Metals-ICP-EPA 200.7

1.0 Scope and Application

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is used to determine metals and some non-metals in solution. This method is applicable to the following analytes:

Aluminum, Antimony, Arsenic, Barium, Beryllium, Boron, Cadmium, Calcium, Cerium, Chromium, Cobalt, Copper, Iron, Lead, Lithium, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Phosphorus, Potassium, Selenium, Silica, Silver, Sodium, Strontium, Thallium, Tin, Titanium, Vanadium, Zinc.

1.2 For use in compliance monitoring, refer to appropriate sections of 40 CFR Parts 136 and 141 and the latest Federal Register announcements.

1.3 ICP-AES can be used to determine dissolved analytes in aqueous samples after suitable filtration and acid preservation. Dissolved solids should be <0.2%.

1.4 With the exception of silver, samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has a turbidity <1 NTU at time of analysis. This total recoverable determination is referred to as direct analysis.

1.5 For determination of total recoverable analytes in aqueous and solid samples a digestion is required prior to analysis when the elements are not in solution.

1.6 When determining boron or silica in aqueous samples, only plastic, PTFE or quartz labware should be used from time of collection to completion of analysis.

1.7 It is recommended that samples be digested prior to the determination of the silver. The total recoverable sample digestion procedure given in this method is suitable for the determination of silver in aqueous samples containing concentrations up to 0.1 mg/L. For the analysis of wastewater samples containing higher concentrations of silver, succeeding smaller volume, well mixed aliquots should be prepared until the analysis solution contains <0.1 mg/L silver.

1.8 The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying and unknown concentrations of sulfate, analysis should be completed as soon as possible after sample preparation.

1.9 Detection limits and linear ranges for the elements will vary with the wavelength selected, the spectrometer, and the matrices. Method detection limits and linear working ranges will be dependent on the sample matrix, instrumentation, and selected operating conditions.

1.10 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must document and have on file the required initial demonstration performance data described in section 9.2 prior to using the method for analysis.

2.0 Summary of Method

2.1 An aliquot of a well mixed, homogeneous aqueous or solid sample is accurately weighed or measured for sample processing. For total recoverable analysis of a solid or an aqueous sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, is mixed and centrifuged or allowed to settle overnight prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is <1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid, and the diluted to a predetermined volume and mixed before analysis.

2.2 The analysis described in this method involves multi-elemental determination by ICP-AES using a simultaneous Thermo Fisher 7400 ICAP Dual View. The instrument measures characteristic atomic-line emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element specific emission spectra are produced by a radio-frequency inductively coupled plasma.

3.0 Definitions of Method

3.1 **Calibration Blank**- A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7. 10. 1).

3.2 **Calibration Standard (CAL)**- A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to the analyte concentration (Section 7.9).

3.3 **Dissolved Analyte**- The concentration of analyte in an aqueous sample that will pass through a 0.45 um membrane filter assembly prior to sample acidification (Section 11.1).

3.4 **Field Reagent Blank (FRB)**- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures/ The purpose of the FRB

is to determine if method analytes or other interferences are present in the field environment (Section 8.5).

3.5 **Instrument Detection Limit (IDL)-** The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank signal at the same wavelength.

3.6 **Instrument Performance Check (IPC) Solution**- A solution of method analytes, used to evaluate the performance of the instrument system with respect to a defined set of method criteria (Sections 7.11 and 9.3.4).

3.7 **Internal Standard**- Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component (Section 11.5).

3.8 **Laboratory Duplicates (LD1 and LD2)**- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.9 **Laboratory Fortified Blank (LFB)**- An aliquot of LRB 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 (Sections 7.10.3 and 9.3.2).

3.10 **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 value in the LFM corrected for background concentrations (Section 9.4).

3.11 **Laboratory Reagent Blank (LRB)**- An aliquot of reagent water of other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus (Sections 7.10.2 and 9.3.1).

3.12 **Linear Dynamic Range (LDR)**- The concentration range over which the instrument response to an analyte is linear (Section 9.2.2).

3.13 **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 (Section 9.2.4).

3.14 **Plasma Solution**- A solution that is used to determine the optimum height above the work coil for viewing the plasma (Sections 7.15 and 10.2.3).

3.15 **Quality Control Sample (QCS)**- A solution of method analytes 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 either laboratory or instrument performance (Sections 7.12 and 9.2.3).

3.16 **Solid Sample**- For the purpose of this method, a sample taken from material classified as either soil, sediment or sludge.

3.17 **Spectral Interference Check (SIC) Solution**- A solution of selected method analytes of higher concentrations which is used to evaluate the procedural routine for correcting known interelement spectral interferences with respect to a defined set of method criteria (Sections 7.13, 7.14 and 9.3.5).

3.18 **Standard Addition**- The addition of a known amount of analyte to the sample in order to determine the relative response of the detector and analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration (Sections 9.5.1 11.5).

3.19 **Stock Standard Solution**- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Section 7.8).

3.20 **Total Recoverable Analyte**- The concentration of analyte determined either by "direct analysis" of an unfiltered acid preserved drinking water sample with turbidity of <1 NTU (Section 11.2.1), or by analysis of the solution extract of a solid sample or an unfiltered aqueous sample following digestion by refluxing with hot dilute mineral acid(s) as specified in the method (Sections 11.2 and 11.3).

3.21 **Water Sample**- For the purpose of this method, a sample taken from one of the following sources: drinking, ground, storm runoff, industrial or domestic wastewater.

4.0 Interferences

4.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

4.1.1 Background emission and stray light can usually be compensated for by subtracting the background emission determined by measurement(s) adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the

analyte regions may indicate not only when alternate wavelengths are desirable because of severe spectral interference, but also will show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by the measured emission on one side or the other. The location(s) selected for the measurement of background intensity will be determined by the complexity of the spectrum adjacent to the wavelength peak. The location(s) used for routine measurement must be free of off-line spectral interference (inter-element or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak.

4.1.2 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated for by equations that correct for inter-element contributions, which involves measuring the interfering elements.

4.1.3 On-going SIC solutions (Section 7.14) must be analyzed to verify the absence of inter-element spectral interference or a computer software routine must be employed for comparing the determinative data to limits files for notifying the analyst when an interfering element is detected in the sample at a concentration that will produce either an apparent false positive concentration, greater than the analyte IDL, or false negative analyte concentration, less than the 99% lower control limit of the calibration blank. When the interference accounts for 10% or more of the analyte concentration, either an alternate wavelength free of interference or another approved test procedure must be used to complete the analysis.

4.2 Physical interferences are effects associated with the sample nebulization and transport process. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by such means as a high-solids nebulizer, diluting the sample, using a peristaltic pump, or using an appropriate internal standard element.

4.3 Chemical interferences include molecular-compound formation, ionization effects, and solute-vaporization effects. Normally, these effects are not significant with the ICP-AES technique.

4.4 Memory interferences result when the analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer, and from the buildup of sample material in the plasma torch and spray chamber.

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 to these compounds should be as low as reasonably achievable. 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 data handling sheets should also be made available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper missing when working with these reagents.

5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

5.4 The inductively coupled plasma should only be viewed with proper eye protection from the ultraviolet emissions.

5.5 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6.0 Equipment and Supplies

6.1 Inductively coupled plasma emission spectrometer: Thermo Fisher 7400 Dual View.

6.1.1 Computer-controlled emission spectrometer with background-correction capability. The spectrometer must be capable of meeting and complying with the requirements described and referenced in Section 2.2.

6.1.2 Radio-frequency generator compliant with FCC regulations.

6.1.3 Argon gas supply- High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

6.1.4 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer.

6.1.5 This instrument is equipped with triple mass flow controllers: Coolant Gas, Auxiliary Gas, and Nebulizer Gas. Monitor nebulizer back pressure and clean it if back pressure goes over 500 KPa.

6.2 Analytical balance, with capability to measure 0.1 mg, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

6.3 Metals Digestion Apparatus: Environmental Express Block Digester with polyethylene digestion vials and filter-mate filters for removing un-dissolved solids after digestion.

6.4 Glassware- Volumetric flasks, graduated cylinders, funnels and filters.

6.5 Assorted calibrated pipettes.

6.6 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with screw closure, 125 mL to 1 L capacities.

6.7 One-piece stem FEP wash bottle with screw closure, 500 mL capacity.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications should be used whenever possible.

7.2 Hydrochloric acid, concentrated (sp.gr. 1.19) – HCl.

7.2.1 Hydrochloric acid (1+1)- Add 500 mL concentrated HCl to 400 mL reagent water and dilute to 1 L.

7.2.2 Hydrochloric acid (1+4) – Add 200 mL concentrated HCl to 400 mL reagent water and dilute to 1 L.

7.2.3 Hydrochloric acid (1+20) – Add 10 mL concentrated HCl to 200 mL reagent water.

7.3 Nitric acid, concentrated (sp.gr. 1.41) – HNO_3

7.3.1 Nitric acid (1+1) – Add 500 mL concentrated HNO_3 to 400 mL reagent water and dilute to 1 L.

7.3.2 Nitric acid (1+2) – Add 100 mL concentrated HNO_3 to 200 mL reagent water.

7.3.3 Nitric acid (1+5) – Add 50 mL concentrated HNO_3 to 250 mL reagent water.

7.3.4 Nitric acid (1+9) – Add 10 mL concentrated HNO_3 to 90 mL reagent water.

7.4 Reagent water. All references to water in this method refer to ASTM Type I grade water.

7.5 Hydrogen peroxide, 30%, stabilized certified reagent grade.

7.6 Standard Stock Solutions- Stock standard solution of each element will be purchased (Environmental Express or equivalent) Calcium, Sodium, Potassium and Magnesium are 10,000 ug/ml and all other elements are 1000 ug/ml concentrations, all in nitric acid.

7.7 Mixed Calibration Standard Solutions- Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in 1000 mL volumetric flasks containing 20 mL (1+1) HNO_3 and 20 mL (1+1) HCl and dilute to volume with reagent water. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities. Refer to appendix i for volumes of each element used for standard preparation.

7.8 Blanks- Four types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, the laboratory fortified blank is used to assess routine laboratory performance and a rinse blank is used to flush the instrument uptake system and nebulizer between standards, check solutions, and samples to reduce memory interferences.

7.8.1 The calibration blank for aqueous samples and extracts is prepared by acidifying reagent water to the same concentrations of the acids as used to the standards. The calibration blank should be stored in a FEP bottle.

7.8.2 The laboratory reagent blank (LRB) must contain all the reagents in the same volumes as used in the processing of the samples. The LRB must be carried through the same entire preparation scheme as the samples including the sample digestion, when applicable.

7.8.3 The laboratory fortified blank (LFB) is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to a suitable concentration using the following recommended criteria: Ag ≤0.1 mg/L, ≥K 5.0 mg/L and all other analytes 0.2 mg/L or a concentration approximately 100 times their respective MDL, whichever is greater. The LFB must be carried though the same entire preparation scheme as the samples including sample digestion, when applicable.

7.8.4 The rinse blank is prepared by acidifying reagent water to the same concentrations of acids as used in the calibration blank and stored in a convenient manner.

7.9 Instrument Performance Check (IPC) Solution- The IPC solution is used to periodically verify instrument performance during analysis. It should be prepared in the same acid mixture as the calibration standards by combining method analytes at appropriate concentrations. Silver must be limited to <0.5 mg/L; while potassium and phosphorus because of higher MDLs and silica because of potential contamination should be at concentrations of 10 mg/L. For other analytes a concentration of 2 mg/L is

recommended. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in an FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.10 Quality Control Sample (QCS) – Analysis of a QCS is required for initial and periodic verification of calibration standards or stock standard solutions in order to verify instrument performance. The QCS must be obtained from an outside source different from the standard stock solutions and prepare in the same acid mixture as the calibration standards. The concentration of the analytes in the QCS solution should be ≥1 mg/L, except silver, which must be limited to a concentration of 0.5 mg/L for solution stability. The QCS solution should be stored in a FEP bottle and analyzed as needed to meet data-quality needs. A fresh solution should be prepared quarterly or more frequently as needed.

7.11 For instruments without inter-element correction capability or when inter-element corrections are not used, SIC solutions (containing similar concentrations of the major components in the samples, e.g., ≥10% of the analyte concentration, the analyte must be determined using a wavelength and background correction location free of the interference or by another approved test procedure. Users are advised that high salt concentrations can cause analyte signal suppressions and confuse interference tests.

7.12 Plasma Solution (used for axial viewing instruments)- The plasma solution is used for determining the optimum viewing height of the plasma above the work coil prior to using the method (Section 10.2). The solution is prepared by adding a 5 mL aliquot from each of the stock standard solutions of arsenic, lead, selenium, and thallium to a mixture of 20 mL (1+1) nitric acid and 20 mL (1+1) hydrochloric acid and diluting to 500 mL with reagent water. Store in a FEP bottle.

7.14 SIC Solution is used to verify the absence of effects at the wavelengths selected. The concentration of the major components should be >= 10 mg/L. If the SIC solution confirms an operative interference that is >= 10% of the analyte concentration, the analyte must be determined using a wavelength free of the interference.

8.0 Sample Collection, Preservation and Storage

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or “direct analysis” to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to six months before analysis.

8.2 For the determination of the dissolved elements, the sample must be filtered through a 0.45 um pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. (Glass or plastic filtering apparatus are recommended to avoid

possible contamination. Only plastic apparatus should be used when the determinations of boron and silica are critical.) Use a portion of the filtered sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH <2.

8.3 For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH <2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of the collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for 16 hours, and then verified to be pH <2 just prior to withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be >2, more acid must be added and the sample held for 16 hours until verified to be pH <2. See Section 8.1.
Non-potable water samples must be acidified at least 24 hours prior to analysis. If the sample must be analyzed within 24 hours of collection is must be acidified in the field at the time of collection.

Note: When the nature of the sample is either unknown or is known to be hazardous, acidification should be done in a fume hood. See Section 5.2.

8.4 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 Quality Control

9.1 The laboratory operates 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 thus generated.

9.2 Initial Demonstration of Performance (mandatory).

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.

9.2.2 Linear dynamic range (LDR) – The upper limit of the LDR must be established for each wavelength utilized. It must be determined from a linear calibration prepared in the normal manner using the established analytical operating procedure for the instrument. The LDR should be determined by analyzing succeeding higher standard

concentrations of the analyte until the observed analyte concentration is no more than 10% below the stated concentration of the standard. Determined LDRs must be documented and kept on file. The LDR which may be used for the analyses of samples should be judged by the analyst from the resulting data. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDRs should be verified annually or whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be re-determined.

9.2.3 Quality control sample (QCS) – When beginning the use of this method, on a quarterly basis, after the preparation of stock or calibration standard solutions or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation of the analyses of a QCS (Section 7.12). To verify the calibration standards the determined mean concentrations from three analyses of the QCS must be within ±5% of the stated values. If the calibration standard cannot be verified, performance of the determinative step of the method is unacceptable. The source of the problem bust be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.4 Method detection limit (MDL) - MDLs must be established for all wavelengths utilized, using reagent water (blank) fortified at a concentration of one to five times the estimated instrument detection limit as outlined in 40 CFR Part 136 using the procedure in the EPA publication entitled "Definition and Procedure for the Determination of the Method Detection Limit, Revision 2" published December 2016. Perform all calculations defined in the method and report the concentration values in the appropriate units. The MDLs must be sufficient to detect analytes at the required levels according to compliance monitoring regulation (Section 1.2). MDLs should be determined annually, when a new operator begins work or whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be re-determined.

9.3 Assessing Laboratory Performance (mandatory)

9.3.1 Laboratory reagent blank (LRB) – The laboratory must analyze at least on LRB (Section 7.10.2) with each batch of 20 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) – The laboratory must analyze at least one LFB (Section 7.10.3) with each batch of samples. If the recovery of any analyte falls outside the required control limits of 85-115%, 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 85-115% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery.

9.3.4 Instrument performance check (IPC) solution – For all determinations the laboratory must analyze the IPC solution (Section 7.9) and a calibration blank immediately following daily calibration, after every 10^{th} sample (or more frequently, if required) and at the end of the sample run. Analysis of the calibration blank should always be < the analyte IDL, but greater than the lower 3-sigma control limit of the calibration blank. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm$5% of calibration with a relative standard deviation <3% from replicate integrations ≥4. Subsequent analyses of the IPC solution must be within $\pm$10% of calibration.

9.3.5 Spectral interference check (SIC) solution – For all determinations the laboratory must periodically verify the interlement spectral interference correction routine by analyzing SIC solutions.

9.4 The laboratory must add a known amount of each 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 and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank. Acceptance criteria for matrix spikes is 70-130%.

9.5 The laboratory must perform duplicate analysis on at least 10 percent of the samples in any given run. Acceptance criteria for relative difference is within 10%.

10.0 Calibration and Standardization

10.1 Specific wavelengths are listed in appendix ii.

Instrument is a Thermo Fisher 7400 Dual View which is a simultaneous spectrometer which utilizes an eschelle grating coupled with a CID camera as the detector.

10.2 Creating a Method

-Open Qtegra software.
-From the homepage click “LabBooks”. When creating a new LabBook select eQUANT , enter a name and select a file location.. LabBooks can be created from blank Templates, existing Templates, imported Templates or from existing LabBooks.
-From “Analytes” use the periodic table to begin selecting elements by pausing cursor over the element symbol to see preferred wavelength list. A left click on the element of

choice will pick from the top of the preferred wavelength list. If the first wavelength is not desired, a right click will bring up a window and allow optional selections. This also will show interfering elements. After all elements have been selected move on to the Measure Modes tab.
-Measure modes such as source parameters, RF power, and gas flows may be adjusted. These setting are left at factory default values.

-The next tab is Acquisition Parameters
These are left at factory default settings. Radial view or axial view may be selected on this screen. Analysis mode should be set to "speed". Set pump speed to 50 rpm and flush speed to 75 rpm.

-Standards
From the Standards window select "New" to add a standard where "Elemental Standard" should be selected. The default concentration can be set prior to creating the standard by clicking on settings and entering the value. Otherwise double click each concentration field and type in values. Repeat this for each standard and QC check required by the Method/LabBook.

-Select the Quality Control tab.
Under Quantification select the box "Use Quality Control".

-Autosampler
On the left hand side of the menu select the Autosampler tab.
This is where uptake time and rinse time may be set. Both should be set to 60 seconds.

10.3 Running a method

-Sample List
Use the Sample List to build a sequence and define the samples to be analyzed. Add lines individually for each of the calibration standards and QC checks. Use the label identifier and sample type drop down list as required. In the far right column identify the autosampler rack number and vial number of each standard and sample.

-Running the analysis
In the toolbar of the LabBook click Run to schedule the LabBook for execution. If there is nothing running at the time the current LabBook will begin execution, otherwise it will be added to the queue and begin after completion of the previous LabBook.

-Auto Peak Adjust
If this is the first time a method or wavelength has been used Auto Peak needs to be run. This will correctly align analyte wavelengths in the center of the sub array measurement window. From the Acquisition Parameters window, select the hot button for "Auto Peak". Introduce the high standard and follow the prompts. Make sure the solution has reached the plasma before analyzing and then click ok.

11.0 Procedure

11.1 Aqueous Sample Preparation – Dissolved Analytes

11.1.1 For the determination of dissolved analytes in ground and surface waters, pipet an aliquot (≥20 mL) of the filtered, acid preserved sample into a 50 mL polypropylene centrifuge tube. Add an appropriate volume of (1+1) nitric acid to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL (1+1) HNO_3 to a 20 mL aliquot of sample). Cap the tube and mix. The sample is now ready for analysis.

11.2 Aqueous Sample Preparation – Total Recoverable Analytes

11.2.1 For the "direct analysis" of total recoverable analytes in drinking water samples containing turbidity <1 NTU, treat an unfiltered acid preserved sample aliquot using the sample preparation procedure described in Section 11.1.1 while making allowance for sample dilution in the data calculation (Section 1.2).

11.2.2 For the determination of total recoverable analytes in aqueous samples (other than drinking water with <1 NTU turbidity) transfer a 50 mL (±0.5 mL) aliquot from a well mixed, acid preserved sample to a digestion vial (Environmental Express) (Sections 1.2, 1.3, 1.6, 1.7, 1.8, and 1.9).

11.2.3 Add 1 mL (1+1) nitric acid and 0.5 mL of (1+1) hydrochloric acid to the tube containing the measured volume of sample. Place the tube into the hot block digester for solution evaporation. The hot block should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85 degrees Celsius.

11.2.4 Reduce the volume of the aliquot to about 10 ml by gentle heating at 85 degree Celsius. DO NOT BOIL. This step takes about two hours for a 50 ml aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 10 ml.

11.2.5 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 minutes. (Slight boiling may occur, but vigorous boiling must be avoided to prevent loss of the HCL-H_2O azeotrope.)

11.2.6 Allow the digestion tube to cool. Dilute the sample solution back to its original 50 ml volume in the digestion tube with reagent water, stopper and mix.

11.2.7 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample

contains suspended solids that would clog the nebulizer, a portion of the sample may be filtered for their removal prior to analysis.)

11.3 Sample Analysis

11.3.1 Prior to daily calibration of the instrument inspect the sample introduction system including the nebulizer, torch, injector tube and uptake tubing for salt deposits, dirt and debris that would restrict solution flow and affect instrument performance. Clean the system when needed or whenever there is evidence of diminished response.

Perform the following steps to start up the instrument and prepare for analysis.

-Turn on power to the chiller, check that coolant level is in safe operating range.
-Plug in blower for plasma torch exhaust.
-Remove caps from vials in the Standard Rack of the autosampler.
-Position the peristaltic pump tubes and tighten the platen tensioners. Any adjustment will be done after torch is lit.
-Argon is always on, make sure there is enough tank pressure (80psi) and the tank level is adequate.
-Open the Qtegra software and select “Dashboard” from the tabs on the left hand side of the screen.
-In the center of dashboard screen, click on the round blue “Get Ready” button. The instrument start up parameters will appear. These settings are factory defaults and typically will not need to be changed.
-Click the “OK” button and instrument will initiate startup sequence. The plasma should ignite and the peristaltic pump should start and position itself in the rinse water for the predefined warm up period.

-To calibrate the instrument and analyze samples, LabBook setup and running the LabBook is described above in section 10.3.

11.3.4 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4. Only for the determination of dissolved analytes or the “direct analysis” of drinking water with turbididty <1 NTU is the sample digestion of the LRB, LFB, and LFM not required.

11.3.5 Determined sample analyte concentrations that are 90% or more of the upper limit of the analyte LDR must be diluted with reagent water that has been acidified in the same manner as calibration blank and reanalyzed (see Section 11.4.8).

12.0 Data Analysis and Calculations

12.1 Sample data should be reported in units of mg/L for aqueous samples and mg/kg dry weight for solid samples.

12.2 For dissolved aqueous analytes (Section 11.1) report the data generated directly from the instrument with allowance for sample dilution. Do not report analyte concentrations below the IDL.

12.3 For total recoverable aqueous analytes (Section 11.2), multiply solution analyte concentrations by the dilution factor 0.5, when 100 mL aliquot is used to produce the 50 mL final solution, and report data as instructed in 12.4.

12.4 For analytes with MDLs <0.01 mg/L, round the data values to the thousandth place and report analyte concentrations up to three significant figures. For analytes with MDLs ≥0.01 mg/L round the data values to the 100th place and report analyte concentrations up to three significant figures.

12.5 MRL,MDL and LDR values for each element are listed in Appendix iii.
MRL values are equivalent to the lowest standard value used in the calibration curves.

13.0 Pollution Prevention

13.1 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 was generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.0 Waste Management

14.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect 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 the Section 14.2.

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known to or suspected to have viable E.coli attached or contained must be sterilized prior to disposal.

14.3 Samples preserved with HCl to pH < 2 are hazardous and must be neutralized before being disposed, or must be handled as hazardous waste.

14.4 For further information on waste management consult the Nashoba Analytical LLC Quality Systems Manual.

15.0 References

USEPA Method 200.7 Revision 4.4 EMMC Version

Appendix i

Calibration Standard Values mg/L:

Element	Stain1A	Stain2A	Stain3A	Stain4A	Stain5A	Stain6A
Aluminum	0.002	0.01	0.05	0.1	0.5	1
Antimony	0.001	0.005	0.025	0.05	0.25	0.5
Arsenic	0.001	0.005	0.025	0.05	0.25	0.5
Boron	0.001	0.005	0.025	0.05	0.25	0.5
Barium	0.001	0.005	0.025	0.05	0.25	0.5
Beryllium	0.0002	0.001	0.005	0.01	0.05	0.1
Cadmium	0.0002	0.001	0.005	0.01	0.05	0.1
Calcium	0.2	1	5	10	50	100
Chromium	0.001	0.005	0.025	0.05	0.25	0.5
Copper	0.004	0.02	0.1	0.2	1	2
Iron	0.004	0.02	0.1	0.2	1	2
Lead	0.001	0.005	0.025	0.05	0.25	0.5
Magnesium	0.1	0.5	2.5	5	25	50
Manganese	0.004	0.02	0.1	0.2	1	2
Molybdenum	0.001	0.005	0.025	0.05	0.25	0.5
Nickel	0.001	0.005	0.025	0.05	0.25	0.5
Phosphorus	0.01	0.05	0.25	0.50	2.5	5.0
Potassium	0.1	0.5	2.5	5	25	50
Selenium	0.001	0.005	0.025	0.05	0.25	0.5
Silica	0.01	0.05	0.25	0.50	2.5	5.0
Silver	0.0002	0.001	0.005	0.01	0.05	0.1
Sodium	0.2	1	5	10	50	100
Strontium	0.001	0.005	0.025	0.05	0.25	0.5
Thallium	0.001	0.005	0.025	0.05	0.25	0.5
Zinc	0.004	0.02	0.1	0.2	1	2

Preparation of "Stain 6A" ICP Standard

	Stock concentration	mls of stock std per 1 Liter of "Stain 6"	Final Concentration, mg/L
Ca, Na	10,000 ug/ml	10	100
K, Mg	10,000 ug/ml	5	50
Cu, Fe, Mn, Zn	1,000 ug/ml	2	2
Al	1,000 ug/ml	1	1
P, Si	1,000 ug/ml	5	5
Ag, Be, Cd	1,000 ug/ml	.1	.1
As and all others	1,000 ug/ml	0.5	0.5

Using class A volumetric glassware prepare by serial dilution of Stain 6, the following standards

	Dilute to 1 L: with reagent water
Stain 5A	500ml
Stain 4A	100ml
Stain 3A	50ml
Stain 2A	10ml
Stain 1A	2ml

Appendix ii

Element	Wavelength
Aluminum	167.079 Axial
Antimony	206.833 Axial
Arsenic	189.042 Axial
Barium	455.403 Axial
Beryllium	313.042 Axial
Boron	249.678 Axial
Cadmium	226.502 Axial
Calcium	315.887 Axial
Chromium	267.716 Axial
Copper	324.754 Axial
Iron	259.940 Axial
Lead	220.353 Axial
Magnesium	279.806 Axial
Manganese	257.610 Axial
Molybdenum	202.030 Axial
Nickel	231.604 Axial
Phosphorus	177.495 Axial
Potassium	766.490 Radial
Selenium	196.090 Axial
Silica	251.611 Axial
Silver	328.068 Axial
Strontium	407.771 Axial
Thallium	190.856 Axial
Sodium	589.592 Radial
Zinc	213.856 Axial

Appendix iii
Values as of Jun 29, 2017

Thermo 7400 ICP EPA 200.7

	MRL	MDL	LDR
Ag	0.007	0.003	10
Al	0.029	0.013	4
As	0.004	0.002	10
B	0.022	0.01	10
Ba	0.002	0.001	5
Be	0.0004	0.0002	5
Ca	0.2	0.1	100
Cd	0.0004	0.0002	10
Cr	0.002	0.001	10
Cu	0.009	0.004	25
Fe	0.009	0.004	25
K	0.9	0.4	200
Mg	0.2	0.1	200
Mn	0.002	0.001	10
Mo	0.007	0.003	10
Na	2.2	1	200
Ni	0.002	0.001	10
P	0.01	0.01	9
Pb	0.004	0.002	10
Sb	0.009	0.004	10

Se	0.009	0.004	1
Si	0.13	0.06	25
Sr	0.002	0.001	3
Tl	0.009	0.004	10
Zn	0.011	0.005	15

Appendix iiii

Instrument Maintenance

Most importantly, keep instrument clean, wipe up any spills right away and clean off any dust that may accumulate. Cleaning should be done with a soft cloth and mild detergent, do not use any solvent based cleaners.

Sample Introduction System Cleaning

Failure to maintain sample introduction can result in erroneous results, poor precision and detection limits and blockages. Peristaltic tubing needs to be replaced frequently, they will usually last 2 maybe 3 days before needing to be advanced to the next usable stop on the tube. The drain pump tubing must also be changed as frequently. The first sign a tubing needs replacement is poor replicates usually accompanied by a failing IPC. If this happens, change the tubing.

The nebulizer is also a critical component to watch. The flow rates are controlled by a mass flow controller, so it is the backpressure that becomes the indicator. A wide-open nebulizer will run at about 300 KPa back pressure. As salts or other deposits buildup the back pressure will increase. When it reaches 500 KPa, the pressure indicator on the control panel will turn red indicating that the nebulizer needs cleaning. We maintain two nebulizers and torches that may be swapped immediately to minimize down time. Clean one set while the other is in operation. To clean the nebulizer, rinse with DI water, dilute acid or organic solvent or aspirate a cleaning solution through it.

To clean the torch, remove from the metal torch mount and soak in a dilute solution of analytical grade surfactant for five minutes. To remove metallic deposits from the torch tip, separate it from the quartz tube and immerse the tip in a 10% acid solution for several hours or until clean.

To clean the spray chamber, soak in analytical surfactant for five minutes.

Check water chiller daily and make sure it is at safe operating level.

Written by:_____

Approved by:_____

Effective Date:_____

Revision #7 Apr 2019

Issued by: QA Officer

SOP – Phosphorus – SM4500-P-E

1. SUMMARY

This method is for the determination of ortho-phosphorus and total phosphorus in drinking water, wastewater and surface water.

2. SAMPLING AND HANDLING

Ortho-phosphorus samples are stored at 4° C and must be analyzed within 48 hours of sampling. Total phosphorus samples are preserved with 1+1 H_2SO_4, are stored at 4° and must be analyzed within 28 days of sampling.

3. APPARATUS

Spectrophotometer capable of reading absorbance at 880 nm
Autoclave

4. CHEMICALS

Sulfuric Acid
Hydrochloric Acid
Sodium Hydroxide
Ammonium Persulfate
Antimony Potassium Tartrate
Ammonium Molybdate
Ascorbic Acid
Potassium Phosphorus- monobasic (KH_2PO_4)

5. REAGENTS

11N Sulfuric Acid; Add 155mL concentrated H_2SO_4 to 250mL deionized water in a 500mL volumetric flask. Allow to cool, and fill to volume with deionized water.

10N Sodium hydroxide; Add 200g NaOH to 300mL deionized water in a 500mL volumetric flask. Dissolve NaOH and allow to cool. Fill to volume with deionized water.

5N Sulfuric Acid; Add 140mL concentrated H_2SO_4 to 500mL deionized water in a 1000mL volumetric flask. Allow to cool, and fill to volume with deionized water.

Antimony Potassium Tartrate solution; Dissolve 1.3715g Antimony Potassium Tartrate in 250mL deionized water is a 500mL volumetric flask. Fill to volume, transfer to a glass-stoppered bottle and store at 4° C.

Ammonium Molybdate solution; Dissolve 20g Ammonium Molybdate in 250 mL deionized water in a 500mL volumetric flask. Fill to volume. Transfer to a glass-stoppered bottle and store at 4° C for no more than 1 week.

0.1M Ascorbic Acid solution; Dissolve 1.76g Ascorbic Acid in 100mL deionized water in a volumetric flask. Store solution at 4° C for no more than 1 week.

Combined reagent; Mix room-temperature reagents in the following order and proportions for 100mL of combined reagent: 50mL 5N H_2SO_4, 5mL Antimony Potassium Tartrate solution, 15mL Ammonium Molybdate solution, 30mL Ascorbic Acid solution. Mix well after the addition of each reagent. If turbidity develops, shake and let stand for several minutes until turbidity disappears before using. Combined reagent is stable for 4 hours.

50mg/L Primary Standard Stock; Dry KH_2PO_4 in a 105° C oven for 2 hours and allow to cool in a desiccator. Dissolve 0.1098g in 500 mL deionized water. Stock solution is stored at 4° C for no more than 6 months.
50mg/L Second Source Stock; Dry KH_2PO_4 in a 105° C oven for 2 hours and allow to cool in a dessicator. Dissolve 0.1098g in 500 mL deionized water. Stock solution is stored at 4° C for no more than 6 months.

5mg/L Intermediate Standard; Measure 50mL Primary Standard Stock (50mg/L) into a 500mL volumetric flask and dilute to volume with deionized water.

Calibration Standards:
0.01 mg/L; Add 0.1mL of Intermediate Standard to flask, dilute to 50 ml final volume.
0.05 mg/L; Add 0.5mL of Intermediate Standard to flask, dilute to 50mL final volume.
0.3 mg/L; Add 3mL of Intermediate Standard to flask, dilute to 50 mL final volume.
0.6 mg/L; Add 6mL of Intermediate Standard to flask, dilute to 50mL final volume.
0.9 mg/L; Add 9mL of Intermediate Standard to flask, dilute to 50mL final volume.

Second Source Check Standard. 0.1mg/L; Add 1mL of Second Source Stock to a 500mL volumetric flask, dilute to volume with deionized water.

6. PROCEDURE

Phosphorus glassware should be kept separate from other glassware to prevent contamination. If needed, clean phosphorus glassware with 1+1 HCl and rinse with deionized water.

Digestion for Total Phosphorus:
Measure 50mL of sample and/or standard into an Erlenmeyer flask. Add 0.4g Ammonium Persulfate and 1mL of 11N H_2SO_4 to each flask. Autoclave all samples and standards for 30 minutes at 121°C and 15-20psi. Allow flasks to cool to room temperature before proceeding.

Turn on spectrophotometer, set wavelength to 880nm and allow to warm up for 15 minutes. For Total Phosphorus, add 2mL of 10N NaOH to each flask. Add 8mL of the combined reagent to each flask and swirl to mix. Allow at least 10 minutes for color development but read within 30 minutes.

Zero the spectrophotometer using prepared blank (50mL deionized water, prepared in same manner as samples and standards), read standards, samples and all associated quality control preparations.

7. CALCULATION

Reported values for samples are calculated from the corresponding absorbance value in relation to the calibration curve. Samples reading below 0.01 mg/L must be reported as BDL (below detection limit).

8. QUALITY CONTROL

The calibration curve must have a correlation coefficient of 0.995 or better. The second source check standard must be read immediately after the calibration standards, after every 10 samples and at the end of the run. Acceptable recovery for the check standard is ± 10%. Sample duplicates are run every 10 samples or at least 1 per run. The allowable Percent Relative Deviation for sample duplicates is ±10%. A blank must also be analyzed after each 10 samples and at the end of each run. Acceptable recovery for the blank is below the detection limit (<0.01 mg/L).

Laboratory Fortified Blanks and Matrix Spikes:
A Lab Fortified Blank (LFB) must be analyzed immediately following calibration. Prepare by adding 0.1 mL of the Intermediate Standard and diluting to 50 mL final volume. This solution should produce a reading of 0.01mg/L. Acceptable recovery for the LFB is ± 10%. Matrix spikes must be analyzed after every 10 samples, or at least 1 per batch of samples. Spike 2 mL of the Intermediate standard to a sample, dilute to a final volume of 50 mL, and analyze. Acceptable recovery for matrix spikes is ± 20%. A blank must be analyzed after every 10 samples and at end of run. Acceptable recovery for the blank is below the detection limit (<0.01),

Written by:_____

Approved by:_____

Effective Date: _____

Revision # 3
Issued by: Q.A. Officer

Reference: *Standard Methods for the Examination of Water and Wastewater, 22nd Ed,* Rice, Baird, Eaton, Clesceri

SOP – Ammonia – SM4500-NH3-B,D

1. SUMMARY

This method is applicable to the measurement of 0.03 to 1400 mg/L NH_3-N in potable, surface waters , and domestic and industrial waste. The standards and samples are both analyzed using an ion specific electrode calibrated for the range of interest. If interferences are present a preliminary distillation step may be required.

2. SAMPLING AND HANDLING

Collect samples in plastic or glass containers. If samples are to be analyzed within 24 hours they may be refrigerated, unacidified at 4 deg C. Otherwise acidify with sulfuric acid to pH < 2, store at 4 deg C. and analyze within 28 days.

3. APPARATUS

-Distillation apparatus- Environmental Express Simple Distillation system
-pH Meter
-Specific Ion Meter
-Ammonia Specific Electrode
-Magnetic Stirrer

4. REAGENTS

-Ammonia Free Water
-Borate Buffer Solution Add 88 ml 0.1N NaOH solution to 500ml approximately 0.025M sodium tetraborate solution (9.5 gm Sodium Tetraborate/L) and dilute to 1 L.
-Sodium Hydroxide 6N (24 gms/100ml deionized water)
-Dechlorinating agent-Dissolve 3.5 gm sodium thiosulfate in 1 Liter deionized water. Prepare fresh weekly. 1ml will remove 1mg/L residual chlorine in 500ml sample.
-Sodium Hydroxide 1N (4 gms/100ml deionized water)
-Sulfuric Acid 1N (28ml Con. Sulfuric per 1 L deionized water)
-Sulfuric Acid 0.04 N (1ml Con. Sulfuric per 1 L deionized water)
-Sodium Hydroxide 10N.
-ISA solution: Dissolve 200gm NaOH in 600 ml water, allow to cool. Add 18.6 gms $Na_4EDTA.4H2O$, allow to dissolve completely. Add 100ml Methanol followed by 0.1gm Thymolphthalein Blue indicator..Dilute to 1000ml final volume with DI water.
-Standard Ammonium chloride solution: Dissolve 3.819 gm anhydrous Ammonium Chloride in deionized water, dilute to 1000ml=1000mg/L= 1mg N/ml=1.22 mg NH_3/ml.
-Second Source Standard Ammonium Chloride solution-prepared as above with a different lot number of chemical.

5. DISTILLATION PROCEDURE

- Dechlorinate samples if necessary.
- Add 50mL of sample or standard, along with a few boiling chips or glass beads to the distillation vessel.
- Add 20mL of 0.04N H_2SO_4 to each distillate trap and attach to distillation vessel. Attach vacuum hose to each trap. Turn on vacuum pump and verify bubbles in both the distillation vessel and the trap.
- Add 2.5mL borate buffer to each vessel, and enough 10N NaOH to raise pH to <9.5SU (confirm with pH meter or indicator paper).
- Turn on hot block, verify temperature setting is at 135ºC (adjust if necessary). Distill for 1 hour.
- After distillation is complete, turn off hot block. Disconnect trap from distillation vessel first, then from vacuum hose. Dilute trap solution to original volume of 50mL. Sample is now ready for analysis.

6. ANALYSIS

Prepare a series of standard solutions covering the range of interest with the lowest standard corresponding to the minimum reporting limit.

- 10.0 mg/L Standard – Add 10mL of the 1000 mg/L standard solution to a 1L volumetric flask and dilute to volume with ammonia free water.
- 1.0 mg/L Standard – Add 1mL of the 1000 mg/L standard solution to a 1L volumetric flask and dilute to volume with ammonia free water.
- 0.1 mg/L Standard – Add 10mL of the 10mg/L working standard to a 1L volumetric flask and dilute to volume with ammonia free water.

Calibrate the meter according to manufacturer's instructions. Starting with the lowest concentration standard, place 50 ml into a 100ml beaker with stirring bar. Place on stirring box, start spinning, and place electrode into solution so that it is slightly angled to prevent the formation of bubbles on the membrane. Add a sufficient volume of ISA solution so that solution turns blue indicating proper pH, if mercury or silver may be present, (0.5 ml) to raise pH above 11. Allow meter to stabilize and press the "yes" or "enter" button when prompted by the meter. Repeat for all standards. Immediately after calibration, analyze a mid range second source check standard.

Analyze the samples in the same manner and dilute if necessary to bring within the calibration range.

7. CALCULATION

Read ammonia concentration directly from meter or construct standard curve on semi log graph paper plotting millivolts on the x-axis versus concentration on the y-axis. Read ammonia concentration from curve and report as mg/L NH_3-N. Samples reading below 0.1 mg/L must be reported as BDL (below detection limit).

8. QUALITY CONTROL

The second source check standard must be read immediately after the calibration standards, after every 10 samples and at the end of the run. Acceptable recovery for the check standard is ± 10%. Sample duplicates are run every 10 samples or at least 1 per run. The allowable Percent Relative Difference for sample duplicates is ±10%. Analyze a blank after calibration, every 10 samples, and at the end of the run.

Laboratory Fortified Blanks and Matrix Spikes:

A Lab Fortified Blank (LFB) must be analyzed immediately following calibration. Prepare by adding 1 mL of the 10.0mg/L Standard and diluting to 100 mL final volume. This solution should produce a reading of 0.1mg/L. Acceptable recovery for the LFB is ± 10%. Matrix spikes must be analyzed after every 10 samples, or at least 1 per run. Spike 2 mL of the Intermediate standard to a sample, dilute to a final volume of 50 mL, and analyze. Acceptable recovery for matrix spikes is ± 20%.

If distilled samples are being analyzed, distill a standard within the range of the calibration curve with an allowable range of +\- 20%. Distill the blank, LFB and MS when distilled samples are being analyzed.

Maintain Quality Control Charts by plotting spike recoveries and percent relative difference of duplicates.

Written by: _____

Approved by: _____

Effective Date: _____
Reference: *Standard Methods for the Examination of Water and Wastewater, 22th Ed,* Rice, Baird, Eaton, Clesceri

SOP – Inorganic Anions by Ion Chromatography – EPA 300.0 Rev 7.0

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of the following inorganic anions:

Chloride	Nitrite
Fluoride	Ortho-Phosphate
Nitrate	Sulfate

1.2 The matrices applicable to this method are as follows: Drinking water, surface water, mixed domestic and industrial wastewaters, groundwater and reagent waters.

1.3 This method is recommended for use only by of under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.4 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of fortified sample matrix covering the anions of interest. This fortification procedure is described in section 11.6

2.0 SUMMARY OF METHOD

2.1 A small volume of sample is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, and conductivity detector.

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 of 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 matricies 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 wither 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 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 matricies 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 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.

3.9 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 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or fortification can be used to solve most interference problems associated with retention times.

4.2 The water dip or negative peak that elutes near, and can interfere with, the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent to 100 mL of each standard and sample.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

4.4 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems.

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 to these compounds should be as low as reasonably achievable. 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 data handling sheets should also be made available to all personnel involved in the chemical analysis.

6.0 EQUIPMENT AND SUPPLIES

6.1 Analytical balances capable of accurately weighing to the nearest 0.0001 g.

6.2 Ion chromatograph

6.2.1 Ion Chromatograph (Dionex ICS-1600), 2 mm system fitted with AG-22 Fast guard column, AS22-Fast analytical column, suppressor, and conductivity detector .

6.2.2 Sample loop, 10 uL

6.2.3 Autosampler (Dionex AS-DV), 5 mL vials and filter caps

6.3 Drying oven (103-105 ^{o}C) and dessicator

7.0 REAGENTS AND STANDARDS

7.1 Sample bottles: Glass or polyethylene of sufficient volume to allow replicate analyses of anions of interest.

7.2 Reagent water: Distilled or deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.3 Eluent stock: (180mM Sodium Carbonate, 170mM Sodium Bicarbonate); Dissolve 3.816g Sodium Carbonate and 2.856g Sodium Bicarbonate in deionized water and dilute to a final volume of 200mL.

7.4 Eluent solution: ; Add 10mL Eluent stock to a 1L volumetric flask and dilute to volume with deionized water.

7.5 Stock standard solutions: All standard solutions are prepared from ACS reagent grade materials, dried at 105^{o}C for 30 minutes

7.5.1: Chloride Standard Stock, 1000mg/L; Dissolve 1.6485g NaCl in deionized water and dilute to 1L.

7.5.2: Fluoride Standard Stock, 1000mg/L; Dissolve 2.2100g NaF in deionized water and dilute to 1L.

7.5.3: Nitrate Standard Stock, 1000mg/L; Dissolve 6.0679g $NaNO_3$ in deionized water and dilute to 1L.

7.5.4: Nitrite Standard Stock, 1000mg/L; Dissolve 4.9257g $NaNO_2$ in deionized water and dilute to 1L.

7.5.5: Ortho-P Standard Stock, 1000mg/L; Dissolve 4.3937g KH_2PO_4 in deionized water and dilute to 1L.

7.5.6: Sulfate Standard Stock, 1000mg/L; Dissolve 1.8141g K_2SO_4 in deionized water and dilute to 1L.

Note: Second source stocks are prepared in same manner as standard stocks, but must be produced from a separate lot of reagent grade material.

8.0 SAMPLING AND HANDLING

8.1 Samples are collected in glass or plastic containers and stored at 4° C. Holding times are as follows: Fluoride, Chloride and Sulfate; 28 days. Nitrite-N and Ortho-phosphate; 48 hours. Nitrate-N samples may be held for 14 days if chlorinated, 48 hours if not chlorinated.

9.0 QUALITY CONTROL

9.1 Initial Demonstration of Performance:

9.1.1 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.

9.1.2 Method Detection Limit (MDL) – MDLs must be established for all analytes, using reagent water fortified at a concentration of two to three times the estimated instrument detection limit. 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.

9.2 Assessing Laboratory Performance

9.2.1 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 of reagent contamination should be suspected and corrective action be taken before continuing the analysis.

9.2.2 Laboratory Fortified Blank (LFB) – The laboratory must analyze at least one LFB with each batch of samples. Required control limits for the LFB are 90-110% recovery.

9.2.3 Instrument Performance Check (IPC) – The laboratory must analyze the IPC (a mid-range check standard) after calibration, at the beginning and the end of a sample batch, and every 10 samples during a sample batch. Acceptable recovery for the IPC is ± 10%.

9.3 Assessing Analyte Recovery and Data Quality

9.3.1 Laboratory Fortified Sample Matrix (LFM) – (Also known as Matrix Spike or MS) – The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LFM 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 used in the laboratory fortified blank.

9.3.11 If the concentration of fortification is less than 25% of the background concentration of the matrix, the matrix recovery should not be calculated.

9.3.2 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%.

9.3.3 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, the recovery problem is judged to be either matrix or solution related, not system related.

9.3.4 The laboratory must perform duplicate analysis on at least 10 percent of the samples in any given run. Acceptance criteria for relative difference is within 10%.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare a calibration blank and calibration standards as follows:

10.1.1 Working Standard #5; Measure 100mL Chloride stock, 2.0mL Fluoride stock, 5.0mL Nitrate stock, 1.0mL Nitrite stock, 10.0mL Ortho-P stock and 100mL Sulfate stock into a 1L volumetric flask and dilute to volume with deionized water.

10.1.2 Working Standard #4; Measure 100mL of Standard 5 into a 200mL volumetric flask and dilute to volume with deionized water.

10.1.3 Working Standard #3; Measure 20mL of Standard 5 into a 200mL volumetric flask and dilute to volume with deionized water.

10.1.4 Working Standard #2; Measure 10mL of Standard 5 into a 200mL volumetric flask and dilute to volume with deionized water.

10.1.5 Working Standard #1; Measure 2mL of Standard 5 into a 200mL volumetric flask and dilute to volume with deionized water.

10.2 Inject the calibration blank and the 5 standards. The software will construct calibration curves for each analyte based on either peak height or area against the concentration. The minimum correlation coefficient allowed is 0.995.

10.3 The calibration curve must be verified on each working day, whenever the anion eluent is changed and after every 10 samples. If the response or retention time for any analyte varies from the expected value by more than 10%, the test must be repeated using fresh calibration standards. If the results are still more than 10%, a new calibration curve must be prepared for that analyte.

10.4 Nonlinear response can happen when the separator column capacity is exceeded. The response of the detector to the sample when diluted 1:1, and when not diluted, should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

11.0 PROCEDURE

11.1 Turn the instrument on by pressing the main power switch on the ICS-1600. Check eluent reservoir – replenish if necessary. Open the Chromeleon 7 Console. On the Pump_ECD tab, turn the pump on and set the flow rate to 0.30 mL/min, turn on the suppressor, verify that the current is set to 8 mA, and set the column oven temperature to 30.0^{o} C. Allow the system to equilibrate before commencing analysis.

11.2 Create an autosampler sequence by either using the New Sequence Wizard, or by editing a previous sequence and saving it with a unique file ID. Either create a new Instrument Method using the wizard, or use an established method.

11.3 Load the autosampler according to the prepared sequence. Fill autosampler vial and press filter cap into vial, making certain that the top of the filter cap is centered within the vial. If necessary, prefilter samples through a 0.45µm filter disc to prolong column life. Add the created sequence to the queue in the Chromeleon console, and press start.

11.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.5 If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

11.6 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

12.0 MANUAL PEAK INTEGRATION

12.1 There are occasions where manual integration of chromatography peaks is warranted, for example: Peak Co-elutions, Leading or Tailing Peaks, Baseline Interferences, Matrix Interference, or Negative Peaks.

12.2 The Thermo Scientific Chromeleon Software is 21CFR part 11 compliant which provides a full audit trail of each analysis. The original chromatographic data is preserved and any manual integrations are also saved. There is no need to save under a new file name.

12.3 Manual Integration must never be used in an attempt to make QC data meet objectives.

12.4 Baseline resolution of each target analyte provides the best quantitative results, but is not always possible to achieve due to limited chromatographic separation ability or sample matrix effects. The laboratory must demonstrate that each target analyte is resolved adequately during calibration and can meet all method quality control criteria.

12.5 Chromatographic Conditions where Manual Integrations may be Warranted:

12.5.1 Peak Co-elutions: Co-eluting peaks with a valley less than half the peak height of the smaller peak may be manually integrated by separating the two peaks with a line drawn from the valley bottom to the projected baseline

12.5.2 Leading or tailing peaks: Severely leading or tailing peaks make it difficult to determine the beginning or ending of a peak from the baseline. Peak symmetry can be measured at 10% peak height as the ration of the forward part of the peak to the rear part of the peak, relative to a tangent drawn at the peak apex. A symmetry ration of 0.90 to 1.2 is considered acceptable for most

chromatography. A leading or tailing peak may be corrected by adjusting the temperature of the column oven, change in flow rate or change in column load.

12.5.3 Baseline Interferences: The manner in which a baseline is drawn will substantially affect the quantitation results of an analysis. Peak areas must also reflect accurately the concentrations of the analytes they represent. If the baseline is too far above or below the contours of each peak, the area attributed to the peaks will not represent the concentration of the corresponding analytes. Baselines may be elevated, drifting, sloped, humped, or excessively noisy. Poor

12.5.3 (cont) baseline quality is sometimes a sign of poor instrument performance. Corrective action for these conditions must be taken to minimize or eliminate baseline abnormalities prior to sample analysis. In the case of a slope, the baseline reference must be projected horizontally under each peak to the next baseline reference point during quantitation.

12.5.4 Matrix Interference: In chromatography the presence of matrix interference frequently appears as extra peaks or as an elevated or sloping background. Additional dilutions or analysis by another technique may be required if the peaks cannot be resolved.

12.5.5 Negative Peaks: Negative peaks (dips) before of after the target peaks are common with some chromatographic instruments. This is frequently seen as a water dip before the fluoride peak in ion chromatography. Negative peaks may cause a significant drop in the chromatographic baseline, leading to serious quantitative errors. For peaks followed by a dip, extend the baseline from the beginning of the peak to the end point of the dip. For peaks preceded by a dip, extend the baseline from the beginning of the dip to the end point of the peak.

13.0 POLLUTION PREVENTION

13.1 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 was generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.0 WASTE MANAGEMENT

14.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect 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 the Section 14.2.

14.2 For further information on waste management consult the Nashoba Analytical LLC Quality Systems Manual.

Written by: _____

Approved by: _____

Effective Date: _____

Revision # 7
Issued by: Q.A. Officer

STANDARD VALUES (mg/L)

Anion	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5
Fluoride	--	0.1	0.2	1.0	2.0
Chloride	1.0	5.0	10	50	100
Nitrite-N	0.02	0.05	0.1	0.5	1.0
Nitrate-N	0.05	0.25	0.5	2.5	5.0
Ortho-P	0.1	0.5	1.0	5.0	10

Sulfate	1.0	5.0	10	50	100

SOP – Total Suspended Solids – SM2540D, E

1. SUMMARY

A well-mixed sample is filtered through a glass-fiber filter and dried to a constant weight at 103-105ºC. The increase in the weight of the filter represents the total suspended solids

2. SAMPLING AND HANDLING

Samples are collected in glass or plastic containers and are stored at 4ºC. Samples may be held no more than 7 days, however, analysis within 24 hours is preferable.

3. APPARATUS

Glass-fiber disks (Whatman grade 934AH or equivalent)
Filtration apparatus, consisting of: Vacuum pump, suction flask and filtration funnel.
Drying oven, for operation at 103-105ºC.
Desiccator
Analytical Balance
Muffle furnace, for operation at 550ºC

4. PROCEDURE

Prepare filters by placing disk wrinkled side up in filtration apparatus. Apply vacuum and wash disk with 3 successive 20mL portions of deionized water. Continue suction to remove all traces of water, and dry disks in the oven for 1 hour at 103-105ºC. If volatile solids are to be measured, ignite at 550ºC for 15 minutes in the muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

Choose sample volume to yield between 2.5 and 200 mg dried residue. Assemble filtering apparatus and begin suction. Wet filter with a small amount of deionized water to seat it. Mix sample, measure appropriate volume and add to filtration apparatus. Wash filter with 3 successive 10mL portions of deionized water and continue suction to remove all traces of water. If complete suction takes more than 10 minutes, reduce sample size. Remove filter from apparatus, and dry at 103-105ºC for at least 1 hour. Allow filter to cool in desiccator to balance temperature, and weigh. Continue cycle of drying, desiccating and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5mg.

5. CALCULATIONS

$$\text{TSS, mg/L} = \frac{(A-B)(1000)}{\text{Sample volume, mL}}$$

Where: A= weight filter and dried residue
B= weight of filter, mg

If volatile solids are to be determined, ignite residue at 550ºC for 15 to 20 minutes. Allow filter to cool partially in air until most of the heat has been dispersed. Transfer to a desiccator and cool to balance temperature before weighing.

$$\text{TVSS, mg/L} = \frac{(A-B)(1000)}{\text{Sample volume, mL}}$$

Where: A= weight of residue and filter before ignition, mg
B= weight of residue and filter after ignition, mg

Results less than 1 mg/L must be reported as below detection limit (BDL).

6. QUALITY CONTROL

Analyze at least one blank (deionized water) per set of samples. Ignite blank along with TVSS samples, if needed. Analyze a sample duplicate for every 10 samples, or at least one per set of samples. Duplicate determinations should agree within 5% of their average weight.

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Approved by: _____

Effective Date: _____

Revision # 3

Issued by: Q.A. Officer

Reference: *Standard Methods for the Examination of Water and Wastewater, 22th Ed*, Rice, Baird, Eaton, Clesceri

SOP- ENZ. SUB. SM9223-Colilert-18

1.0 Scope and Application

1.1 This Presence/Absence method is for the simultaneous detection of Total Coliforms and *E. coli* bacteria in water. The presence of Coliform bacteria in water is one of the principal indicators of the suitability of a water supply for drinking or other uses. *E.coli* is a common inhabitant of the intestinal tract of warm-blooded animals, and its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens. Colilert-18 can also be used with the IDEXX Quantitray system for enumeration of Total and Ecoli in potable waters and non-potable waters, and enumeration of fecal coliform in wastewaters.

1.2 This enzyme substrate coliform test is approved for the analysis of Total Coliform and *E. coli* in drinking water for compliance with the Total Coliform Rule and the Groundwater Rule.

2.0 Principle/Summary of Method

2.1 Colilert-18 either simultaneously detects total coliforms and E.coli, or fecal coliforms in water. It is based on IDEXX's proprietary Defined Substrate Technology. When total or fecal coliforms metabolize Colilert-18's nutrient indicator, ONPG, the sample turns yellow. When E.coli metabolize Colilert-18's DST nutrient indicator, MUG, the sample also fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 cfu/100ml within 18 hours even with as many as 2 million heterotrophic bacteria per 100ml present.

3.0 Interferences/Limitations

3.1 Water samples containing humic or other material may be discolored. If there is background color, compare inoculated samples to a control sample containing only water sample. In certain waters, high calcium salt content can cause precipitation but this should not affect the reaction. This enzyme substrate test should not be used to verify presumptive coliform cultures of membrane filter colonies, because the substrate may be overloaded by the heavy inoculum of weak β–D-galactosidase-producing noncoliforms, causing false-positive results. Colilert-18 is a primary water test. Colilert-18 performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

4.0 Safety

4.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory setting while preparing, using and disposing of cultures, reagents, and materials.

4.2 Mouth-pipetting is prohibited.

5.0 Equipment and Supplies

5.1 Thermometer(s) for incubator checked against a NIST traceable certified thermometer.

5.2 Incubator maintained at 35.0ºC ± 0.5ºC. Monitored and recorded twice daily.

5.3 Water Bath maintained at 44.5 deg C +/- 0.2, monitored and recorded twice daily.

5.4 Long-wavelength U.V. Lamp- 6-watt, 365-nm

5.5 Sample bottles, pre-sterilized, clear, colorless, non-fluorescent, 120-mL capacity containing sodium thiosulphate if sampling from chlorinated source.

6.0 Reagents and Standards

6.1 Colilert-18 media is in granular form and is purchased in single use disposable ampoules (snap packs) as prepared by IDEXX Laboratories Inc. Media to be stored at temperature of 2-25ºC. Protect from light. Shelf life is up to 12 months from date of manufacture.

6.2 Control Cultures

6.2.1 Positive Control:
Stock cultures of *E.coli* ATCC 11775 or equivalent that gives typical response. (for *E. coli* check)
Stock cultures of *Enterobacter aerogenes* ATCC 13048 or equivalent. (for total coliform check)

Negative Control:
Stock Cultures of *Pseudomonas aeruginosa* ATCC 10145, ATCC 27853, or equivalent **nonfluorescent** species. (for noncoliform check)

6.3 Phosphate Buffered Dilution/Rinse Water

6.3.1 See SOP "Preparation of Sterile Buffered Rinse Water"

7.0 Sample Collection, Preservation and Storage

7.1 Sampling procedures are described in detail in the Nashoba Analytical QA/QC Manual and the *Standard Methods for the Examination of Water & Wastewater 20th Edition 1998*, Section 9060. Adherence to sample preservation procedures and holding time limits is critical to the production of valid data. Samples shall not be analyzed if these conditions are not met.

7.1.1 Storage Temperature and Handling Conditions

Ice or refrigerate bacteriological samples at a temperature of <10 deg C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that samples are not totally immersed in water during transit or storage.

7.1.2 Holding Time Limitations

Samples should be tested as soon as possible. If analysis cannot be done within 1 hour of collection, samples should be placed on ice or stored in a refrigerator at 2-8º C for a maximum holding time of 30 hours for potable water samples, 8 hours for wastewater, source waters and ambient sources.

8.0 Quality Control

8.1 Perform general quality control procedures as outlined in the Nashoba Analytical Quality Systems Manual.

8.2 The following quality control procedure is required for each lot of Colilert-18 media received:

8.2.1 Inoculate three sample bottles filled with 100-mL each of sterile Phosphate Buffered Dilution/Rinse Water with each of the positive and negative Control Cultures listed in Section 6.2. Also include a sterile buffered water control. Avoid using a heavy inoculum.

8.2.2 Follow the test procedure outlined in Section 10.0.

8.2.3 Read and record results. Results should match those outlined in Section 11.0.

9.0 Calibration and Standardization

9.1 Check temperature of incubator twice daily to ensure operation at 35º ± 0.5ºC, and of the water bath at 44.5 deg C +/- 0.2
Record in bound log book for each day incubator is in use.

9.2 Check thermometers every six months that they are in use against a NIST certified thermometer.

10.0 Procedure

10.1 Sampling for this method should be done in the field with correct bottles for the analysis so that sample does not have to be transferred in the laboratory.

10.2 100-mL of sample is required for this analysis. Check sample level using calibrated line on sample bottle. If greater than 100-mL, volume may be adjusted to 100-mL by pouring some to waste *after* sample has been thoroughly shaken. If there is not enough sample, do not proceed but request another sample with adequate volume.

Presence/Absence Procedure

10.3 **Aseptically** add preweighed contents of one Colilert-18 snap-pack to a 100-mL water sample in a sterile, transparent, **non-fluorescing** vessel:

10.4 Recap the sample bottle and shake the sample to dissolve the granules completely.

10.5 If sample is not already at 33-38 degrees then place sample in a 35 deg water bath for 20 minutes or, alternatively a 44.5 deg water bath for 7-10 minutes.

10.6 Incubate at 35 +/- 0.5 for the remainder of the 18 hours.

10.7 Read results according to Result Interpretation table below.

Quanti-Tray Enumeration Procedure

10.8 **Aseptically** add preweighed contents of one Colilert-18 snap-pack to a 100-mL water sample in a sterile, transparent, **non-fluorescing** vessel:

10.9 Recap the sample bottle and shake the sample to dissolve the granules completely

10.10 Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray 2000 and seal in an IDEXX Quanti-Tray sealer.

10.11 Place the sealed tray in a 35 +/- 0.5 incubator for Total/Ecoli or 44.5 +/-0.2 water bath for fecal coliforms. For incubation in a water bath submerge the quanti-tray as-is below the water level using a weighted ring.

10.12 Read results according to the Result interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

11.0 Interpretation of Results

11.1 After the minimum proper incubation period, examine the sample vessels for the appropriate color change. See table in Section 11.2 below.

11.2

Appearance	Result
Less Yellow than the comparator when incubated at 35+/-0.5 or 44.5+/- 0.2	**Negative** for total coliforms and *E. coli, negative for fecal coliforms*
Yellow equal to or greater than the comparator when incubated at 35----- When incubated at 44.5----------	**Positive** for total coliforms **Positive** for Fecal Coliform
Yellow and fluorescence equal to or greater than the comparator at 35 deg.	**Positive** for *E. coli*

Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and toward the sample.

Colilert-18 results are to be read after 18 hours of incubation.

However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total) to allow the color and/or fluorescence to intensify.

Positives observed before 18 hours and negatives observed after 22 hours are also valid.

In addition, laboratories may incubate samples for additional time (up to 22 hours total) for their convenience.

12.0 Reporting Results

Presence/Absence

12.1 Report results as Present or Absent for total coliforms per 100ml and Present or Absent for *E. coli* per 100ml.

Enumeration

12.2 Report results as MPN/100ml based on tables provided with Colilert-18

13.0 Pollution Prevention

13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

14.0 Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known to or suspected to have viable total coliform or *E.coli* bacteria attached or contained must be sterilized prior to disposal.

14.3 For further information on waste management, consult the Nashoba Analytical LLC Quality Systems Manual.

15.0 References

Section 9223 Enzyme Substrate Coliform Test, *Standard Methods for the Examination of Water & Wastewater 23rd ed. 2017.*

Colilert-18 Test Kit Instruction Pamphlet, 2019 IDEXX Laboratories, Inc.

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