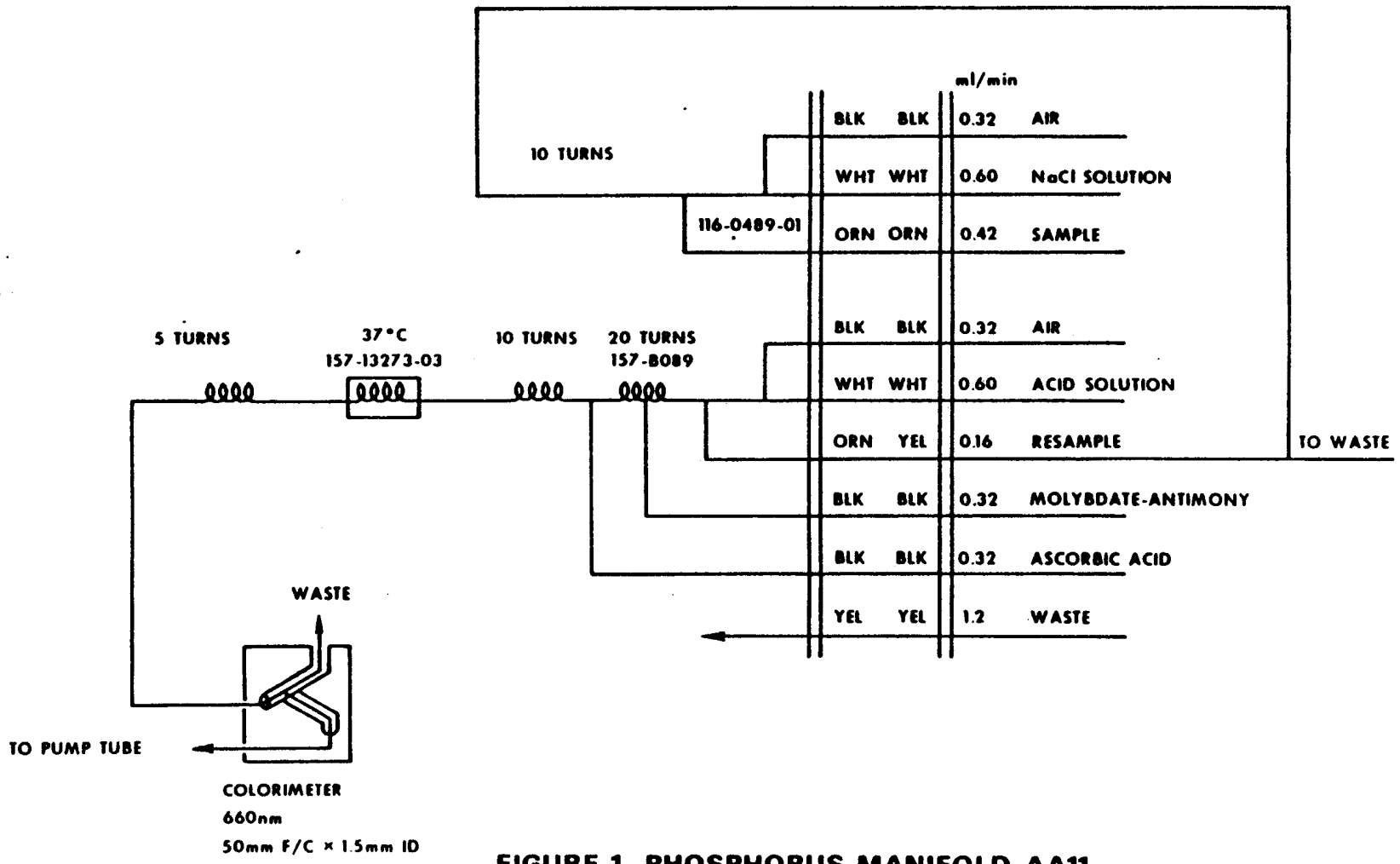

FIGURE 1. PHOSPHORUS MANIFOLD AA11