Title: Simplified TKN

P1. IDENTIFICATION OF THE TEST METHODS

1.1 Total Kjeldahl Nitrogen by HACH Method 10242

P2. APPLICABLE MATRIX OR MATRICES

2.1 This method is for the measurement of Total Kjeldahl Nitrogen (TKN) in surface water and waste waters.

P3. DETECTION LIMIT

3.1 The limit of detection (LOD) for this analysis is determined according to 40 Code of Federal Regulations Part 136 Appendix B, *Definition and Procedure for the Determination of Method Detection Limit* (Revision 2) and re-verified annually. The limit of quantitation for this analysis is targeted to be 0.5 mg/L; however, HACH Method 10242 specifies a measurement range from 1.0 to 16.0 mg/L.

P4. SCOPE AND APPLICATION

4.1 This standard operating procedure (SOP) describes the preparation and analysis of samples for TKN. This test method is intended for use in the measurement of VPDES-compliant wastewater discharges.

P5. SUMMARY OF METHOD

5.1 In simplified TKN, inorganic (NH_3, NH_4^+, NO_2^-) and organic nitrogen are oxidized to nitrate by digestion with peroxodisulfate. The nitrate ions react with 2,6-dimethylphenol in solution of sulfuric and phosphoric acid to form a nitrophenol. Oxidized forms of nitrogen in the original sample (nitrite + nitrate) are determined in a second test vial and then subtracted, resulting in TKN.

P6. DEFINITIONS

6.1 **Certified Reference Material**: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

6.2 **Calibration Verification** (CV): A calibration standard that is analyzed periodically to ensure instrument performance has not changed significantly since initial calibration.

6.3 **Laboratory Fortified Matrix** (LFM): A sample aliquot that is “spiked” with a known amount of the analyte of interest. The LFM is used to access the effects of the sample matrix on the accuracy of the analytical method.

6.4 **Laboratory Fortified Matrix Duplicate** (LFMD): A duplicate aliquot prepared of an LFM. The LFMD is used to access the effects of the sample matrix on the accuracy and precision of the analytical method.

6.5 **Limit of Detection** (LOD): The lowest analyte concentration that produces a signal significantly differentiable from the blank.

6.6 **Limit of Quantitation** (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

6.7 **Method Blank** (MB): A sample of method appropriate pure water containing no target analyte that is taken through the entire sampling and analytical procedure.

6.8 **Safety Data Sheets** (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

6.9 **Total Kjeldahl Nitrogen** (TKN): The sum of organic and free ammonia.

P7. INTERFERENCES

7.1 High levels of oxidizable organic substances (COD) affect the reagent color and give high results.

7.2 Nitrite concentrations greater than 2.0 mg/L can cause high-bias results. To combat this, add 50 mg of sulfamic acid to 5.0 mL of sample, allow to fully dissolve and acclimate for about 10 minutes.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples should protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter and clean bench surfaces before and after testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels

8.5 Always put tops back on containers when not in use.

P9. TOOLS AND EQUIPMENT

9.1 Class A volumetric flasks and serological pipettes

9.2 Fume hood

9.3 Beakers

9.4 HACH DRB200 Reactor with 20-mm wells

9.5 20-mm Reaction Tubes

9.6 HACH DR3900 spectrophotometer

9.7 Pipettors (1000 µL and 10 mL)

9.8 Pipette tips

9.9 KimTech Wipes

P10. REAGENTS AND STANDARDS

10.1 Deionized water (SOP-WI-35, *Deionized Water System*)

10.2 TNTplus Simplified TKN Reagents (TNT 880)

10.3 Concentrated Sulfuric Acid (min 98%)

10.4 Ammonia Standard Solutions (i.e., 1.0mg/L, 10.0 mg/L)

10.5 Sodium Hydroxide (for pH adjustment)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE

11.1 Sampling should be performed as specified by a field sampling plan or based on compliance-based sampling requirements.

11.2 Samples must be iced or refrigerated at 4°C ± 2°C, and should not be frozen. If analysis cannot be performed immediately following sample collection, preserve samples with sulfuric acid to less than pH 2 (2 mL concentrated sulfuric acid per 1 L sample). If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.3 The maximum recommended holding time for wastewater samples requiring TKN testing is 28 days. Holding time for composite samples is defined as the time from the end of compositing to the beginning of sample analysis. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

12.2.1 To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for TKN obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.2.2 Sign in the proficiency test in the Sample Receiving Logbook. Be sure to record the sample ID number on the sample container.

12.2.3 Follow the PT provider's included instructions for preparing the sample and analyze according to P14.

12.3 Initial Demonstration of Capability (IDC)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 The analyst should prepare certified reference material provided by the qualified analyst according to the manufacturer's specifications.

12.3.4 The analyst then shall carry out four replicate analyses as per P14. The interpretation of the analyst's results is discussed in P18.

12.4 On-going Demonstration of Capability (ODC)

12.4.1 Analysts are required to re-verify their capability in this field of testing through the on-going demonstration of capability. Prior to completing the on-going demonstration of capability, the analyst must review this SOP and sign off on the SOP Signature Sheet.

12.4.2 The analyst should prepare a certified reference material according to the manufacturer's specifications.

12.4.3 The analyst shall carry out the analysis of this sample and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Method Blank (MB)

12.5.1 Per method requirement, the laboratory shall perform analysis of a blank sample, consisting solely of deionized water, taken through all sample preparation and analysis steps, per batch. Interpretation of method blank analyses is discussed in P18.

12.6 Laboratory Fortified Matrix (LFM)

12.6.1 Per method requirement, the laboratory shall perform analysis of an LFM and LFMD pair, prepared by spiking a sample with a known concentration of analyte, per batch. Interpretation of LFM and LFMD analyses is discussed in P18.

12.7 Calibration Verification (CV)

12.7.1 Per method requirement, the laboratory shall perform analysis of two CV samples, prepared from a certified reference material and taken through all sample preparation and analysis steps, per ten samples analyzed or per batch, whichever is greater. Preparation of CV standards is discussed further in P13. Interpretation of CV analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 Calibration

13.1.1 The Hach DR Series spectrophotometers have a built-in calibration that is automatically used when the TNTplus nitrate vial is inserted into the apparatus. No initial calibration is needed.

13.2 Standards

13.2.1 CV

13.2.1.1 The analyst shall prepare CV standards (as specified in P12.7) from a primary source ammonium chloride standard. (1.0 mg/L and 10 mg/L NH_3-N standards are recommended to verify the instrument is accurately measuring TKN.)

P14. PROCEDURE

14.1 Follow the manufacturer's instructions for setting up the DR3900.

14.2 Turn on the DRB200 reaction block and allow instrument to heat to 100°C.

14.3 Using the TNT880 Kit

14.3.1 Add **1.3 mL of sample** to a dry 20-mm reaction tube, followed by **1.3 mL of Solution A** and **1 tablet of Reagent B** in quick succession. Close the tube immediately and **DO NOT INVERT**. (*Do not get rid of excess sample, will need later in the analysis*)

14.3.2 Insert reaction tube in the hot block and close plastic hood and heat for one hour.

14.3.3 After one hour, remove the reaction tube from the hot block and allow to cool to room temperature (15-20°C). While waiting for the reaction tube to cool, align and label **Test Vial 1 (red cap)** and **Test Vial 2 (green cap)** for each sample.

14.3.4 Once at room temperature (can verify by IR thermometer), add **1 Micro Cap C capsule** to the reaction tube. Cap the reaction tube and invert at least 3 times until no more white streaks or particles are seen.

14.3.5 Remove cap from **Test Vial 1** and **Test Vial 2**, carefully placing them in order with the opening face down on the surface of the work bench.

14.3.6 Pipette **0.5 mL** of the **digested** sample from the reaction tube into **Test Vial 1 (red cap)**.

14.3.7 Pipette **0.2 mL** of **Solution D** into the same **Test Vial 1 (red cap)**. Quickly cap the vial and invert 2-3 times until no more streaks can be seen. Immediately proceed to next step with no pause or lag in between.

14.3.8 Pipette **1.0 mL** of **undigested sample** (*the excess sample from earlier*) into a **Test Vial 2 (green cap)**.

14.3.9 Pipette **0.2 mL** of **Solution D** into the **Test Vial 2 (green cap).** Quickly cap and invert 2-3 times till no more streaks are seen. Be **cautious** when doing this, as the vial will produce **heat**!

14.3.10 Set timer for 15 minutes to allow the reactions to take place

14.3.11 After the 15 minutes, clean **Test Vial 1 (red cap)** with a dry KimTech Wipe and insert the vial into the spectrophotometer. It will then read the bar code and display **E1**. While waiting for the E1 reading, have **Test Vial 2 (green cap)** cleaned and waiting.

14.3.12 Once E1 is displayed, remove **Test Vial 1 (red cap)** and insert **Test Vial 2 (green cap)**. The spectrophotometer will then read the bar code and give you the data output.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 The sample result will be displayed in mg/L Total N, mg/L NO_3-N + NO_2-N and mg/L TKN. Record all displayed values in the appropriate spaces on the Simplified TKN Bench Sheet.

15.2 Average

15.2.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.2.2 Applicable Formulas

15.2.2.1 $Average(X) = \sum \frac{X_i}{n},$

15.3 Relative Standard Deviation (RSD)

15.3.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $Standard\ Deviation(X) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$

15.3.2.2 $RSD = \frac{Standard\ Deviation}{Average} * 100\%$

15.4 Percent Recovery

15.4.1 The percent recovery of analyte measured in the CV samples.

15.4.2 Applicable Formulas

15.4.2.1 $\%\ Recovery = \frac{x_{measured}}{x_{theoretical}} * 100\%$

15.5 Spike Recovery

15.5.1 The percent recovery of analyte measured in LFM and LFMD samples.

15.5.2 Applicable Formulas

15.5.2.1 $\%\ Recovery = \frac{[LFM]_{measured} * V_{LFM} - [Sample]_{measured} * V_{sample}}{[Spike\ Standard]_{theoretical} * V_{spike\ standard}} * 100\%$

15.6 Relative Percent Difference (RPD)

15.6.1 The relative percent difference between the measured values of the LFM and LFMD.

15.6.2 Applicable Formulas

15.6.2.1 $RPD = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} *$
$100\%\ where\ X_1\ and\ X_2\ are\ the\ concentrations\ of\ the\ LFM\ and\ LFMD$

15.7 Significance

15.7.1 TKN values should be reported to 3 significant figures or to two decimal points, whichever is less significant.

P16. METHOD PERFORMANCE

16.1 The current limit of detection and limit of quantitation are posted in the laboratory. Additionally, they are included as an attachment to this document and as an appendix in the VELAP Quality Manual.

16.2 Accuracy and precision requirements are established by control charting.

P17. POLLUTION PREVENTION

17.1 All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Proficiency test results must fall within the range established by the provider.

18.3 Initial Demonstration of Capability

18.3.1 Average must fall within the acceptance range specified by the provider, if given.

18.3.2 RSD must be less than 20%.

18.4 Ongoing Demonstration of Capability

18.4.1 Result must fall within the range established by the provider.

18.5 P12.5: Method Blank

18.5.1 Result must be less than half the LOQ (should also be below the LOD).

18.6 P12.6: Laboratory Fortified Matrix

18.6.1 Spike recovery must be between 80% and 120%.

18.6.2 RPD must be less than 20%

18.7 P12.7: Calibration Verification

18.7.1 Percent recovery must be between 90% and 110%.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL OR UNACCEPTABLE DATA

19.1 If any of the quality control procedures indicates that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measures, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting the analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 The wastes generated in this method are hazardous because of a high final pH. The samples may be neutralized by the addition of water in an accumulation container (e.g., a large beaker or flask) or by slowly pouring down the drain with the faucet running followed by a triple rinse of the container to remove residual corrosives.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

22.1 2009 TNI Standard (Volumes 2 and 4)

22.2 40 CFR 136, Appendix B.

22.3 Water Analysis Handbook," Hach Company, 5th Edition, 2008.

22.4 Nitrogen, Simplified TKN (s-TKN™), DOC316.53.01258, Hach Company, Loveland, Colorado USA. http://www.hach.com/quick.search-quick.search.jsa?keywords=DOC316.53.01258.

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline		

SOP-WI-1

Alkalinity as $CaCO_3$

Revision 4

Approval:

Marianne Simko — 2-4-19

Environmental Programs Manager — Date

2-4-19

Laboratory Director — Date

2-4-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

Title: Alkalinity as $CaCo_3$

P1. IDENTIFICATION OF THE TEST METHOD

Determination of Alkalinity as $CaCO_3$. Standard Method 2320 B

P2. APPLICABLE MATRIX OR MATRICES

Alkalinity of a sample is determined by titrating a sample to a specific pH end-point value. Alkalinity is significant in many uses and treatment of natural waters and wastewaters.

P3. DETECTION LIMITS

The limit of quantitation (LOQ) is 20 mg/L as specified in Standard Method 2320 B.

P4. SCOPE AND APPLICATION

Alkalinity is a measure of the acid-neutralizing capacity of a water sample and is the sum of all the titratable bases. Alkalinity is an aggregate property and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. Although many materials may contribute to the alkalinity, the major contributor of alkalinity in natural water is a function of carbonate, bicarbonate, and hydroxide content. Other constituents, borates, phosphates, silicates, and/or other bases, may contribute to alkalinity, but in most situations, these substances are insignificant and may be ignored. The measured alkalinity value can vary significantly depending on the selected titration end-point.

P5. SUMMARY OF METHOD

This method has been modified within the flexibility allowed in 40 CFR 136.6. The term alkalinity encompasses several different chemical components of water but can be best viewed as the ability of the water to resist a drop in pH, or in another sense, its buffering capacity. The test is performed by slowly titrating a sample with sulfuric acid to a colorimetric endpoint corresponding to a specific pH. Phenolphthalein Alkalinity is determined by titrating to a pH of 8.3. Total Alkalinity is determined by the titration to a pH range between 3.7 and 5.1, which includes all carbonate, bicarbonate, and hydroxide species. The selected indicator dye changes color within the desired pH range for alkalinity determination. For most analyses, *bromcresol green-methyl red* is used to indicate a pH endpoint of 4.5 for water containing elevated levels of phosphate. Values less than 20 mg/L may only be reported if determined by a low-alkalinity method as described in standard methods 2320 B4d.

P6. DEFINITIONS

Alkalinity: Alkalinity refers to the buffering capacity of a water sample and is determined by the sum of carbonate and bicarbonate concentrations.

Alkalinity, $[CaCO_3] = [OH^-] + 2[CO_3^{-2}] + [HCO_3^-] - [H^+]$
Where:
$[OH^-]$ = hydroxide ion concentration

$[CO_3^{-2}]$ = carbonate ion concentration

$[HCO_3^-]$ = bicarbonate ion concentration

$[H^+]$ = hydrogen ion concentration

All concentrations are expressed in $\frac{mg}{L}$

Duplicate (DUP): Two aliquots of the same sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

Laboratory Fortified Blank (LFB): Sometimes referred to as a standard, the LFB is a blank that has the analyte of interest added so that the sample has a known concentration. The LFB is prepared/extracted/digested and analyzed exactly like the field samples and is used to determine bias (accuracy) of a method.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations. The LOQ is generally 3 to 5 times the LOD unless specified by the method.

Method Blank (MB): A blank of deionized water or extraction blank of the same matrix as the samples and standards used. The method blank is prepared/extracted/digested and analyzed exactly like the field samples. Its purpose is to assess contamination introduced during sample preparation activities.

Quality Control Sample (QCS): An externally generated sample containing analytes of interest at unknown concentrations. Sometimes referred to as a **Proficiency Test** (PT), the QCS is used to determine laboratory and method competence. It is obtained from an external lab semi-annually and results are compared to that labs acceptance results. If results do not meet acceptance criteria, investigate why, take corrective action, and run a new QCS.

Phenolphthalein Alkalinity: Measurement of alkalinity to the phenolphthalein alkalinity endpoint (pH 8.3). Phenolphthalein Alkalinity is used to predict, (primarily) hydroxide and carbonate portions of alkalinity.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Total Alkalinity: Measurement of the alkalinity to the Bromcresol Green-Methyl Red endpoint (pH 4.3-5.1).

P7. INTERFERENCES

7.1 Highly colored or turbid samples may mask the color change at the endpoint—use a pH meter to determine the endpoint.

7.2 Chlorine may interfere with the indicators. Add one drop of Sodium Thiosulfate (0.1N) to eliminate this effect.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Wear Personal Protective Equipment (PPE) such as lab coat, safety glasses, and gloves when performing Alkalinity as $CaCO_3$ analysis.

8.3 Refer to SDS for all chemicals and reagents before beginning analysis for more safety information.

P9. EQUIPMENT AND SUPPLIES

9.1 Apparatus

- Buret clamp, double
- Buret, Class A, 50 mL
- Erlenmeyer flask, 250 mL
- Graduated Cylinder
- Support stand
- Magnetic stir plate
- Stir bar
- Beakers

P10. REAGENTS AND STANDARDS

10.1 Reagents

- Sulfuric Acid Standard Solution, 0.020 N
- Alkalinity Voluette Ampule Standard Solution, 0.500 N
- Bromcresol Green-Methyl Red Indicator Powder Pillows
- Phenolphthalein Indicator Powder Pillows
- Alkalinity Standard Solution Sodium Carbonate
  Note: This method has been modified within the flexibility allowed in 40 CFR 136.6.

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Collect the samples in clean glass or HDPE plastic container with zero headspace. Place samples on ice. Avoid excessive agitation or prolonged exposure to air.

11.2 If residual chlorine is present, dechlorinate sample. Record residual chlorine on chain of custody and add one drop of Sodium Thiosulfate (0.1N) to eliminate this effect.

11.3 Preserve samples by storing at 4^0C. Recommended to analyze within 24 hours, regulatory holding time is 14 days.

P12. QUALITY CONTROL

12.1 Record the analysis date and time, as well as starting and ending titrations for this analysis in the alkalinity log book.

12.2 *Annually perform two Quality control samples (QCS)/Proficiency Test Studies (PT).* Follow the procedures for preparing the sample, and obtaining test results. Proficiency testing will be performed semi-annually using samples from an outside source.

12.2.1 To demonstrate method performance, the laboratory analyzes a QCS twice per year or otherwise as required for each method where QC samples are available. Quality control samples (QCS) are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in. QCS are not analyzed multiple times unless routine environmental samples are analyzed multiple times.

12.3 *Perform Initial demonstration of capability* (for any new laboratory personnel)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

12.3.2 Carry out four analyses using 50 mL samples for each.

12.3.3 Calculate the average and standard deviation of the values.

12.3.4 The standard deviation should be less than or equal to 20% of the average value. If it is not, evaluate the procedures to identify sources of error.

12.4 *Method Blank*

12.4.1 Analyze a reagent water test blank with each batch.

12.5 *Laboratory Fortified Blank*

12.5.1 Quality control samples are used to document the validity of data and to control data quality within acceptance limits. QC samples are processed through the system in the same manner as any other sample, except when the analyst is aware of the source, concentration, and acceptance ranges of target analytes and calculates analyte recoveries to evaluate method performance in real time.

12.5.2 For each laboratory fortified blank (LFB) determine the percent recovery using equation 1. The percent recovery should be within 10% of the true value.

$$\%\ Recovery = \frac{found\ value}{true\ value}\ x\ 100\% \qquad \text{Equation 1)}$$

12.6 *Duplicate (Dup)*

12.6.1 Carry out a duplicate analysis on a sample. At least 5% of samples or one per batch are analyzed in duplicate every time analysis is performed, whichever is more frequent. Calculate the precision (standard deviation) involved with each duplicate sample set.

12.6.2 The relative percent difference (RPD) between the two alkalinity values should be ≤ 20% of their average value and is calculated using equation 2.

$$RPD = \frac{|(sample - dup)|}{[(sample + dup)/2]}\ x\ 100\% \qquad \text{Equation 2)}$$

12.7 *Accuracy Check*

12.7.1 *Standard Additions Method*
Use the standard additions method to find if the sample has an interference with each sample batch.

- Alkalinity Standard Solution, 0.500 N (25-g/L as CaCO3)
- Ampule Breaker
- Pipet, TenSette, 0.1–1.0 mL and pipet tips

1. Use the test procedure to measure the concentration of the sample.

2. Use a TenSette pipet to add 0.1 mL of the standard solution to the titrated sample.

3. Titrate the spiked sample to the endpoint. Record the mL of titrant added.

4. Add one more 0.1-mL addition of the standard solution to the titrated sample.

5. Titrate the spiked sample to the endpoint. Record the mL of titrant added.

6. Add one more 0.1-mL addition of the standard solution to the titrated sample.

7. Titrate the spiked sample to the endpoint. Record the mL of titrant added.

8. Compare the actual result to the correct result. The correct result for this titration is 2.5 mL of titrant for each 0.1-mL addition of the standard solution. If much more or less titrant was used, there can be a problem with user technique, reagents, apparatus or an interference.

12.7.2 *Standard Solution Method*

Complete the following test when reusing titrant to confirm the analytical technique and reagent performance with each sample batch.

Procedure for use with the **0.020 N** titrant:

1. Standardize the Sulfuric Acid titrant by adding 10 ml of 0.02 N Standard Sodium Carbonate and 90 ml CO2-free water into a 250 ml beaker.
2. Titrate with sulfuric acid according to the pH endpoint procedure below and use the following equation to determine the normality of the sulfuric acid solution.

   NV = N'V' where N is the normality of the standard
   V is the volume of the standard (ml)
   N' is the normality of the titrant
   V' is the volume of the titrant (ml)

The standardized Sulfuric Acid titrant should be approximately 0.02N. If higher, this may be adjusted to 0.02N and confirmed as above. Store in a dated and labeled glass bottle; standardized Sulfuric Acid titrant can be used for 1 year.

12.7.3 The Lab Supervisor shall review the results to ensure accuracy and precision. In addition, the lab supervisor shall initial the results to indicate that the calculations have been reviewed and are accurate.

P13. CALIBRATION AND STANDARDIZATION

13.1 Standards and reagents are commercially prepared. Reagents are restandardized with each analysis by comparing the normality against the expected value and adjusted to fit the experimental value (see *Standard solution method 12.8.2*).

13.2 Laboratory fortified blanks are run at the beginning of each batch to verify method.

13.3 Duplicates are run with each batch to ensure reliability of procedure.

P14. PROCEDURE

14.1 Complete the following documents for each sample or set of samples submitted for analysis:

14.1.1 Chain-of-Custody sheet

14.1.2 Laboratory Bench sheet (See Attachment 1)

14.1.2.1 Sample information

14.1.2.2 Calculation

14.2 Titration Procedure

1. Check for freshness of reagents.

2. Prepare a blank. One blank must be run with each set of samples. With every batch perform a blank, LFB, and dup.

3. For samples with expected acidities less than 1000 mg $CaCO_3$/L use 50 mL of sample and 0.020 N sulfuric acid standard solution as the titrant. For samples with a greater acidity (rarely) use 25 mL of sample with 0.1 N sulfuric acid standard solution as the titrant.

4. After sample is equilibrated to room temperature, measure appropriate volume (typically 50 mL) of sample using a graduated cylinder. Transfer sample to a 250-mL Erlenmeyer flask equipped with a stir bar.

5. Add contents of one Phenolphthalein Indicator Powder Pillow and set sample to stir.

6. Fill a 50-mL buret to the zero mark with 0.020 or .1 N Sulfuric Acid Standard Solution according to step 3 above. Be sure to let the air out of the buret tip. Record the actual starting volume prior to starting titration.

7. While sample is stirring, slowly add sulfuric acid with delivery knob and titrate to a colorless endpoint. Titrate the sample until the solution color changes from pink to colorless (pH 8.3). If the solution is colorless before titrating with sulfuric acid, then the phenolphthalein alkalinity is zero. Record the titrant volume required to get to the colorless endpoint in the phenolphthalein alkalinity space on the data sheet. Do not zero burette. Calculate the phenolphthalein alkalinity as per equation 3 found in *P15 Calculations* below and record the value on the data sheet.

8. Add contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the titrated sample and continue to stir.

9. Begin slowly adding sulfuric acid with delivery knob. Continue the titration until a light pink end point (pH 4.5) is reached and record the total amount of titrant added in the total level

space on the data sheet. Calculate the total alkalinity as per equation 3 found in *P15 calculations* below and record on the data sheet.

10. Add more titrant, as required, and rinse stir bar before each subsequent sample.

P15. CALCULATIONS

$$alkalinity, \frac{mg\ CaCO_3}{L} = \frac{A\ x\ N\ x\ 50{,}000}{mL\ sample}$$ Equation 3)

Where: A = mL standard acid solution used
N = normality of standard acid solution

P16. METHOD PERFORMANCE

This method was validated through inter-laboratory studies. LJT & Associates, Inc. participates in Proficiency Testing (PT) Program for VELAP certification.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Method blanks (MB) should be $\leq$ LOD.

18.2 Quality Control Samples (QCS) must meet the criteria of the lab where they were purchased.

18.3 Laboratory fortified blanks (LFB) should exhibit a percent recovery of less than a 10% variation from the known value.

18.4 Duplicate samples should exhibit a RPD $\leq$20% of the average value of the samples.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

If any of the quality control procedures indicates that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measures, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique

prior to conducting the analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 The samples may be neutralized by the addition of water in accumulation container (e.g., a large beaker or flask). Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- 22nd ed. Standard Methods for the Examination of Water and Wastewater 2320 B, 2011

P23. TABLES, DIAGRAMS AND FLOWCHARTS

Tables and flowcharts can be found within this document

ALKALINITY as $CaCO_3$
Method SM 2320 B

SAMPLE ID	DATE/TIME ANALYZED	Sulfuric Acid Solution Normality	Int. Buret Level	Phenolph. Level	Total Level	Phenolph. Alk (mg/L)	Total Alk (mg/L)	ANALYST

Sulfuric Acid Solution: Lot #_____ Expiration _____

Phenolphthalein Indicator Pillows: Lot # _____ Expiration _____

Bromcresol – Methyl Pillows: Lot # _____ Expiration _____

Alkalinity Ampule Standard: Lot # _____ Expiration _____

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	8/24/09	Switched from using ASTM D 1067-92 method to 18th Ed. SM2320B.
B	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
3	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition
	06/24/13	Latest version 22nd ed. SM. Updated reference - method title- to the latest Standard Method edition. With every batch perform a blank, duplicate, spike, and QC.
	04/01/14	Contract changed from URS/EG&G to LJT & Associates, Inc.
	12/18/14	MSDS changed to SDS. Formatting and Quality Control revised.
	4-28-15	This method has been modified within the flexibility allowed in 40 CFR 136.6 to allow calcium carbonate standard solutions in replacement of sodium carbonate standards. Quality control titration reagents re-standardized once a month or when in use.
	05-15-15	Quality control section revised: Re-standardize reagents with each sample batch. Interference section revised: remove Chlorine by sample pretreatment.
4	01-31-2017	Grammatical and formatting changes. Calibration Verification was removed from method. Changes to procedure to reflect internal audit findings. Accumulation of minor changes under a single revision warrants a revision change.

SOP-WI-2

AMMONIA as N

Revision 5

Approval:

Marianne Smith — 1/31/19

Environmental Programs Manager — Date

1-31-19

Laboratory Director — Date

1-31-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 1/31/2019

Title: Ammonia as N

P1. IDENTIFICATION OF THE TEST METHOD

Ammonia as N

P2. APPLICABLE MATRIX OR MATRICES

This test method is suitable for the quantification of ammonia as nitrogen in surface and wastewaters.

P3. DETECTION LIMIT

The limit of detection (LOD) for this analysis is determined according to 40 Code of Federal Regulations Part 136 Appendix B, *Definition and Procedure for the Determination of Method Detection Limit* (Revision 2) and re-verified annually. The limit of quantitation for this analysis is targeted to be 1 mg/L; however, the actual calculated limit of quantitation, as well as the current limit of detection, are posted in the laboratory.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test method for the analysis of samples for Ammonia as N. This test method is intended for use in the measurement of ammonia in surface waters and domestic and industrial wastewaters.

P5. SUMMARY OF METHOD

This procedure described here is certified under SM 4500-NH_3-D, which describes the determination of ammonia. Ammonia is calculated from potentiometric measurements from an ion-selective probe relative to standards created from certified reference materials using log-linear regression.

P6. DEFINITIONS

Calibration Standard: A solution prepared from a stock solution of known concentration that is used to calibrate the instrument response with a known analyte concentration.

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Calibration Verification (CV): A calibration standard that is analyzed periodically to ensure instrument performance has not changed significantly since initial calibration.

Laboratory Fortified Blank (LFB): A standard prepared from a certified reference material of a different lot than is used to for creation of the primary standard. The LFB is used as a second-source accuracy verification of the calibration curve.

Laboratory Fortified Matrix (LFM): A sample aliquot that is "spiked" with a known amount of the analyte of interest. The LFM is used to access the effects of the sample matrix on the accuracy of the analytical method.

Laboratory Fortified Matrix Duplicate (LFMD): A duplicate aliquot prepared of an LFM. The LFMD is used to access the effects of the sample matrix on the accuracy and precision of the analytical method.

Limit of Detection (LOD): The lowest analyte concentration that produces a signal significantly differentiable from the blank.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

Method Blank (MB): A sample of method appropriate pure water containing no target analyte that is taken through the entire sampling and analytical procedure.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7. INTERFERENCES

7.1 Volatile amines act as a positive interference. This effect is enhanced by acidification.

7.2 Mercury interferes by forming a strong complex with ammonia. Thus the samples cannot be preserved with mercuric chloride.

7.3 Residual chlorine interferes with ammonia determination.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples should protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter and clean bench surfaces with disinfectant before and after bacteriological testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

P9. EQUIPMENT AND SUPPLIES

- Volumetric flasks
- Pipettes and/or micropipettors
- Ammonia Ion Selective Electrode and meter
- Magnetic stirrer and stir bars

P10. REAGENTS AND STANDARDS

10.1 Deionized water (SOP-WI-35, *Deionized Water System*)

10.2 Ionic Strength Adjuster, ISA (lithium hydroxide and EDTA)

10.3 Electrode filling solution

10.4 Ammonium Chloride certified reference materials

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE

11.1 Sampling should be performed as specified by a field sampling plan or based on compliance-based sampling requirements.

11.2 Samples should be iced or refrigerated at 4°C or less but should not be frozen. If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.3 The maximum recommended holding time for wastewater samples requiring ammonia testing is 24 hours. This can be extended to 7 days by acidification with sulfuric acid. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on a fresh copy of the Nitrogen Analysis Template before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for ammonia obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.3 Initial Demonstration of Capability

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 Analyze four aliquots of a certified reference material, prepared according to manufacturer's instructions, with all required batch quality control.

12.3.3 Include any required preparation steps as a line item in the Standard Logbook.

12.3.4 Interpretation of initial demonstration of capability results is discussed in P18.

12.4 On-going Demonstration of Capability

12.4.1 Similarly to P12.3, analyze a single aliquot of a certified reference material with all required batch quality control. Unlike an initial demonstration of capability, customer samples can be batched with on-going demonstrations of capability. Interpretation of on-going demonstration of capability results is discussed in P18.

12.5 Method Blank

12.5.1 Per method requirement, the laboratory shall perform analysis of a blank sample, consisting solely of deionized water, taken through all sample preparation and analysis steps, per batch. Interpretation of method blank analyses is discussed in P18.

12.6 Calibration Standards

12.6.1 Per method requirement, the laboratory shall perform analysis of at least three calibration standards, per batch, prepared from a certified reference material and taken through all sample preparation and analysis steps. Preparation of calibration standards is discussed further in P13. Interpretation of the resulting calibration curve is discussed in P18.

12.7 Laboratory Fortified Blank (LFB)

12.7.1 Per method requirement, the laboratory shall perform analysis of an LFB, prepared from a secondary source certified reference material and taken through all sample preparation and analysis steps, per batch. Preparation of LFB standards is discussed further in P13. Interpretation of LFB analyses is discussed in P18.

12.8 Laboratory Fortified Matrix *(*LFM*)*

12.8.1 Per method requirement, the laboratory shall perform analysis of an LFM and LFMD pair, prepared by spiking a sample with a known concentration of analyte, per batch. Preparation of LFM and LFMD standards is discussed further in P14. Interpretation of LFM and LFMD analyses is discussed in P18.

12.9 Calibration Verification (CV)

12.9.1 Per method requirement, the laboratory shall perform analysis of a CV, prepared from the primary source certified reference material and taken through all sample preparation and analysis steps, per ten samples analyzed or per batch, whichever is greater. Preparation of CV standards is discussed further in P13. Interpretation of CV analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 Ammonia ISE Probe

13.1.1 Setup

13.1.1.1 The Ammonia ISE probe is stored with the glass bulb immersed in a soaker bottle containing Ammonia Electrode Storage Solution. The membrane module is typically stored in the dessicator between uses.

13.1.1.2 To reassemble the probe, first remove the soaker bottle from the cap by unscrewing. This will relieve the pressure, allowing for safe removal of the soaker bottle.

13.1.1.3 Remove a membrane module from the dessicator and put 12 drops of Ammonia Electrode Filling Solution in the cap.

13.1.1.4 Screw the membrane module into the probe body.

13.1.1.5 If not already done, install the cable of the Ammonia ISE Probe into the back of a compatible multimeter.

13.1.1.6 Attach probe to probe arm, making sure to route cable behind the probe using the notches on the probe arm.

13.1.2 Calibration

13.1.2.1 While capable, *SM 4500 NH_3-D* does not allow for the use of the multimeter for calibration record retention. Therefore, the probe is only used to capture voltage and temperature measurements.

13.1.3 Slope Check

13.1.3.1 Prior to use, the response of the probe must be checked.

13.1.3.2 Measure 100 mL of deionized water and add 3 mL of ISA solution.

13.1.3.3 To this solution, add 1 mL of 1000 mg/L NH_3 as N standard and measure the millivolt reading. Record this measurement in the "+1 mL" field on the Nitrogen Analysis Benchsheet under the "Probe Decade Response" header.

13.1.3.4 Additionally, add 10 mL of 1000 mg/L NH_3 as N standard and measure the millivolt reading. Record this measurement in the "+10 mL" field on the Nitrogen Analysis Benchsheet under the "Probe Decade Response" header. The difference should produce a "Slope Check Value" between -54 and -60 mV (acceptance criteria may vary depending on the probe used).

NOTE: This step can also be accomplished using measurements of the calibration standards that exhibit a ten-fold difference (for example, 1 mg/L and 10 mg/L standards).

13.2 Thermometers

13.2.1 Calibrated, NIST-traceable thermometers are kept in the sample refrigerator. Thermometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.2.2 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.2.3 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

13.3 Standards

13.3.1 Calibration Standards

13.3.1.1 The analyst shall prepare, at least, three calibration standards from an ammonium chloride certified reference material spanning the range of expected concentrations (e.g., 100, 10, 1, and 0.5 mg/L) and one of which is run at or below the LOQ. For greater linearity, standards should be prepared by serial dilution.

13.3.2 LFB

13.3.2.1 The analyst shall prepare one LFB standard from a secondary source ammonium chloride standard at a concentration within the log-linear range of the calibration curve. At least quarterly, an LFB should be prepared at one to two times the LOQ.

13.3.3 CV

13.3.3.1 The analyst shall prepare CV standards (as specified in P12.9) from the primary source ammonium chloride standard at a concentration within the log-linear range of the calibration curve.

P14. PROCEDURE

14.1 Chlorine Check

14.1.1 Samples should be checked for residual chlorine prior to the beginning of sample analysis. If detected, samples must be dechlorinated using a freshly-prepared sodium thiosulfate solution.

14.2 Verify that samples are within proper hold conditions (P11).

14.3 Preparation of the LFM and LFMD

14.3.1 Prepare a spike with target concentration inside the range of the calibration curve. Specify the volume of sample and volume of spike standard used as a line item in the “Sample Notes” table.

NOTE: Sample matrix volume should not be altered by more than 5% when spiking.

14.4 Standard Curve

14.4.1 Use the sample volume and the volume ISA indicated on the “Batch Analysis Conditions” table to generate the standard curve.

NOTE: The volume of ISA indicated by the sheet can be changed by right-clicking the tab name on the bottom of the screen and left-clicking “Unprotect Sheet”. This can be helpful if samples require additional ISA to achieve the method-required pH of 11 (indicated by the blue color of the ISA solution).

14.4.2 Indicate the sample ID and target concentration in the “Regression Table”.

14.4.3 Prior to analyzing curve standards, it is highly recommended to allow equilibration to constant temperature conditions in a room temperature water bath for, at least, 30 minutes.

14.4.4 For each standard, pour designated sample volume into an appropriate container (i.e., 150 mL beaker) with a magnetic stirrer for measurement.

14.4.5 While stirring, immerse the membrane cap into the solution to be measured. Ensure that no air bubbles form on the membrane. If bubbles form, slow the stir speed and gently flick the probe body to release.

14.4.6 After addition of indicated ISA volume, record the stabilized potential (mV response) and temperature reading of each calibration standard in the appropriate spaces in the “Regression Table”. Interpretation of the calibration curve in its entirety is discussed in P18.

14.5 Samples

14.5.1 Indicate the sample ID, location, and QC type and target concentration (if applicable) in the “Samples” table. If any sample requires additional clarification (i.e., preparatory steps of an LFM), select its sample ID from the drop-down menu provided in the “Sample Notes” table and provide the clarification in the adjacent “Note” field.

14.5.2 Prior to sample analysis, it is highly recommended to allow equilibration to constant temperature conditions in a room temperature water bath for, at least, 30 minutes.

14.5.3 For each sample, pour designated sample volume into an appropriate container (i.e., 150 mL beaker) with a magnetic stirrer for measurement.

14.5.4 While stirring, immerse the membrane cap into the solution to be measured. Ensure that no air bubbles form on the membrane. If bubbles form, slow the stir speed and gently flick the probe body to release.

14.5.5 After addition of indicated ISA volume, record the stabilized potential (mV response) and temperature reading of each calibration standard in the appropriate spaces in the “Regression Table”.

14.5.6 If required, indicate a dilution factor in the provided field on the "Samples" table; otherwise, leave the field empty or simply type the number "1". An example of when this field may be used is that an analyst measures 80 mL of sample with 20 mL of deionized water (instead of the standard 100 mL) and the standard 3 mL of ISA. In this case, for completion, the analyst should type in the dilution factor as "=100/80" or "1.25".

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Ammonia

15.1.1 The Nitrogen Analysis Benchsheet uses log linear regression, accounting for dilution, to automatically calculate ammonia concentrations by the following formula:

15.1.2 Applicable Formulas

15.1.2.1 $$Ammonia\ as\ N, mg/L = d.f.* [NH_3]$$
$$where\ d.f. is\ the\ dilution\ factor,$$
$$[NH_3]\ is\ the\ concentration\ of\ ammonia\ fron\ the\ calibration\ curve$$

15.2 Ammonia (Average)

15.2.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.2.2 Applicable Formulas

15.2.2.1 $$Average(X) = \sum \frac{X_i}{n},$$
$$where\ X\ contains\ n\ values\ X_i$$

15.3 Ammonia (RSD)

15.3.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $$Standard\ Deviation(X) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$
$$where\ X\ contains\ n\ values\ X_i\ with\ average\ \bar{X}$$

15.4 Ammonia (% Recovery)

15.4.1 The percent recovery of analyte measured in LFB and CV samples and calibration standards.

15.4.2 Applicable Formulas

15.4.2.1 $$\%\,Recovery = \frac{x_{measured}}{x_{theoretical}} * 100\%$$

15.5 Ammonia (% Recovery, Spike)

15.5.1 The percent recovery of analyte measured in LFM and LFMD samples.

15.5.2 Applicable Formulas

15.5.2.1 $$\%\,Recovery = \frac{[LFM]_{measured} * V_{LFM} - [Sample]_{measured} * V_{sample}}{[Spike\ Standard]_{theoretical} * V_{spike\ standard}} * 100\%$$

$$where\ brackets\ denote\ concentrations, in\ \frac{mg}{L}, and\ V\ denotes\ volumes, in\ mL$$

15.6 Ammonia (RPD)

15.6.1 The relative percent difference between the measured values of the LFM and LFMD.

15.6.2 Applicable Formulas

15.6.2.1 $$RPD = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} * 100\%$$

$$where\ X_1\ and\ X_2\ are\ the\ concentrations\ of\ the\ LFM\ and\ LFMD$$

15.7 Significance

15.7.1 Ammonia values should be reported to 3 significant figures or to two decimal points, whichever is less significant.

P16. METHOD PERFORMANCEE

16.1 The current Limit of Quantitation and Limit of Detection are posted on the laboratory wall. The ideal LOQ for this analysis is approximately 1 mg/L.

16.2 Accuracy and precision requirements are established by control charting.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Ammonia as N: Proficiency test results should fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Ammonia (Average) should fall within the acceptance range specified by the provider, if given.

18.3.2 If analyzing four aliquots of a LFB to meet this requirement, Ammonia (% Recovery) should be calculated using Ammonia (Average). This Ammonia (% Recovery) should be between 85% and 115%.

18.3.3 Ammonia (RSD) should be less than 20%.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 Ammonia should fall within the range established by the provider.

18.5 P12.5: Method Blank

18.5.1 Ammonia should be less than half the LOQ (ideally also below the LOD).

18.6 P12.6: Calibration Standards

18.6.1 Ammonia (% Recovery) should be between 90% and 110%.

18.7 P12.7: Laboratory Fortified Blank

18.7.1 Ammonia (% Recovery) should be between 85% and 115%.

18.8 P12.8: Laboratory Fortified Matrix

18.8.1 Ammonia (% Recovery, Spike) should be between 80% and 120%.

18.8.2 Ammonia (RPD) should be less than 20%

18.9 P12.9: Calibration Verification

18.9.1 Ammonia (% Recovery) should be between 90% and 110%.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL OR UNACCEPTABLE DATA

If any of the quality control procedures indicates that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measures, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting the analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 The wastes generated in this method are hazardous because of a high final pH. The samples may be neutralized by the addition of water in an accumulation container (e.g., a large beaker or flask) or by slowly pouring down the drain with the faucet running followed by a triple rinse of the container to remove residual corrosives.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- 22nd Ed Standard Methods for the Examination of Water and Wastewater 4500-NH_3 D, 2011

P23. TABLE, DIAGRAMS, AND FLOWCHARTS

- The *Nitrogen Analysis Benchsheet* can be located in the SOW 7 drive at the following extension: \\wiims.wff.nasa.gov\wiccII\SOW 7\Chem Lab\WW-DW\Bench Sheets\Nitrogen Analysis

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
2	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition. Need to note volume of ISA because it is required for subsequent calculations.
	06/24/13	Latest version 22nd ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	12/18/14	MSDS changed to SDS. Formatting and Quality Control to reflect current Standard Method edition.
	05/15/2015	Interference section revised; remove Chlorine by sample pretreatment.
3	01-31-2017	Grammatical and formatting changes. Updated Method Blank requirements to reflect Corrective Action. Accumulation of minor changes under a single revision warrants a revision change.
4	05-16-2018	Removed old benchsheets and linked to current benchsheet. Revised P18 with appropriate acceptance criteria from SM 4020 B. Added note to procedure to check that samples are within hold time. Restructured SOP to group analytical steps.
5	1/29/2019	Revised header and P1 to resolve SOP name disparity. Included guidance in P14 for temperature equilibration of samples prior to analysis. Re-included erroneously removed formulae in P15. Restructured P18 to be consistent with new round of SOPs. Revised P21 to be more in-line with laboratory practice.

SOP-WI-3

Biochemical Oxygen Demand

Revision 18

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

4-2-19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

4-2-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

4-2-19
Date

LJT & Associates, Inc.

Effective Date: 04/02/2019

SOP-WI-3

Biochemical Oxygen Demand

Revision 18

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Marianne Simko	Marianne Simko	4-2-19	new
Jason Freischlag		4-2-19	new revision
Marga Morris		04-02-19	new
Mark Culver		4-2-19	new rev
Brian Cornwell		04-03-19	revision

Title: Biochemical Oxygen Demand

P1. IDENTIFICATION OF THE TEST METHOD

Biochemical Oxygen Demand

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for the determination of the relative oxygen requirements of municipal and industrial wastewaters.

P3. DETECTION LIMIT

The limit of detection for this analysis is not significant in its performance of this analysis, the description of which is left to SM 5210 B. The limit of quantitation for BOD and CBOD is set at 2.0 mg/L by NPDES reporting requirements.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test method for determining five-day biochemical oxygen demand (BOD) and carbonaceous BOD (CBOD) in non-potable water samples using a luminescent dissolved oxygen meter.

P5. SUMMARY OF METHOD

The procedure described here is certified under SM 5210 B, which describes the determination of BOD and CBOD. Appropriate dilution series are prepared for each sample and incubated for 5 days at 20°C. BOD and CBOD are calculated using the reduction in the dissolved oxygen concentration during the incubation period and the dilution series of the sample.

P6. DEFINITIONS

Biochemical Oxygen Demand (BOD)**:** Empirical measure of the dissolved oxygen used by microorganisms to oxidize the organic matter present in solution. Allows calculation of the effect of the discharges on the oxygen resources of the receiving waters.

Carbonaceous Biochemical Oxygen Demand (CBOD)**:** A BOD test in which a nitrification inhibitor is added. Many biological wastewater treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. In these samples, chemical inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand. This test is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

Glucose-Glutamic Acid (GGA): A solution of equal parts glucose and glutamic acid with a predictable BOD used to assess the accuracy of the total analytical method, including all preparation and analysis steps.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

Lot Quality Control (LQC): Quality control procedure performed on incoming commercially-prepared reagents to ensure usability.

Method Blank (MB): A sample of method appropriate pure water containing no target analyte that is taken through the entire analytical procedure.

Nitrification: An aerobic process in which bacteria oxidize ammonia and organic nitrogen (e.g. proteins) into oxidized nitrogen species (usually nitrate).

Nutrient: Any chemical used by living things that promotes growth.

Pellet: Solid mass eluted at the bottom of a solution due to gravimetric separation. As an action, refers to gravimetric separation of dense particles from an otherwise homogenous solution.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Seeding: The process of inoculating a sample with live bacteria.

Supernatant: Homogenous solution remaining after pelleting.

P7. INTERFERENCES

Certain constituents present in a water sample can inhibit biochemical oxidation and interfere with the BOD analysis. Interferences in the BOD analysis include caustic alkalinity or acidity, the presence of residual chlorine, or the presence of toxic elements, including: trace elements such as copper, lead, chromium, mercury, and arsenic, or compounds such as cyanide.

P8. SAFETY PRECAUTIONS

8.1 Adhere to standard laboratory safety procedures. Review the Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose ~~some~~ threat of infection. Laboratory personnel who are

routinely exposed to such water samples should protect themselves from waterborne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter. Clean bench surfaces with disinfectant before and after testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

8.6 This analytical procedure uses a vacuum pump for the dissolved oxygen adjustment step. There is a risk of implosion under some circumstances. The analyst should ensure that the receiving flask is free from cracks or other imperfections.

P9. TOOLS, EQUIPMENT AND SUPPLIES

- Opaque air incubator capable of maintaining temperature at 20 ± 1°C
- Single-use plastic flared-mouth BOD bottles, 300 mL
- Glass stoppers (for use with BOD bottles)
- Plastic caps (to cover glass stoppers on BOD bottles)
- Sterile pipettes and/or micropipettes with sterile tips
- Graduated cylinders
- Beakers
- Clean carboy, >18 L with spigot
- Filter flask (with rubber stopper)
- Vacuum pump
- GGA ampule breaker
- Scissors
- Deionized water wash bottle
- HQ440d HACH portable meter with DO & pH probes
- Pocket Colorimeter or Spectrophotometer (to measure residual chlorine)

P10. REAGENTS AND STANDARDS

10.1 Commercial seed source (Polyseed®)

10.2 BOD Nutrient Buffer Pillows

10.3 BOD Nutrient Buffer Pillows, Small (one per 300 mL solution)

10.4 GGA ampules, 6 mg each

10.5 Nitrification Inhibitor, Formula 2533 with “shot top” (CBOD)

10.6 Dilute acid (H_2SO_4) and base (NaOH) solutions

10.7 Deionized water used to prepare dilution water (SOP-WI-35, *Deionized Water System*)

10.8 Solid sodium sulfite (chlorine titration)

10.9 Potassium iodide solution, 100 g/L (chlorine titration)

10.10 Starch solution, 1% (chlorine titration)

10.11 Sulfuric acid, 0.020 N (chlorine titration)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Sampling should be performed as specified by a field sampling plan or based on compliance-based sampling requirements.

11.2 Samples should be iced or refrigerated at 4°C ± 2°C, and should not be frozen. If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.3 The maximum recommended holding time for wastewater samples requiring BOD testing is 2 days. Holding time for composite samples is defined as the time from the end of compositing to the beginning of sample analysis. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for BOD and CBOD obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.2.1 Sign in the proficiency test in the Sample Receiving Logbook. Be sure to record the sample ID number on the sample container.

12.2.2 Follow the PT provider’s included instructions for preparing the sample and analyze according to P14.

12.3 Initial Demonstration of Capability (IDC)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 The analyst should prepare certified reference material provided by the qualified analyst according to the manufacturer's specifications.

12.3.4 The analyst then shall carry out four replicate analyses per analyte as per P14. The interpretation of the analyst's results is discussed in P18.

NOTE: SM 5210 B requires a minimum of three bottles per dilution series for samples; therefore, an analyst performing an IDC for both BOD and CBOD will need at least 24 bottles for their batch samples, in addition to the other required batch QC.

12.4 On-going Demonstration of Capability (ODC)

12.4.1 Analysts are required to re-verify their capability in this field of testing through the on-going demonstration of capability. Prior to completing the on-going demonstration of capability, the analyst must review this SOP and sign off on the SOP Signature Sheet.

12.4.2 The analyst should prepare a certified reference material according to the manufacturer's specifications.

12.4.3 The analyst shall carry out the analysis of this sample and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Method Blank (MB)

12.5.1 Per method requirement, the laboratory shall perform analysis of, at least one but not exceeding three, blank sample(s) consisting solely of dilution water. Interpretation of method blank analyses is discussed in P18.

12.6 Polyseed® (POL)

12.6.1 Per method requirement, the laboratory shall perform analysis of a three-bottle dilution series with varying concentrations of the seed formulation used throughout the batch. Interpretation of Polyseed® analyses is discussed in P18.

12.7 GGA

12.7.1 Per method requirement, the laboratory shall perform three analyses of commercially prepared 1:1 glucose-glutamic acid (GGA) standards inoculated with the seed formulation used throughout the batch. The laboratory shall analyze three additional commercially prepared GGA standards for CBOD when batches contain samples requiring CBOD analysis. Interpretation of GGA analyses is discussed in P18.

12.8 Duplicates

12.8.1 Per method requirement, the laboratory shall perform a duplicate analysis, per batch, on a random sample requiring analysis for BOD or CBOD. Interpretation of duplicate analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 Dissolved Oxygen Probe

13.1.1 Daily Calibration

13.1.1.1 All water needs to be removed from probe prior to calibration. Blot the probe dry with a Kimwipe to remove the water.

13.1.1.2 If not already filled, fill the glass BOD calibration bottle ¾ full with deionized water.

NOTE: If water has not been changed in calibration bottle recently, consider cleaning and replacing water.

13.1.1.3 Check probe cap to see if it needs to be replaced. This is indicated on a recurring basis by the attached meter or if the probe cap is severely damaged (i.e., scratched, chipped).

13.1.1.4 Supersaturate the vapor phase of the calibration bottle with water by placing a stopper in the flared mouth and shaking vigorously for approximately 30 seconds.

13.1.1.5 Calibrate the probe according to the manufacturer's instructions. Record applicable parameters in the spaces provided on the DO Meter Calibration Log.

13.1.2 Routine Maintenance

13.1.2.1 The probe cap of the Dissolved Oxygen Probe needs to be routinely changed (conditions as described in P13.1.1.3). Follow the manufacturer's instructions in the replacement of the probe cap and the iButton.

13.2 pH Probe

13.2.1 Daily Calibration

13.2.2.1 Calibrate the probe according to the manufacturer's instructions. Record applicable parameters in the spaces provided on the pH Meter Calibration Logs.

13.3 Thermometers

13.3.1 Calibrated, NIST-traceable thermometers are kept in the air incubator and the sample refrigerator. Thermometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.3.2 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.3.3 The air incubator is maintained at ~20°C. The temperature is checked twice daily, at least four hours apart, per business day, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. Weekend temperature ranges are also recorded in the Temperature Log by recording minima and maxima retained by a NIST-traceable min/max thermometer. The acceptable temperatures of this incubator are 20.0 ± 1.0°C.

13.3.4 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, at least four hours apart, per business day, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

P14. PROCEDURE

14.1 Polyseed Setup

14.1.1 Determine the volume of dilution water to use in setting up the seed. This should be done using historical data for the current lot and/or the manufacturer's instructions, referencing the laboratory's control chart for the current lot.

14.1.2 Record this volume in the Polyseed section of the BOD Bench Sheet. If the laboratory is opting to supplement its seed, include a descriptive notation of the seed augment used.

14.1.3 Using a graduated cylinder, measure half the total dilution water volume for seed into a clean 1L beaker.

NOTE: Dilution water for seed formulation can be prepared using individual buffer packs (1 per 300 mL, rounded up).

14.1.4 Empty the contents of one Polyseed® capsule into the beaker.

14.1.5 Add the remaining half of the dilution water to the beaker, rinsing the sides of the beaker with deionized water to ensure no bran remains adhered to the glass.

14.1.6 Stir seed, using magnetic stir plate and PTFE stir bar, for one to six hours with Kimwipe on top, to prevent aerosolization. Record the start and stop stir times in the Polyseed section of the BOD Bench Sheet. To allow for more consistent trending, it is recommended to limit the Polyseed stir time to 1-2 hours.

14.1.7 When ready to use, let seed bran settle for 5-15 minutes by angling the beaker at approximately a 45° angle with the lip facing downward. The seed supernatant will appear cloudy when ready.

14.1.8 Decant the supernatant into a 150 mL beaker, taking care not to agitate the pellet.

14.1.9 Use supernatant promptly after decanting. Keep supernatant stirring to ensure uniform distribution to samples.

14.2 Sample Adjust

14.2.1 Record sample parameters, as available, in the table on the bottom of the BOD Bench Sheet. Transcribe sample IDs from this table, as well as the QC samples required, in the Sample ID column of the BOD Bench Sheet, using a line-down to signify a single dilution series.

NOTE: If the client provides pH and dissolved oxygen measurements that they are using for NPDES reporting purposes, these values may be used for the initial pH and DO parameters in the table. If contrary to laboratory measurement of these parameters, use the laboratory parameter over the client-provided one.

14.2.2 Temperature

14.2.2.1 If unspecified, take an initial temperature reading for each sample and record in the Initial Temp column on the BOD Bench Sheet. An IR thermometer is an acceptable instrument for this determination.

14.2.2.2 After sample is allowed to adjust to room temperature (20.0°C ± 3.0°C) using the water bath, record adjusted temperature from P14.2.3 or P14.2.4, preferably whichever step is performed last. Use an IR thermometer, if P14.2.3 and P14.2.4 are not required.

14.2.3 Dissolved Oxygen

14.2.3.1 If unspecified, determine the initial DO for each sample and record in the Initial DO column of the BOD Bench Sheet.

14.2.3.2 If the initial DO is greater than the DO saturation point at 20°C (9.07 mg/L), adjust by agitating an aliquot of the sample under vacuum and recombining. Record the adjusted DO in the Adjusted DO column of the BOD Bench Sheet.

14.2.4 pH

14.2.4.1 If unspecified, determine the initial pH for each sample and record in the Initial pH column of the BOD Bench Sheet.

14.2.4.2 If the initial pH is not between 6.0 and 8.0, adjust between 7.0 and 7.2 by minimal addition of dilute acid and base solutions, while stirring. Record the adjusted pH in the Adjusted pH column of the BOD Bench Sheet.

NOTE: While adjusting the sample pH, take care as to not increase the total sample volume by more than 0.5% (5 mL/L sample). If required, perform dechlorination procedures prior to pH adjustment, as chlorinating agents drastically affect sample pH.

14.2.5 Residual Chlorine

14.2.5.1 If unspecified, determine the initial chlorine by SOP-WI-29, *Total Residual Chlorine*, for each sample and record the presence or absence of chlorine in the appropriate space under the “Final Chlorine” header.

14.3 Dechlorination

14.3.1 If no samples contain residual chlorine, these steps may be omitted.

14.3.2 Retrieve the BOD Dechlorination Workbook from the Chem Lab shared drive (one form to be completed per sample).

14.3.3 Fill in sample, analyst, and lot information in the spaces requested.

14.3.4 Specify volume to be titrated on the sheet (at least 50 mL is preferred).

14.3.5 The sheet will then calculate how much 0.020 N sulfuric acid, 10 g/100 mL KI, and starch indicator is required. Combine these four ingredients in an appropriately sized beaker with a magnetic stir bar. The solution should turn a blue-green color.

14.3.6 Using a sodium sulfite solution prepared from solid (1.575 g/L is method-recommended; however, similar concentrations will work as long as they produce a measurable response

during the titration), titrate the solution created in the previous step to a clear color. Record the initial and final buret readings in the appropriate spaces on the sheet. Also, record the remaining sample volume, after titration, in the appropriate space on the sheet.

14.3.7 The sheet will then calculate the volume of the sodium sulfite solution created in the previous step is required to dechlorinate the sample. Add the specified volume of sodium sulfite to the remaining sample and re-check the chlorine residual after 15 minutes to ensure complete dissipation.

14.3.8 Print and staple spreadsheet attachment to the pertinent page of the BOD Logbook.

14.4 Dilution Water

14.4.1 Determine required volume of dilution water required for batch preparation.

TIP: A good rule of thumb to follow is to make one liter per three effluents and one liter for each other dilution series .

14.4.2 Dispense the next-highest multiple of six liters of the determined required volume into the BOD carboy.

TIP: To reduce risk of contamination, it is recommended to wash the BOD carboy regularly (i.e., twice a month) in a dishwasher with Citrajet. Flush the BOD carboy with ~20 liters of deionized water with the spigot set to a slight drizzle.

14.4.3 Per six liters, add one large BOD Nutrient Buffer pillow. After the BOD carboy is filled, cap and shake vigorously for one minute to mix and ensure proper oxygenation.

14.4.4 Check the dissolved oxygen of dilution water to ensure its initial value is greater than 7.50 mg/L. If the initial DO is less than 7.50 mg/L, re-perform the previous step.

14.4.5 Record the temperature of the method blank in the dilution water temperature field on the BOD Bench Sheet. Temperature of dilution water should be room temperature (20.0°C ± 3.0°C).

14.5 Setup Read – Bottles

14.5.1 On a rolling cart, set up the appropriate number of plastic BOD bottles and label them, minimally, with a truncated sample ID number and/or QC identifier, sample location (if applicable), and dilution volume.

NOTE: Bottles are randomly assorted within each box and should not be chosen preferentially.

14.5.2 For each labeled bottle, record the last three digits of the manufacturer’s serial number in the Bottle # column on the BOD Bench Sheet. Any unlabeled or otherwise flawed (i.e., containing dents) bottles should be discarded.

14.6 Setup Read – Dilutions

14.6.1 Specify the sample volume used in the sample bottles for each dilution series in the Sample Amount column of the BOD Bench Sheet. For sites requiring recurring analyses, use historical data in determining sample volumes.

TIP: Most effluents must be run with one bottle at 300 mL for NPDES reporting requirements. For samples with no historical data, running a COD analysis first can suggest a proper dilution series, as BOD is approximately one-third the value of COD. If time doesn't permit a full COD analysis, analysis of an unheated COD vial can give an order-of-magnitude estimation of the sample BOD.

14.6.2 Add determined sample volumes and dilution water to each sample bottle such that they are approximately 2/3 full. If determined sample volume is greater than 200 mL, add volume to approximately 2/3 of total bottle capacity. To inoculate GGA, fill the BOD bottle with dilution water until the water level is approximately ¼ inch up the neck. Align the glass ampule in the breaker, crack the vial atop the sample bottle, and allow it to fall into the BOD bottle. If air bubbles attach to the ampule, remove by gently swirling.

14.7 Setup Read - Inoculation

14.7.1 Specify inoculation volume for sample containers to be seeded in the Seed Amount & F2533 Amt column of the BOD Bench Sheet. Samples requiring seed inoculation include effluent wastewaters and those samples requiring pH adjustment and/or dechlorination. For samples submitted for CBOD analysis, add a notation in this column of "0.16 g", corresponding to 2 shots of the Formula 2533 Nitrification Inhibitor.

14.7.2 Add specified Polyseed volume to each applicable sample container. If nitrification inhibitor is indicated, put "two shots" of the Formula 2533 into each applicable sample container. Additionally, if dilution sample volume is greater than 200 mL, add a small nutrient buffer pillow to the sample container and add remaining sample volume.

14.7.3 Fill non-filled sample bottles up to 300 mL using dilution water.

14.8 Setup Read – Initial Readings

14.8.1 Fill out all parameters in the DO meter calibration section of the BOD Bench Sheet.

14.8.2 For each sample bottle, read its initial DO according to manufacturer's instructions, and record in the Initial DO column. Record the time reading the first sample bottle as the Analysis Start Time on the top of the BOD Bench Sheet.

NOTE: The DO probe does not have to be rinsed between sample measurements when reading less diluted bottles in a series (increasing sample volume).

14.8.3 Push a glass stopper into the flared mouth of each read bottle, making sure no air is trapped within the bottle and that there is sufficient water to form a good water seal. Replace any displaced contents with dilution water to form an adequate seal if necessary.

14.8.4 Cap each bottle with plastic overcap to minimize evaporation.

14.9 Incubation Start

14.9.1 Since the preceding steps are often performed by multiple analysts, specify the initials of the analysts responsible for setup in the Analyst field on the top of the page. Each analyst must write their own initials.

14.9.2 Transfer all sample bottles to the BOD incubator. Record the incubation start date, time, and temperature in the appropriate spaces in the Incubation Dates/Times/Temps section of the BOD Bench Sheet.

14.10 Five Day Read

14.10.1 After five days (±6 hours), transfer all sample bottles from the BOD incubator onto a rolling cart. Record the incubation stop date, time, and temperature in the appropriate spaces in the Incubation Dates/Times/Temps section of the BOD Bench Sheet.

14.10.2 For each sample bottle, read its final DO according to manufacturer's instructions, and record in the Final (5 Day) DO column.

14.10.3 Calculations for the Depletion, Seed Control Factor, Net Depletion/Calc, Sample BOD5, and Average BOD5 columns are discussed in the next section.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Depletion

15.1.1 Difference between initial and final DO readings.

15.1.2 Applicable Formulas

15.1.2.1 $$Depletion = Initial\ DO - Final\ DO$$

15.2 Seed Control Factor

15.2.1 Depletion attributable to inoculated seed. The seed control factor calculation in 15.2.2.1 is performed on each bottle of the Polyseed dilution series that meets the 2:1 rule (15.6)

and the arithmetic mean of these values is used in subsequent calculations to determine the BOD.

15.2.2 Applicable Formulas

15.2.2.1 $Seed\ Control\ Factor = Depletion * \frac{Seed\ Amount}{Sample\ Amount}$

15.3 Net Depletion

15.3.1 Difference between initial and final DO readings, accounting for seeding.

15.3.2 Applicable Formulas

15.3.2.1 $Net\ Depletion = Depletion$ (for unseeded samples)

15.3.2.2 $Net\ Depletion = Depletion - Seed\ Control\ Factor$ (for seeded samples)

15.4 BOD

15.4.1 Concentration of dissolved oxygen associated with 5-day biological oxidation of water in each sample bottle. Reportable BOD or CBOD value is calculated as the arithmetic mean of all Sample BOD5 values (15.4) associated with bottle numbers meeting the 2:1 Rule (15.6).

15.4.2 Applicable Formulas

15.4.2.1 $Sample\ BOD5 = Net\ Depletion * \frac{300}{Sample\ Volume}$

15.5 2:1 Rule

15.5.1 For a sample bottle to meet the 2:1 Rule, it must have both:

15.5.1.1 Depletion (15.1) of, at least, 2 mg/L.

15.5.1.2 Final DO of, at least, 1 mg/L.

15.5.2 If all sample bottles in a dilution series fail according to 15.5.1.1, calculate using the data associated with the least dilute bottle. If this aliquot is not diluted, the sample may be reported as “<2” with no required corrective action.

15.5.3 If all sample bottles in a dilution series fail according to 15.5.1.2, calculate using the data associated with the most dilute bottle and report data using a “>” sign.

15.6 Average

15.6.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.6.2 Applicable Formulas

15.6.2.1 $$Average(X) = \sum \frac{X_i}{n}$$

where X contains n values X_i

15.7 Relative Standard Deviation (RSD)

15.7.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.7.2 Applicable Formulas

15.7.2.1 $$Standard\ Deviation(X) = \sqrt{\sum \frac{(X_i - \bar{X})^2}{n-1}}$$

where X contains n values X_i with average $\bar{X}$

15.7.2.2 $$RSD = \frac{Standard\ Deviation(X)}{Average(X)} * 100\%$$

15.8 % Recovery

15.8.1 The percent recovery of analyte measured in BOD GGA samples.

15.8.2 Applicable Formulas

15.8.2.1 $$\%\ Recovery(x) = \frac{x}{x_{true}} * 100\%$$

where x has true value x_{true}

15.9 Relative Percent Difference (RPD)

15.9.1 The relative percent difference between a sample and its duplicate.

15.9.2 Applicable Formulas

15.9.2.1 $$RPD(X_1, X_2) = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2}{2}} * 100\%$$

where X_1 and X_2 are results of samples analyzed in duplicate

15.10 Significance

15.10.1 BOD values are generally significant to 3 significant figures or to two decimal points,

whichever is less significant.

P16. METHOD PERFORMANCE

16.1 Limit of Quantitation: 2.0 mg/L.

16.2 Precision: RPD ≤ 30%

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. Adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Proficiency test results must fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Average BOD and CBOD must fall within the acceptance range specified by the provider.

18.3.2 RSD must be less than 30% for BOD and CBOD.

18.4 P12.4: On-going Demonstration of Capability

18.4.1 BOD and CBOD must fall within the range established by the provider.

18.5 P12.5: Method Blank

18.5.1 Depletion must not exceed 0.20 mg/L (ideally, 0.10 mg/L).

18.6 P12.6: Polyseed

18.6.1 Seed control factor should fall between 0.6 and 1.0 mg/L.

18.7 P12.7: GGA

18.7.1 BOD and CBOD must fall between 167.5 and 228.5 mg/L.

18.7.2 BOD (% Recovery) and CBOD (% Recovery) must fall between 84.6 and 115.4 %.

18.8 P12.8: Duplicates

18.8.1 BOD (RPD) or CBOD (RPD), whichever is applicable, must be less than 30%.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 Sample cannot be reanalyzed because of the 48 hour sample holding time and the 5 day incubation required for the analysis.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

20.3 Troubleshooting

20.3.1 Blank failure

20.3.1.1 Deionized water contamination.

20.3.1.2 Bad nutrient buffer.

20.3.1.3 Bottles not clean.

20.3.1.4 Contaminated carboy (and/or spigot).

20.3.1.5 Probe not clean.

20.3.2 GGA failure

20.3.2.1 Seed strength not correct (too strong or too weak).

20.3.2.2 Toxic chemicals in seed. This can be postulated if RSD of bottle determinations is shown to be greater than 30%.

20.3.3 No depletion

20.3.3.1 No microorganisms present.

20.3.3.2 No biochemical material to metabolize.

20.3.3.3 DO probe not working.

P21. WASTE MANAGEMENT

The wastes generated in this method are not hazardous. They can be discarded in the laboratory sink with the tap running.

P22. REFERENCES

- 2009 TNI V1M4
- 22 SM 5210 B

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	1/12/04	Revised Sections P3, P7 & P9. Added Section P10 & P11.
B	3/30/04	According to DEQ inspector, Steve Thomas, during an on- site inspection on 3/30/04: GGA Procedure Problem: Only 4 mL of the HACH GGA solution is being used in the analysis. 6 mL of GGA Solution must be used in this procedure.
C	5/26/04	Stopped adding inhibitor to GGA bottle. According to manufacturer, Polyseed® does not contain nitrifying bacteria.
D	10/27/05	According to DEQ inspector, Steve Thomas, during an on- site inspection on 10/27/05: 1) Lab should consider deleting 5 mL Polyseed dilution and add a 25 mL dilution instead. 2) Change dilutions for Raw composite BOD.
E	8/9/07	Steve Thomas, DEQ Inspector informed lab of new BOD guidelines: A duplicate BOD should be performed on 5% of samples. Mr. Thomas stated that the lab does not need to run duplicate analysis on the in-process control BOD.
F	8/29/07	Currently, the lab uses HACH GGA ampoules which contain 300 mg/L Glucose and 300 mg/L Glutamic Acid. To set up a GGA Standard, the lab uses 3mLs of GGA with 2 mL of Polyseed®. The GGA standard was coming out in the middle range. Now, the standard is on the low range, low to the point that it will go out of range. The blanks are coming out good and we do not see any changes with our samples. Wayne Staples DEQ, recommended adding a little more seed (3mL to samples and GGA). If this does not work, he advised purchasing a different source of GGA that does not require manipulation (150 mg/L Glucose and 150 mg/L Glutamic Acid) or dilution (vials contain exactly 6 mL). Lab switched to Environmental Express GGA and did not experience further problems.
G	10/31/07	According to memo from Steve Thomas, DEQ Inspector, lab needs to run one duplicate sample per week of BOD analysis. Prepare identical dilution series for the two samples. Calculate the BOD for each sample and then take the average. No additional blanks, seed series or GGAs need to be set-up to analyze duplicate BODs.
H	11/20/07	Seed correction did not fall between 0.6 and 1.0 mg/L and the 5mL and 10 mL dilutions did not deplete by 2 mg/L. Wayne Staples DEQ, recommended making the seed material up as a stronger solution. Instead of preparing the seed material in 500 mL, we could use the same amount of material in 400 mL. He also recommended to use less of the seed material in the samples to obtain a better seed correction.
I	1/24/08	According to DEQ inspector, Steve Thomas, during an on- site inspection on 1/24/08. The first dilution (5mL) for seed control can be eliminated for a 5 day BOD test if it is an outlier (BOD results for the first dilution are not consistent with the results for the other three dilutions 10mL, 15mL and 20mL).
J	2/27/08	Switched from using HACH prepared GGA to Environmental Express prepared GGA for BOD analysis. According to manufacturer, the final BOD of the GGA solution must be calculated by dividing lab result by 6.
K	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria. Latest version 22 edition SM.
L	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
M	06/10/13	Three glucose-glutamic acid checks performed with each batch of samples. Duplicate analyzed with each batch or on a 5 percent basis. Latest Version 22nd ed. SM. SOP-WI-3 and 4 were combined as one to SOP-WI-3.
N	09/16/13	Changes made to reflect new equipment in use (e.g. HACH DO meter) and changes to methodology and equipment sections.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.

	10/07/14	Minor grammatical errors fixed, Quality Control Sample removed from definitions for redundancy, MSDS changed to SDS, section 14.7.15.1 added, 14.7.10 changed from 1/3 and 160 to 2/3 and 200, steel toed boots removed from required PPE, 14.6 changed to reflect improved technique and added section about adjusting concentration and added amount of seed suspension to acquire appropriate DO range for GGA standard test.
	10/31/14	Limit of detection updated from 5.0 to 2.0 mg/L, minor grammatical errors fixed, 14.7.25 changed from “false positive" to false results and additional information added to this section, 14. 7.1 sample storage temp changed from 1-4ºC to ≤6ºC, 4.7.18 added section requiring immediate analysis of rapidly reacting samples.
15	04/02/15	Method was revised to reflect changes from the Internal Audit.
	05/15/2015	Interference section revised; remove Chlorine by sample pretreatment. Run a duplicate sample for each matrix type daily or with each bath of 20 or fewer samples.
	07/01/2015	Section 12.4 changed from 10% basis to 5% basis based on Standard Methods 22nd ed.
	05/09/2016	Minor grammatical changes; addition to the interferences section about chlorination.
	12/08/16	Grammatical changes. Removed requirement of bottle QC and dilution water pre-incubation (due to temperature controlled laboratory). Added ability to run multiple blanks.
16	01-31-2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
	08-10-2017	Alternative Polyseed Preparation Procedure
17	1/31/2019	Incorporated modifications from working copy. Reorganized for increased clarity. Removed lot QC requirements, as volume modification by control charting is more effective in resolving noncompliant QC. Added feedback from Environmental Standards to use min/max thermometer for weekend monitoring of incubator temperature.
18		Added requirement for CBOD GGA. Removed hyperlinks, as all VELAP documents are now accurately hyperlinked on the Master List of Documents.

SOP-WI-5

Chemical Oxygen Demand

Revision 3

Approval:

Marianne Simpson — 2-1-19

Environmental Programs Manager — Date

2-1-19

Laboratory Director — Date

2-1-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

Title: Chemical Oxygen Demand

P1. IDENTIFICATION OF THE TEST METHOD

Determination of Chemical Oxygen Demand, or COD: HACH Method 8000

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for determining the relative COD of water, wastewater, and seawater.

P3. DETECTION LIMIT

The limit of detection (LOD) is 5 mg/L. The limit of quantitation (LOQ) is 15 mg/L.

P4. SCOPE AND APPLICATION

The purpose of this procedure is to provide a method for analysis of water for chemical oxygen demand. The low range method is applicable for a COD range of 3 to 150 mg/L. The high range method is applicable for a COD range of 20 to 1500 mg/L.

P5. SUMMARY OF THE TEST METHOD

Organic materials are oxidized in strong acid and mercury catalyst and measured spectrophotometrically compared to a standard curve.

P6. DEFINITIONS

Chemical Oxygen Demand (COD): A measure of the amount of organic pollutants in a sample of water. COD works on the principle that nearly all organic compounds can be fully oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions.

Calibration Standard: A solution prepared from a stock solution of known concentration that is used to calibrate the instrument response with a known analyte concentration.

Calibration Verification (CV): A calibration verification is a known standard that is analyzed periodically to ensure instrument performance has not changed significantly since initial calibration. CVs are run after every ten samples or at the end of every batch, whichever is more frequent. For a CV to be considered valid, the result must be within 10% of the known value.

Limit of Detection (LOD): The lowest analyte concentration that can be distinguished from the absence of that analyte with 99% confidence.

To calculate the LOD:

a. Prepare a solution with the concentration of COD at 1 to 3 times the estimated LOD

b. Analyze seven potions of this solution over a period of at least three days
c. Include all sample processing steps in the determination
d. Calculate the standard deviation (s)
e. Using a one-sided T table for 99% confidence find the value of t that matches the number of measurements. For 7 measurements this value is 3.14
f. 3.14 (or other t value if 7 samples are not used) times s (standard deviation) is equal to the LOD.

Laboratory Fortified Blank (LFB): Sometimes referred to as a standard, the LFB is a blank that has the analyte of interest added so that the sample has a known concentration. The LFB is prepared/extracted/digested and analyzed exactly like the field samples and is used to determine bias (accuracy) of a method.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations. The LOQ is generally 3 to 5 times the LOD unless specified by the method.

Method Blank (MB): A blank of deionized water or extraction blank of the same matrix as the samples and standards used. The method blank is prepared/extracted/digested and analyzed exactly like the field samples. Its purpose is to assess contamination introduced during sample preparation activities.

Quality Control Sample (QCS): An externally generated sample containing analytes of interest at unknown concentrations. Sometimes referred to as a **Proficiency Test** (PT), the QCS is used to determine laboratory and method competence. It is obtained from an external lab semi-annually and results are compared to that labs acceptance results. If results do not meet acceptance criteria, investigate why, take corrective action, and run a new QCS.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7. INTERFERENCES

7.1 Chloride can cause additive interference in saline waters where chloride concentrations exceed 2000 mg/L. Mercuric sulfate may be complexed with the sample prior to refluxing to remove the interferences caused by chloride ions.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. COD vials contain Sulfuric Acid and mercury. If spilled on skin or eyes, wash area with water for 15 minutes. If irritation or burning persists, seek medical attention immediately.

8.2 Impact resistant safety glasses, latex or rubber gloves, and a laboratory jacket should be worn during all phases of this procedure.

8.3 A protective shield should be in place around the reactors during digestion.

8.4 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- COD Reactor with safety shield
- COD Low Range and High Range Digestion Reagent Vials
- DR3 Spectrophotometer (or similar)

P10. REAGENTS AND STANDARDS

COD Standard solutions prepared by manufacturer.

P11. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

11.1 Sample Containers - Collect samples in glass bottles. Plastic bottles may be used only if known to be free of organic contamination.

11.2 Sample Holding Time: Analyze samples as soon as possible after collection. If immediate analysis is not possible, then acidify samples to a pH of less than 2 with sulfuric acid (at least 2 mL per liter of sample) and refrigerate samples (~4^0C). Samples required to be held must be acidified the same day sample as collected. The acidified sample can be held up to 28 days.

11.3 Reactor Preparation- Turn on the COD Reactor. It takes 30-40 minutes to reach a temperature of 150^0C. Place a plastic shield in front of the reactor. Ensure that the safety shield is in place to protect analyst from splattering in the event that a reagent leaks or a tube breaks.

11.4. Sample Preparation

11.4.1 If the sample does not contain suspended solids, omit step 11.14.2.

11.4.2 Homogenize sample in a blender to permit representative sampling analysis. Blending samples ensures distribution of solids and improves accuracy and reproducibility.

11.4.2.1 Add 100 mL of sample to the blender and homogenize for 2 minutes.

11.4.2.2 Pour the homogenized sample into a 250-mL beaker and stir with a magnetic stirrer.

P12. Quality Control

12.1 In order to be certain that analytical values obtained using these test methods are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing COD.

12.2 *Annually perform two Quality control samples (QCS)/Proficiency Test Studies (PT).* Follow the procedures for preparing the sample, and obtaining test results. Proficiency testing will be performed semi-annually using samples from an outside source (NSI Solutions).

12.2.1 To demonstrate method performance, the laboratory analyzes a QCS twice per year or otherwise as required for each method where QC samples are available. Quality control samples (QCS) are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in. QCS are not analyzed multiple times unless routine environmental samples are analyzed multiple times.

12.3 *Perform Initial demonstration of capability* (for any new laboratory personnel)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

12.3.2 Carry out four analyses using 2 mL samples for each.

12.3.3 Calculate the average and standard deviation of the values.

12.3.4 The standard deviation (precision) should be less than or equal to 20% of the average value. If it is not, evaluate the procedures to identify sources of error.

12.4 *Method Blank*

12.4.1 Analyze a reagent water test blank with each batch.

12.5 *Laboratory Fortified Blank*

12.5.1 Laboratory fortified blanks are used to document the validity of data and to control data quality within acceptance limits. Laboratory fortified blank samples are processed through the system in the same manner as any other sample, except when the analyst is aware of the source, concentration, and acceptance ranges of target analytes and calculates analyte recoveries to evaluate method performance in real time.

12.5.2 For low range (3 to 150 mg/L) samples use a 30 mg/L LFB. For high range samples (20 to 1500 mg/L) use a 100 mg/L LFB.

12.5.3 For each laboratory fortified blank (LFB) determine the percent recovery using equation 1. The percent recovery should be within 10% of the true value.

$$\%\ Recovery = \frac{found\ value}{true\ value}\ x\ 100\% \qquad \text{Equation 1)}$$

12.6 *Accuracy Check*

The Lab Supervisor shall review the results to ensure accuracy and precision. In addition, the lab supervisor shall initial the results to indicate that the calculations have been reviewed and are accurate.

P13. CALIBRATION AND STANDARDIZATION

13.1 Check instrument accuracy using the standards from section P10.

P14. PROCEDURE

14.1 Sample Analysis

1. Remove the cap of a COD Digestion Reagent Vial for the appropriate range: (selecting the appropriate range is based on worker experience and trial and error) Sample Concentration Range (mg/L COD Digestion Reagent Vial Type)

3-150	Low Range
20-1,500	High Range

2. Hold the vial at a 45-degree angle. Pipette 2.0 ml of sample into the Reagent Vial.

3. Replace the vial cap tightly.

4. Hold the vial by the cap and over a sink. Invert gently several times to mix the contents. The vial will become very hot during mixing. Place the vial in the preheated COD Reactor at 150^0C.
5. Prepare a blank by adding 2.0 ml of deionized water in a COD tube. One blank must be run with each set of samples. All tests (samples and blanks) should be run with the same lot of vials. The lot number is located on the container label.

6. Run a standard for each range. Prepare standards according to manufacturer recommended procedures.

7. Heat the vials for 2 hours. Push the timer button on the reactor and turn the dial to 120 minutes. The reactor will shut off automatically after 2 hours.

8. Wait about 20 minutes for the vials to cool to 120^0C or less.

9. Invert each vial several times while still warm. Place the vials in a rack. Wait until the vials have cooled to room temperature. If a pure green color appears in the reacted sample, the reagent capacity may have been exceeded. Measure the COD and, if necessary, repeat the test with a diluted sample or in a different range.

14.2 Colorimetric Determination Using the HACH Spectrophotometer

1. Enter the stored program number for COD, low range,430, or for COD high range, 435, store program and press CLEAR.

   NOTE: While not common, program numbers may change with updated equipment. Please see provided documentation with the in-use instrument for actual program information.

2. Place the COD vial adapter into the cell holder with the marker to the right and press CLEAR.

3. Clean the outside of the blank sample with a towel. Wiping with a damp towel, followed by a dry one will remove fingerprints or other marks and smudges.

4. Place the blank into the adapter with the HACH logo facing the front of the instrument. Place the cover on the adapter.

5. Press ZERO; CONCENTRATION and AUTODATE lights will come on and a concentration of 0 will appear in the display.

6. Clean the outside of the sample vial with a towel as in step 5.

7. Place the sample vial into the adapter in the same manner as the blank and place the cover on the adapter and read mg/L from the display.

Note: In the constant-on mode, pressing READ/ENTER is not required. Wait will not appear. When the display stabilizes, read the result.

14.3 Record the sample number or label and mg/L COD value measured for each vial/cuvette in a personal logbook.

P15. DATA ANALYSIS AND CALCULATIONS

All relevant equations found above.

P16. METHOD PERFORMANCE

This method was validated through inter-laboratory studies. The LJT & Associates, Inc. Lab participates in Proficiency Testing (PT) Program for VELAC certification.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. All sample vials should be disposed of in appropriate waste container. Volumes of reagents made should mirror the number of samples being analyzed to reduce excess waste.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness (e.g., calculations).

18.2 Method blanks (MB) should be $\leq$ 0.5 x LOQ.

18.3 Quality Control Samples (QCS) must meet the criteria of the lab where they were purchased.

18.4 Laboratory fortified blanks (LFB) should exhibit a percent recovery (accuracy) of less than a 10% variation from the known value.

18.5 The data will be reviewed by the QA/QC Officer.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL OR UNACCEPTABLE DATA

19.1 Quality control charts will be created for charting precision (standard deviation) of duplicate samples and accuracy (average) of LFB values.

19.2 If an accuracy value exceeds ±10% for LFBs then the analyst should note that the data set is out- of-control in the data report given to the QA/QC manager.

19.3 If a MB value exceeds 0.5 x LOQ then the analyst should note that the data set is associated with a MB value that exceeds the detection limit in the data report given to the QA/QC manager.

19.4 If there is sufficient volume of sample remaining for all samples, the analytical batch may be re-analyzed. Quality control data from the first run must be recorded on the control charts.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL DATA

20.1 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 **The pre-mix digestion solution contains sulfuric acid, chromic acid, mercuric sulfate, silver sulfate, and are toxic.** The hazardous waste generated by this procedure shall be handled and disposed of in accordance with state and federal regulations.

P22. REFERENCES

- 22nd ed. Standard Methods for the Examination of Water and Wastewater 5220 D, 2011
- 8000 HACH COD analysis procedures, EPA approved method

P23. TABLES, DIAGRAMS, AND FLOWCHARTS

23.1 See Attachment 1 for Chemical Oxygen Demand bench sheet.

Attachment 1

Chemical Oxygen Demand (COD)

HACH Method 8000

Check	LR (3-150 mg/L)		Sample ID:						
or	HR(200-1500 mg/L		Sample Date:						
Test Date:			Sample Time:						
Heat Start Time:			Sample location:						
Heat End Time:			Sample Type:						
Block Temp:			Sampled By:						
Block ID:			Preserved pH <2?						
Analyst:			Result:						
Digestion Vials:			Notes:						

Lot #: _____

Exp : _____

Standard Solution:

Lot #: _____

Exp: _____

Attachment 1

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
1	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
	06/24/13	Removed KHP reagent and standard preparation. COD standard are prepared by manufacturer. Quality Control removed Calibration Verification and Matrix Spike. Spikes not possible with COD analysis. Perform accuracy check with each batch.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	01/07/2015	MSDS changed to SDS. Formatting and Quality Control revised.
	09/17/2015	Quality Control revised.
2	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
3	02/01/2019	Revision to change reference spectrometer from old 2800 to 3900.

SOP-WI-9

Ignitability

Revision 6

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

5-16-19
Date

Jason Freischlag
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5-16-19
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5-16-19
Date

LJT & Associates, Inc.

Effective Date: 5/16/2019

SOP-WI-9

Ignitability

Revision 6

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Jason Freischlag		5-16-19	new revision, adding barometric corrections
Marianne Simko	Marianne Simko	5-16-19	new revision
Mark Cuter		5-16-19	new rev

Title: Ignitability

P1. IDENTIFICATION OF THE TEST METHOD

Ignitability by EPA 1010 (ref ASTM D93-08)

P2. APPLICABLE MATRIX OR MATRICES

Petroleum and biodiesel products.

P3. DETECTION LIMIT

Not applicable

P4. SCOPE AND APPLICATION

This test method describes the flash point determination of the flash point of petroleum products in the temperature range from 40 to 360°C and the determination of the flash point of biodiesel in the temperature range of 60 to 190°C by automated Pensky-Martens closed-cup apparatus.

P5. SUMMARY OF METHOD

A brass test cup of specified dimensions, filled to the inside mark with test specimen and fitted with a cover of specified dimensions, is heated and the specimen stirred at specified rates, using one of the defined procedures (A or B). An ignition source is directed into the test cup at regular intervals with simultaneous interruption of the stirring, until a flash is detected.

P6. DEFINITIONS

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration or physical response characteristic, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Flash point: The lowest temperature, corrected to a barometric pressure of 101.3 kPa (760 mmHg), at which application of an ignition source causes the vapor of a specimen to ignite under specified conditions of the test. (ASTM D93-08)

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

P7. INTERFERENCES

7.1 Erroneously high flash points may be obtained if precautions are not taken to avoid the loss of volatile material. Do not open containers unnecessarily to prevent the loss of volatile material or possible introduction of moisture, or both. Avoid storage of samples at temperatures in excess of 35°C (95°F). Samples for storage shall be capped tightly with inner seals. Do not make a transfer unless the sample temperature is at least the equivalent of 18°C (32°F) below the expected flash point. The results of flash point determinations can also be affected if the sample volume is less than 50% of sample container capacity.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

8.3 Keep bench areas free of clutter and clean bench surfaces before and after testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

8.6 The operator must exercise appropriate safety precautions during the initial application of the ignition source, since samples containing low-flash material may give an abnormally strong flash when the ignition source is applied.

8.7 When performing flash point analysis, increment the expected flash point appropriate to material specifications as to avoid hazardous flashes associated with overestimation.

P9. TOOLS, EQUIPMENT, AND MATERIALS

- FP93 5G2 Pensky-Martens Automated Flash Point Analyzer

P10. REAGENTS AND STANDARDIZATION

- Acetone, ACS grade
- Certified Reference Material (i.e., Ignitability standard from NSI Lab Solutions)

P11. SAMPLE HANDLING AND PRESERVATION

11.1 Ensure at least 75 mL of sample is available so there is sufficient material for the test. When obtaining a sample of residual fuel oil, the sample container must be 85-95% full. For other types of samples, ensure that sample container is 50-85% full.

11.2 Do not store samples in plastic (polyethylene, polypropylene, etc.) containers, since volatile material may diffuse through the walls of the enclosure. Samples in leaky containers are suspect and not a source of valid results.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on a fresh copy of the Flash Point Template before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for ignitability obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.3 Initial Demonstration of Capability

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 Analyze four aliquots of a certified reference material, prepared according to manufacturer's instructions, with all required batch quality control.

12.3.3 Interpretation of initial demonstration of capability results is discussed in P18.

12.4 On-going Demonstration of Capability

12.4.1 Similarly to P12.3, analyze a single aliquot of a certified reference material with all required batch quality control. Unlike an initial demonstration of capability, customer samples can be batched with on-going demonstrations of capability. Interpretation of on-going demonstration of capability results is discussed in P18.

12.5 Laboratory Control Standard (LCS)

12.7.1 Per method requirement, the laboratory shall perform analysis of an LCS on an annual basis. Interpretation of LCS analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 The automated flash point apparatus is externally calibrated annually and the Certificate of Calibration is retained in the laboratory's records.

13.2 Barometer

13.2.1 A calibrated, NIST-traceable barometer is maintained adjacent to the flashpoint apparatus. Barometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.2.2 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

P14. PROCEDURE

14.1 Instrument Diagnostics

14.1.1 Ensure the instrument is situated on a level surface in a draft-free environment.

14.1.2 Ensure all parts of the test cup and its accessories are thoroughly cleaned with acetone before starting the test.

14.1.3 Turn on the FP93 5G2 Analyzer. The toggle switch is located on the back, left corner of the instrument. Place the test cup in the heating block and the test cover on the test cup.

14.1.4 Once the analyzer is on, the instrument information screen is displayed. Press "ENT" on the keypad to exit from this.

14.1.5 The Run Screen is now displayed. Press the <Exit> button on the bottom left. Choose <Main Menu> on the bottom left. Choose <Access> on the left. Choose Level 2 Access. Enter the password 6216 and press "ENT." Press <Exit> on the bottom left, this will return the analyzer to the main menu.

14.1.6 Go to the "Service" tab. Choose "Diagnostics." Select "Stirrer." From the stirrer tab, the operator should position the stirrer drive arm, make the test cup stir, and remove the stirrer drive arm from the test cover and cup assembly. This ensures that the analyzer will stir the sample correctly. Exit from the stirrer tab by pressing <Exit> on the bottom left to go back to the diagnostics tab.

14.1.7 Select “Flame” from the diagnostics tab. From the flame tab, the operator should position the igniter presentation arm, turn the glow plug on, execute a flame test, turn the glow plug off, and remove the igniter presentation arm. This ensures that the analyzer will execute flame tests correctly. Exit from the flame tab by pressing <Exit> on the bottom left to go back to the diagnostics tab.

14.1.8 Press <Exit> until the analyzer is back to the main menu. Press <Access> on the left. Choose <Back to Level 0,> and <Exit> back to the main menu.

14.1.9 This concludes the instrument diagnostic checks. <Exit> from the main menu, and choose <Run Menu> to begin running flashpoint analyses.

14.2 Flash Point Determination

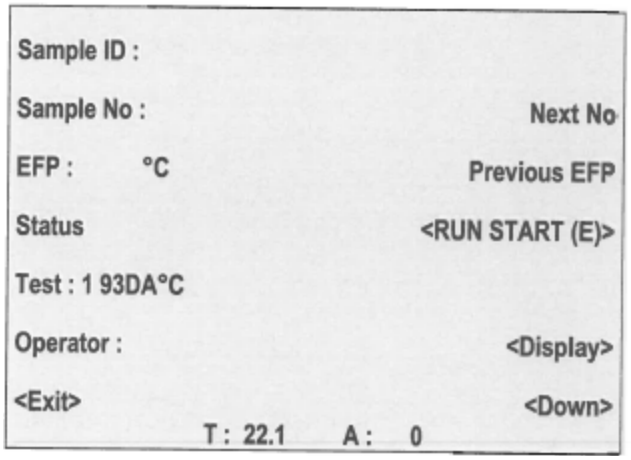
14.2.1 Fill the test cup with sample to the filling mark inside of the test cup. If too much has been added, use a transfer pipet to remove the excess.

14.2.2 Place the cup in the heating block. The 2 protruding notches of the heating block are used as guides for the 2 slots on the top rim of the cup.

14.2.3 Place the test cover on the test cup.

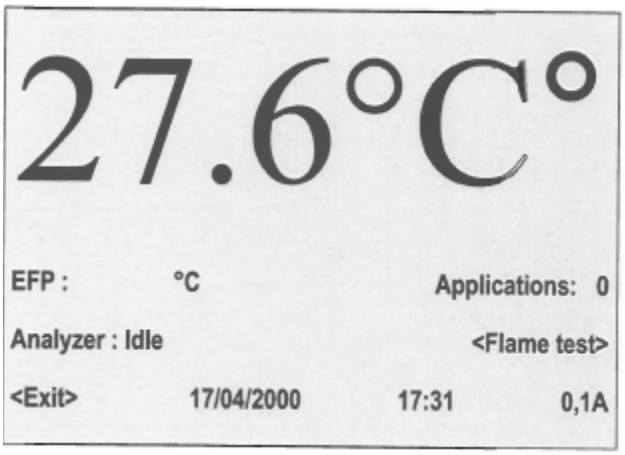
14.2.4 Turn the cover handle clockwise to lock the assembly in position. Make sure that the locking device is correctly engaged.

14.2.5 Press “ENT,” select <Run menu>, and the following screen is displayed:

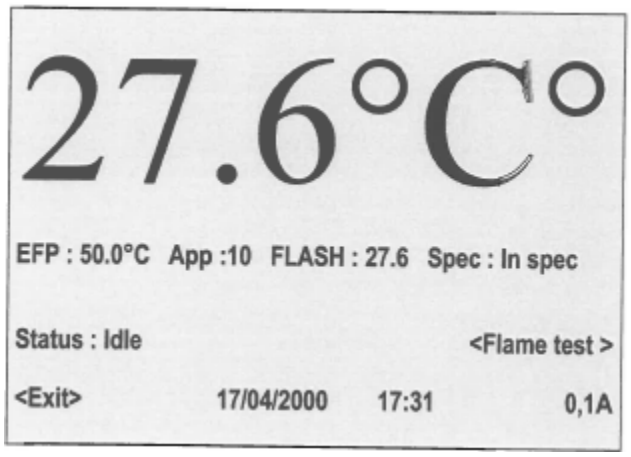


14.2.6 Select “EFP” and enter the Expected Flash Point. Pay attention to the units when selecting a flashpoint! Press “ENT.” It is also possible to use the “Previous EFP” function so as to reselect the EFP for the previous test run.

14.2.7 When, the “RUN START(E)” function is selected, the following screen is displayed and the flash point apparatus will begin heating and incrementally checking the sample for flash:



14.2.8 When the flash point of the sample is detected, an intermittent audio alarm is triggered. To stop the alarm press the **<<STOP ALARM>>** button on the keyboard. The “End of Test” screen will be displayed. Select **<Quit>**. Quitting gives a screen that is similar to the “Test running” screen and is called the “End of test” screen:



14.2.9 If the flash point of the sample is not detected, the expected flash point should be raised by 5-10°C (14.2.6), the sample aliquot should be allowed to cool by 15°C and the procedure repeated from 14.2.7.

14.2.10 Record flash point measurement, in degrees Celsius, and barometric pressure, in hPa, on the Flash Point Bench Sheet.

P15. CALCULATIONS

15.1 Flash Point (Corrected)

15.1.1 The Flash Point Benchsheet uses a correction factor, based on the barometric pressure, to calculate the corrected flash point.

15.1.2 Applicable Formulas

15.1.2.1 $$Flash\ Point, °C = Flash\ Point\ (measured) + 0.025\ (1013 - Pressure\ (hPa))$$

15.2 Flash point (Average)

15.2.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.2.2 Applicable Formulas

15.2.2.1 $$Average(X) = \sum \frac{X_i}{n},$$
$$where\ X\ contains\ n\ values\ X_i$$

15.3 Flash point (RSD)

15.3.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $$Standard\ Deviation(X) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$
$$where\ X\ contains\ n\ values\ X_i\ with\ average\ \bar{X}$$

P16. METHOD PERFORMANCE

16.1 Accuracy: N/A

16.2 Precision: 10%.

P17. POLLUTION PREVENTION

After cooling to ambient temperatures, all wastes from these procedures shall be collected and waste disposed in accordance with state and federal regulations. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Flash point: Proficiency test results should fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Flash point (Average) should fall within the acceptance range specified by the provider.

18.3.2 Flash point (RSD) should be less than 10%.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 Flash point should fall within the range established by the provider.

18.5 P12.5: Laboratory Control Standard

18.7.1 Flash point should be within the range established by the provider.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL OR UNACCEPTABLE DATA

If any of the quality control procedures indicates that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measures, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

20.1 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting the analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 Collect all waste from fuel testing in a suitable closed container (approved fuel can) in the satellite accumulation area. Placing fuel sample cups and bottles upside down in a funnel facilitates draining excess fuel or test chemicals into the waste container.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- Flash Point By Pensky-Martens Closed Tester[1] – EPA 1010 (9/86)
- ASTM D93-08 Standard Test Methods for Flash Point by Pensky-Martens Closed Cup Tester
- FP93 5G2 Pensky-Martens automated flash point analyzer user and maintenance manual

P23. TABLES, DIAGRAMS AND FLOWCHARTS

Not applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
B	09/24/12	New Instrument and guidelines
C	01/18/13	Automated Flash Point Analyzer malfunctioned. Equipment repaired. Notation: EQUIPMENT NEEDS TO BE MONITORED AT ALL TIMES DURING OPERATION WHILE PERFORMING AN ANALYLSIS. DO NOT WALK AWAY AND LEAVE UNATTENDED.
	05/01/13	A certified standard shall be analyzed with each batch when performing an analysis.
4	09/18/13	Revised to include cleaning procedure, instrument diagnostic checks, and annual quality control requirements.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	11/21/2014	MSDS changed to SDS. Formatting revised.
	12/07/2016	Revised standardization and quality control to analyze a certified standard monthly to ensure instrument performance.
5	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
6	5/16/2019	Revised to include barometric pressure requirement. Formatting changes to fit with SOP format.

SOP-WI-10

Nitrate as N

Revision 5

Approval:

Marianne Smith — 1-31-19

Environmental Programs Manager — Date

1-31-19

Laboratory Director — Date

1-31-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 1/31/2019

Title: Nitrate as N

P1. IDENTIFICATION OF THE TEST METHOD

Nitrate as N

P2. APPLICABLE MATRIX OR MATRICES

This test method is suitable for the quantification of nitrate as nitrogen in surface and wastewaters.

P3. DETECTION LIMIT

The limit of detection (LOD) for this analysis is determined according to 40 Code of Federal Regulations Part 136 Appendix B, *Definition and Procedure for the Determination of Method Detection Limit* (Revision 2) and re-verified annually. The limit of quantitation for this analysis is targeted to be 2 mg/L; however, the actual calculated limit of quantitation, as well as the current limit of detection, are posted in the laboratory.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test method for the analysis of samples for Nitrate as N. This test method is intended for use in the measurement of nitrate in surface waters and domestic and industrial wastewaters.

P5. SUMMARY OF THE TEST METHOD

This procedure described here is certified under SM 4500-NO_3-D, which describes the determination of nitrate. Nitrate is calculated from potentiometric measurements from an ion-selective probe relative to standards created from certified reference materials using log-linear regression.

P6. DEFINITIONS

Calibration Standard: A solution prepared from a stock solution of known concentration that is used to calibrate the instrument response with a known analyte concentration.

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Calibration Verification (CV): A calibration standard that is analyzed periodically to ensure instrument performance has not changed significantly since initial calibration.

Laboratory Fortified Blank (LFB): A standard prepared from a certified reference material of a different lot than is used to for creation of the primary standard. The LFB is used as a second-source accuracy verification of the calibration curve.

Laboratory Fortified Matrix (LFM): A sample aliquot that is "spiked" with a known amount of the analyte of interest. The LFM is used to access the effects of the sample matrix on the accuracy of the analytical method.

Laboratory Fortified Matrix Duplicate (LFMD): A duplicate aliquot prepared of an LFM. The LFMD is used to access the effects of the sample matrix on the accuracy and precision of the analytical method.

Limit of Detection (LOD): The lowest analyte concentration that produces a signal significantly differentiable from the blank.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

Method Blank (MB): A sample of method appropriate pure water containing no target analyte that is taken through the entire sampling and analytical procedure.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7. INTERFERENCES

7.1 The nitrate electrode responds to numerous interfering anions.

7.2 Temperature changes affect electrode potentials. Therefore, standards and samples must be equilibrated at the same temperature (± 1 °C).

7.3 The user should be aware of the potential for interferences from colloidal substances and that, if necessary, the samples should be filtered. If the samples are filtered, the associated method blanks must also be filtered.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are

routinely exposed to such water samples should protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter and clean bench surfaces with disinfectant before and after bacteriological testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

P9. EQUIPMENT AND SUPPLIES

- Volumetric flasks
- Pipettes and/or micropipettors
- Nitrate Ion Selective Electrode and meter
- Magnetic stirrer and stir bars

P10. REAGENTS AND STANDARDS

10.1 Deionized water (SOP-WI-35, *Deionized Water System*)

10.2 Interference Suppression Solution, ISS (silver and aluminum sulfates, boric and sulfamic acids)

10.4 Potassium Nitrate certified reference materials

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE

11.1 Sampling should be performed as specified by a field sampling plan or based on compliance-based sampling requirements.

11.2 Samples should be iced or refrigerated at 6°C or less but should not be frozen. If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.3 The maximum recommended holding time for wastewater samples requiring nitrate testing is 48 hours. This cannot be extended by acidification with sulfuric acid. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on a fresh copy of the Nitrogen Analysis Template before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for nitrate obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.3 Initial Demonstration of Capability

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 Analyze four aliquots of a certified reference material, prepared according to manufacturer's instructions, with all required batch quality control.

12.3.3 Include any required preparation steps as a line item in the Standard Logbook.

12.3.4 Interpretation of initial demonstration of capability results is discussed in P18.

12.4 On-going Demonstration of Capability

12.4.1 Similarly to P12.3, analyze a single aliquot of a certified reference material with all required batch quality control. Unlike an initial demonstration of capability, customer samples can be batched with on-going demonstrations of capability. Interpretation of on-going demonstration of capability results is discussed in P18.

12.5 Method Blank

12.5.1 Per method requirement, the laboratory shall perform analysis of a blank sample, consisting solely of deionized water, taken through all sample preparation and analysis steps, per batch. Interpretation of method blank analyses is discussed in P18.

12.6 Calibration Standards

12.6.1 Per method requirement, the laboratory shall perform analysis of at least three calibration standards, per batch, prepared from a certified reference material and taken through all sample preparation and analysis steps. Preparation of calibration standards is discussed further in P13. Interpretation of the resulting calibration curve is discussed in P18.

12.7 Laboratory Fortified Blank (LFB)

12.7.1 Per method requirement, the laboratory shall perform analysis of an LFB, prepared from a secondary source certified reference material and taken through all sample preparation and analysis steps, per batch. Preparation of LFB standards is discussed further in P13. Interpretation of LFB analyses is discussed in P18.

12.8 Laboratory Fortified Matrix *(*LFM*)*

12.8.1 Per method requirement, the laboratory shall perform analysis of an LFM and LFMD pair, prepared by spiking a sample with a known concentration of analyte, per batch. Preparation of LFM and LFMD standards is discussed further in P14. Interpretation of LFM and LFMD analyses is discussed in P18.

12.9 Calibration Verification (CV)

12.9.1 Per method requirement, the laboratory shall perform analysis of a CV, prepared from the primary source certified reference material and taken through all sample preparation and analysis steps, per ten samples analyzed or per batch, whichever is greater. Preparation of CV standards is discussed further in P13. Interpretation of CV analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 Nitrate ISE Probe

13.1.1 Setup

13.1.1.1 The Nitrate ISE probe is stored dry, ideally under dessication.

13.1.1.2 Prior to use, immerse the probe in a Nitrate as N standard at the lowest calibration concentration for at least 15 minutes prior to use. Best results may be obtained when immersion occurs overnight.

13.1.1.3 If not already done, install the cable of the Nitrate ISE Probe into the back of a compatible multimeter.

13.1.1.4 Attach probe to probe arm, making sure to route cable behind the probe using the notches on the probe arm.

13.1.2 Calibration

13.1.2.1 While capable, *SM 4500* NO_3*-D* does not allow for the use of the multimeter for calibration record retention. Therefore, the probe is only used to capture voltage and temperature measurements.

13.1.3 Slope Check

13.1.3.1 Prior to use, the response of the probe must be checked.

13.1.3.2 Measure 100 mL of deionized water and add 10 mL of ISS solution.

13.1.3.3 To this solution, add 1 mL of 1000 mg/L NO_3 as N standard and measure the millivolt reading. Record this measurement in the "+1 mL" field on the Nitrogen Analysis Benchsheet under the "Probe Decade Response" header.

13.1.3.4 Additionally, add 10 mL of 1000 mg/L NO_3 as N standard and measure the millivolt reading. Record this measurement in the "+10 mL" field on the Nitrogen Analysis Benchsheet under the "Probe Decade Response" header. The difference should produce a "Slope Check Value" between -54 and -60 mV (acceptance criteria may vary depending on the probe used).

NOTE: This step can also be accomplished using measurements of the calibration standards that exhibit a ten-fold difference (for example, 1 mg/L and 10 mg/L standards).

13.2 Thermometers

13.2.1 Calibrated, NIST-traceable thermometers are kept in the sample refrigerator. Thermometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.2.2 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.2.3 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

13.3 Standards

13.3.1 Calibration Standards

13.3.1.1 The analyst shall prepare, at least, three calibration standards from an potassium nitrate certified reference material spanning the range of expected concentrations (e.g., 100, 10, 1, and 0.5 mg/L) and one of which is run at or below the LOQ. For greater linearity, standards should be prepared by serial dilution.

13.3.2 LFB

13.3.2.1 The analyst shall prepare one LFB standard from a secondary source potassium nitrate standard at a concentration within the log-linear range of the calibration curve. At least quarterly, an LFB should be prepared at one to two times the LOQ.

13.3.3 CV

13.3.3.1 The analyst shall prepare CV standards (as specified in P12.9) from the primary source potassium nitrate standard at a concentration within the log-linear range of the calibration curve.

P14. PROCEDURE

14.1 Chlorine Check

14.1.1 Samples should be checked for residual chlorine prior to the beginning of sample analysis. If detected, samples must be dechlorinated using a freshly-prepared sodium thiosulfate solution.

14.2 Verify that samples are within proper hold conditions (P11).

14.3 Preparation of the LFM and LFMD

14.3.1 Prepare a spike with target concentration inside the range of the calibration curve. Specify the volume of sample and volume of spike standard used as a line item in the "Sample Notes" table.

NOTE: Sample matrix volume should not be altered by more than 5% when spiking.

14.4 Standard Curve

14.4.1 Use the sample volume and the volume ISS indicated on the "Batch Analysis Conditions" table to generate the standard curve.

14.4.2 Indicate the sample ID and target concentration in the "Regression Table".

14.4.3 Prior to analyzing curve standards, it is highly recommended to allow equilibration to constant temperature conditions in a room temperature water bath for, at least, 30 minutes.

14.4.4 For each standard, pipette designated sample volume into an appropriate container (i.e., 50 mL beaker) with a magnetic stirrer for measurement.

14.4.5 While stirring, immerse the membrane cap into the solution to be measured. Ensure that no air bubbles form on the membrane. If bubbles form, slow the stir speed and gently flick the probe body to release.

14.4.6 After addition of indicated ISS volume, record the stabilized potential (mV response) and temperature reading of each calibration standard in the appropriate spaces in the "Regression Table". Interpretation of the calibration curve in its entirety is discussed in P18.

14.5 Samples

14.5.1 Indicate the sample ID, location, and QC type and target concentration (if applicable) in the "Samples" table. If any sample requires additional clarification (i.e., preparatory steps of an LFM), select its sample ID from the drop-down menu provided in the "Sample Notes" table and provide the clarification in the adjacent "Note" field.

14.5.2 Prior to sample analysis, it is highly recommended to allow equilibration to constant temperature conditions in a room temperature water bath for, at least, 30 minutes.

14.5.3 For each sample, pipette designated sample volume into an appropriate container (i.e., 50 mL beaker) with a magnetic stirrer for measurement.

14.5.4 While stirring, immerse the membrane cap into the solution to be measured. Ensure that no air bubbles form on the membrane. If bubbles form, slow the stir speed and gently flick the probe body to release.

14.5.5 After addition of indicated ISS volume, record the stabilized potential (mV response) and temperature reading of each calibration standard in the appropriate spaces in the "Regression Table".

14.5.6 If required, indicate a dilution factor in the provided field on the "Samples" table; otherwise, leave the field empty or simply type the number "1". An example of when this field may be used is that an analyst measures 8 mL of sample with 2 mL of deionized water (instead of the standard 10 mL) and the standard 10 mL of ISS. In this case, for completion, the analyst should type in the dilution factor as "=10/8" or "1.25".

P15. DATA ANALYSIS AND CALCULATION

15.1 Nitrate

15.1.1 The Nitrogen Analysis Benchsheet uses log linear regression, accounting for dilution, to automatically calculate nitrate concentrations by the following formula:

15.1.2 Applicable Formulas

15.1.2.1 $Nitrate\ as\ N, mg/L = d.f. * [NO_3^-]$

$$where\ d.f.is\ the\ dilution\ factor,$$
$$[NO_3^-]\ is\ the\ concentration\ of\ ammonia\ fron\ the\ calibration\ curve$$

15.2 Nitrate (Average)

15.2.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.2.2 Applicable Formulas

15.2.2.1
$$Average(X) = \sum \frac{X_i}{n},$$
$$where\ X\ contains\ n\ values\ X_i$$

15.3 Nitrate (RSD)

15.3.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1
$$Standard\ Deviation(X) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$
$$where\ X\ contains\ n\ values\ X_i\ with\ average\ \bar{X}$$

15.4 Nitrate (% Recovery)

15.4.1 The percent recovery of analyte measured in LFB and CV samples and calibration standards.

15.4.2 Applicable Formulas

15.4.2.1
$$\%\ Recovery = \frac{x_{measured}}{x_{theoretical}} * 100\%$$

15.5 Nitrate (% Recovery, Spike)

15.5.1 The percent recovery of analyte measured in LFM and LFMD samples.

15.5.2 Applicable Formulas

15.5.2.1
$$\%\ Recovery = \frac{[LFM]_{measured} * V_{LFM} - [Sample]_{measured} * V_{sample}}{[Spike\ Standard]_{theoretical} * V_{spike\ standard}} * 100\%$$
$$where\ brackets\ denote\ concentrations, in\ \frac{mg}{L}, and\ V\ denotes\ volumes, in\ mL$$

15.6 Nitrate (RPD)

15.6.1 The relative percent difference between the measured values of the LFM and LFMD.

15.6.2 Applicable Formulas

15.6.2.1 $$RPD = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} * 100\%$$

$$where\ X_1\ and\ X_2\ are\ the\ concentrations\ of\ the\ LFM\ and\ LFMD$$

15.7 Significance

15.7.1 Nitrate values should be reported to 3 significant figures or to two decimal points, whichever is less significant.

P16. METHOD PERFORMANCEE

16.1 The current Limit of Quantitation and Limit of Detection are posted on the laboratory wall. The ideal LOQ for this analysis is approximately 2 mg/L.

16.2 Accuracy and precision requirements are established by control charting.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Nitrate as N: Proficiency test results should fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Nitrate (Average) should fall within the acceptance range specified by the provider, if given.

18.3.2 If analyzing four aliquots of a LFB to meet this requirement, Nitrate (% Recovery) should be calculated using Nitrate (Average). This Nitrate (% Recovery) should be between 85% and 115%.

18.3.3 Nitrate (RSD) should be less than 20%.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 Nitrate should fall within the range established by the provider.

18.5 P12.5: Method Blank

18.5.1 Nitrate should be less than half the LOQ (ideally also below the LOD).

18.6 P12.6: Calibration Standards

18.6.1 Nitrate (% Recovery) should be between 90% and 110%.

18.7 P12.7: Laboratory Fortified Blank

18.7.1 Nitrate (% Recovery) should be between 85% and 115%.

18.8 P12.8: Laboratory Fortified Matrix

18.8.1 Nitrate (% Recovery, Spike) should be between 80% and 120%.

18.8.2 Nitrate (RPD) should be less than 20%

18.9 P12.9: Calibration Verification

18.9.1 Nitrate (% Recovery) should be between 90% and 110%.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL OR UNACCEPTABLE DATA

If any of the quality control procedures indicates that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measures, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting the analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 The wastes generated in this method are hazardous because of a low final pH. The samples may be neutralized by the addition of water in an accumulation container (e.g., a large beaker or flask) or by slowly pouring down the drain with the faucet running followed by a triple rinse of the container to remove residual corrosives.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- 22nd Ed Standard Methods for the Examination of Water and Wastewater 4500-NO_3 D, 2011

P23. TABLES, DIAGRAMS AND FLOWCHARTS

- The *Nitrogen Analysis Benchsheet* can be located in the SOW 7 drive at the following extension: \\wiims.wff.nasa.gov\wiccII\SOW 7\Chem Lab\WW-DW\Bench Sheets\Nitrogen Analysis

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
2	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	06/24/13	Latest version 22nd ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	12/09/2014	MSDS changed to SDS. Formatting and quality control revised.
3	01/31/2017	Grammatical and formatting changes. Updated Method Blank requirements to reflect Corrective Action. Accumulation of minor changes under a single revision warrants a revision change.
4	5-16-2018	Removed old benchsheets and linked to current benchsheet. Revised P18 with appropriate acceptance criteria. Added note to procedure to check that samples are within hold time.
5	1/31/2019	Revised header and P1 to resolve SOP name disparity. Included guidance in P14 for temperature equilibration of samples prior to analysis. Re-included erroneously removed formulae in P15. Restructured P18 to be consistent with new round of SOPs. Revised P21 to be more in-line with laboratory practice.

SOP-WI-13

3 point pH Calibration

Revision 4

Approval:

Marianne Sinclair — 2-4-19

Environmental Programs Manager — Date

2-4-19

Laboratory Director — Date

2-4-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

Title: 3 point pH Calibration

P1. IDENTIFICATION OF THE TEST METHOD

Determination of pH

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for the determination of pH in a wastewater or drinking water sample.

P3. DETECTION LIMIT

The detection limit for this method is 0.1 SU.

P4. SCOPE AND APPLICATION

This method has been developed for analysis of pH in water samples (i.e., drinking water, surface waters, wastewater and groundwater). It has been found to meet data quality criteria with all waters of these types for which it has been tested.

P5. SUMMARY OF METHOD

Reproduced below is a simple, step-by-step outline of our pH method for quick reference.

Summary of Procedure for pH Measurement

1. The pH of the sample should be taken at room temperature.
2. Turn on pH meter.
3. Calibrate with pH 4, 7, and 10 buffer solutions.
4. Record slope.
5. Check calibration with a 2nd source pH 7 buffer solution. The result must fall within the range of 7.0 ± 0.1 SU. Recalibrate if necessary.
6. Measure the pH of samples and record results in log book.
7. Turn off the meter and store electrode in storage solution.

P6. DEFINITIONS

Initial Demonstration of Capability (IDC): A procedure to establish the ability of the analyst to generate data of method acceptable accuracy and precision. (NELAC)

Duplicate (Dup): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

P7. INTERFERENCES

Not Applicable.

P8.0 SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Wear Personal Protective Equipment (PPE) such as lab coat, safety glasses, and gloves when performing pH analysis.

8.3 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

9.1 Apparatus

- pH Selective Electrode
- Electrometer (pH meter) with expanded mV scale or a specific ion meter
- Magnetic stirrer and stirring bars
- Styrofoam cooler or other suitable thermal insulator, if necessary
- Beaker, 250 mL
- Support stand
- Magnetic stir plate
- Stir bar

9.2 Materials

- Lab Coat
- Disposable Rubber Gloves
- Safety Glasses

P10. REAGENTS AND STANDARDS

- Manufactured prepared, pH 4, 7, and 10 buffers

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Sample collection is done with clean glass or plastic bottles.

11.2 Measurement should be performed in the field at the time of collection. However, if

measurement is performed in the laboratory, collect the samples in container with zero headspace. Avoid excessive agitation or prolonged exposure to air. Holding time is 15 minutes.

P12. QUALITY CONTROL

12.1 *Annually perform two Quality control samples (QCS)/Proficiency Test (PT).* Follow the procedures for preparing the sample, and obtaining test results. Proficiency testing will be performed annually using samples from an outside source

12.1.1 To demonstrate method performance, the Analyst analyzes a QCS at least once per year or otherwise as required for each method where QC samples are available. Quality control samples (QCS) are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in. QCS are not analyzed multiple times unless routine environmental samples are analyzed multiple times.

12.2 Initial Demonstration of Laboratory Capability

12.2.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

12.2.2 Carry out four analyses using 100 mL samples for each.

12.2.3 Calculate the average and standard deviation of the values.

12.2.4 The standard deviation should be less than or equal to 20% of the average value. If it is not, evaluate the procedures to identify sources of error.

12.3 Duplicate Analysis

12.3.1 Carry out two replicates on the same sample matrix with each batch of 10 or fewer sample every time analysis is performed.

P13. CALIBRATION AND STANDARDIZATION

13.1 Instrument Calibration

13.1.1 Check electrode slope by performing calibration recommended by instrument Manufacturer every day the instrument is in use. The slope should be within instrument manufacturer’s recommended range.

13.1.2 After calibration of the pH electrode, analyze 2nd source pH 7 buffer. The result must fall within the range of 7.0 ± 0.1 SU. Recalibrate if necessary.

13.1.3 Refer to instrument manual for additional information related to pH meter calibration, troubleshooting, and maintenance. Document calibration results in appropriate logbooks.

P14. PROCEDURE

Complete the following documents for each sample or set of samples submitted for analysis:

14.1 Three-Point Calibration

14.1.1 Set up meter according to meter instruction manual.

14.1.2 Standardize the meter and electrode system according to manufacturer's instructions.

14.1.3 Calibrate using pH 4, 7, and 10 commercial, NIST traceable buffers.

14.1.4 Fill marked falcon tubes with each pH calibration standard. Immerse the electrode in the first buffer and prompt the meter to take a reading on its calibration screen. Repeat until all 3 calibration standard are read into the meter. Rinse the electrode between each reading. Record pH and temperature in logbook of each calibration standard.

14.1.5 If all steps are preformed correctly, and the slope is between 92 and 102 percent, proceed to measure pH of sample(s).

14.1.6 After calibration, a pH 7.0 buffer is analyzed as a check to sample to verify that calibration is correct. Agreement should be within ± 0.1 SU.

NOTE: For meters with ATC that also have temperature display, the thermometer needs to be annually verified.

If any of the above procedures do not work, refer to troubleshooting guide in the pH electrode instruction manual.

14.2 pH Measurement

14.2.1 Calibrate pH meter daily before use.

14.2.2 Calibrate the electrode as described in one of the previous sections. (Permits or technical guidance documents may specify the calibration method to use.)

14.2.3 Rinse the electrode with distilled water.

14.2.4 Immerse the electrode in the sample.

14.2.5 When the display is stable, record sample pH to the nearest 0.1 SU and the sample temperature in degrees Celsius..

14.2.6 Rinse the electrode with distilled water and soak electrode in pH electrode storage solution after use (if recommended by instrument manufacturer).

14.2.7 Record data on appropriate logbooks or analysis sheets.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 There are no calculations for this method.

15.2 Report pH concentrations to the nearest 0.1 SU.

15.3 Reporting

15.3.1 Discharge Monthly Report (DMR)

15.3.2 If a duplicate sample is analyzed, the reported value for that sampling event is the average concentration of the sample and the duplicate for the reported value on the DMR.

P16. METHOD PERFORMANCE

16.1 The desired performance criteria for this measurement are:

16.1.1 Detection limit: 0.1 SU

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 The desired detection limit is 0.1 SU.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 The samples cannot be reanalyzed because of the 15 minute sample holding time.

P20. CONTINGENCIES FOR HANDLLING OUT-OF-CONTROL DATA

20.1 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

P21. WASTE MANAGEMENT

21.1 The samples may be neutralized by the addition of water in accumulation container (e.g., a large beaker or flask). Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- SM 4500-H^+ B 22nd ED 2011

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	03/04/2009	pH method EPA 150.1 is no longer acceptable. Lab is currently using 20th Ed. SM 4500-H^+ B.
B	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
3	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	11/20/13	Latest version 22nd ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	10/22/14	MSDS changed to SDS. Formatting revised.
4	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.

SOP-WI-16

Total Suspended Solids

Revision 8

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

6/7/19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

6-7-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

6-7-19
Date

LJT & Associates, Inc.

Effective Date: 06/07/2019

SOP-WI-16

Total Suspended Solids

Revision 8

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Mark Cuhel	[illegible]	6-7-19	new rev
Jason Freischlag	[illegible]	6-7-19	new revision

Title: Total Suspended Solids

P1. IDENTIFICATION OF THE TEST METHOD

1.1 Total Suspended Solids

1.2 Total Volatile Suspended Solids

P2. APPLICABLE MATRIX OR MATRICES

2.1 These methods are suitable for the determination of total and volatile suspended solids in non-potable waters.

P3. DETECTION LIMIT

3.1 The limit of detection is not well defined for these analyses. The limit of quantitation for TSS is set, assuming a one-liter sample, at 1.0 mg/L. The limit of quantitation for TVSS is set, assuming a 100 mL sample, at 10 mg/L.

P4. SCOPE AND APPLICATION

4.1 This standard operating procedure (SOP) describes the test method for the analysis of samples for total suspended solids (TSS) and/or total volatile suspended solids (TVSS) in surface waters and domestic and industrial wastewaters.

P5. SUMMARY OF METHOD

5.1 The TSS procedure described here is certified under SM 2540 D, which describes the gravimetric determination of TSS by vacuum filtration. TSS is calculated as a concentration using the mass of deposited material and the volume of sample flowed through a pre-weighed filter paper.

5.2 The TVSS procedure described here is certified under EPA 160.4 and largely complies with SM 2540 E, which describes the gravimetric determination of TSS before and after ignition at 550°C. TVSS is calculated as a concentration using the mass of deposited material before and after ignition as well as the volume of sample flowed through a pre-weighed filter paper.

P6. DEFINITIONS

6.1 **Certified Reference Material**: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

6.2 **Deionized Water Blank**: A method blank prepared using rinse-grade deionized water that does not contain any suspended solids.

6.3 **Limit of Quantitation** (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

6.4 **Lot Quality Control** (LQC): Quality control procedure performed on incoming commercially-prepared reagents to ensure usability.

6.5 **Safety Data Sheets** (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

6.6 **Total Suspended Solids** (TSS): The total amount of solids from a sample that deposit on a filter paper. TSS analyses are important in the control of wastewater treatment processes and for assessing compliance with discharge regulations.

6.7 **Volatile Suspended Solids** (TVSS, VSS): The total solids lost on ignition at 550°C. TVSS are useful to the treatment plant operator because they can be used to calculate operational parameters, such as sludge age and food-to-microorganism ratio.

P7. INTERFERENCES

7.1 Measurements can include both positive errors (occluded water and waters of crystallization) and negative errors (decomposition and volatilization of minerals such as carbonates, as well as loss of solids small enough to pass through the filter).

P8. SAFETY PRECAUTIONS

8.1 Adhere to standard laboratory safety procedures. Review the Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples should protect themselves from waterborne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter. Clean bench surfaces with disinfectant before and after testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

8.6 This analytical procedure uses a vacuum pump for the filtration steps. There is a risk of implosion under some circumstances. The analyst should ensure that the receiving flask/carboy is free from cracks or other imperfections.

8.7 Determination of TVSS requires the use of a muffle furnace at 550°C, which is capable of inflicting severe burns. Always use appropriately-rated thermal gloves when transporting samples to and from the muffle furnace.

P9. TOOLS, EQUIPMENT AND SUPPLIES

9.1 Filtration Apparatus

9.1.1 Pall 3-piece aluminum filter funnel manifold

9.1.2 Plastic funnels with magnetic adhesive strips

9.1.3 Tygon Tubing

9.1.4 Carboy with tube fitting (for filtrate waste)

9.1.5 Vacuum Pump

9.2 Permanent marker

9.3 Forceps or tweezers

9.4 Wash bottle

9.5 “TD” Graduated cylinders

9.6 Aluminum dish tongs

9.7 Drying oven capable of maintaining temperature at 103 – 105°C

9.8 Desiccator (with water-reactive color indicator or digital moisture gauge)

9.9 Analytical balance, capable of weighing to 0.1 mg.

9.10 NIST traceable reference weights

9.11 Muffle furnace capable of maintaining temperature of 550°C

9.12 Crucible tongs

P10. REAGENTS AND STANDARDS

10.1 Deionized water (SOP-WI-35, Deionized Water System)

10.2 ProWeigh® pre-weighed filter paper (47mm) for TSS with aluminum dish

10.3 ProWeigh® pre-weighed filter paper (47mm) for Volatiles with aluminum dish

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Sampling should be performed as specified by a field sampling plan or based on compliance-based sampling requirements.

11.2 Samples should be iced or refrigerated at 4°C or less but should not be frozen. If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.3 The maximum recommended holding time for wastewater samples requiring TSS and TVSS testing is 7 days. The storage temperature and sampling time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis. Document LQCs using the template document(s) listed in the relevant section.

12.2 Proficiency Testing

12.2.1 To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for TSS and TVSS obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in).

12.2.2 Sign in the proficiency tests in the Sample Receiving Logbook. Be sure to record the sample ID number on the sample container.

12.2.3 Follow the PT provider's included instructions for preparing the sample and analyze according to P14.

12.3 Initial Demonstration of Capability (IDC)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 The analyst then shall carry out the analysis of four sample aliquots as per P14. The interpretation of the analyst's results is discussed in P18.

12.4 On-going Demonstration of Capability (ODC)

12.4.1 Analysts are required to re-verify their capability in this field of testing through the on-going demonstration of capability. Prior to completing the on-going demonstration of capability, the analyst must review this SOP and sign off on the SOP Signature Sheet.

12.4.2 The analyst shall carry out the analysis of a single sample and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Duplicates (DUP)

12.5.1 Per method requirement, the laboratory shall perform duplicate analyses on, at minimum, 10% of all samples received. Interpretation of duplicate analyses is discussed in P18.

12.6 Pro-Weigh® Filter LQC

12.6.1 For each new lot of filters, analyze a filter according to P14 with approximately one liter of deionized water as the sample. Interpretation of Pro-Weigh® Filter LQC analyses is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

13.1 Analytical Balance

13.1.1 Ensure the balance is clean and is in calibration prior to use.

13.1.2 Each day of use, the balance is to be verified against NIST-traceable weights. These measurements are recorded on the Daily Scale Reading bench sheet. Interpretation of scale readings is discussed in P18.

13.2 Thermometers

13.2.1 Calibrated, NIST-traceable thermometers are kept in the drying oven and the sample refrigerator. Thermometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.2.2 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.2.3 The drying oven is maintained at ~104°C. The temperature is checked twice daily, at least four hours apart, while in use, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this oven are 103.0°C to 105.0°C.

13.2.4 The muffle furnace is maintained at 550°C. The temperature is checked twice daily, at least four hours apart, while in use, by reading the digital display on the furnace. The

temperature is transcribed onto the Temperature Log. The acceptable temperatures of this oven are ± 550°C.

13.2.5 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, at least four hours apart, per business day, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

P14. PROCEDURE

14.1 Filtration Phase

14.1.1 Bring samples to ambient temperature prior to start of filtration phase. This is easiest to accomplish with a room temperature water bath.

14.1.2 Using an indelible marker, record the sample ID and the QC type (if applicable) on each aluminum tray. If analyzing for TVSS, engrave the last two digits of the sample ID number into the tray using a pen tip, as marker ink is volatile at 550°C.

NOTE: While labeling, check filter papers for imperfections (i.e., small punctures) and discard, if found.

14.1.3 Record each pre-determined weight in the corresponding row of the bench sheet.

14.1.4 Check to ensure the vacuum pump is connected to the vacuum port of the hood, the collection bottle is connected to the vacuum port, and the collection bottle is connected to the filtration manifold.

14.1.5 Turn on the vacuum pump.

14.1.6 Detach plastic funnel from filtration manifold.

14.1.7 With the stopcock closed (horizontal), transfer filter paper right side up (rough side) from aluminum tray to the platform on the filtration manifold and reattach plastic funnels using the magnetic strips.

14.1.8 Seat the filter paper with a small amount of rinse-grade deionized water and turn the stopcock on (vertical).

14.1.9 After thoroughly mixing sample, measure appropriate volume of sample using a graduated cylinder.

NOTE: Per NPDES reporting requirements, 1000 mL, or entire provided sample volume, must be used when analyzing effluents. If this volume will not filter after 10 minutes, make a notation on the applicable row.

14.1.10 Slowly pour measured aliquot through the filter paper. Record the filtration start time for each sample in the corresponding spot on the bench sheet.

14.1.11 Continue filtering measured aliquot until complete or until filtration speed notably slows. Record the filtered volume for each sample in the corresponding spot on the bench sheet.

14.1.12 If entire aliquot is filtered, rinse graduated cylinder with rinse-grade deionized water and pour through the filter paper.

14.1.13 After complete filtration of the prior rinse step, rinse filter paper and the plastic funnel with three successive ~10 mL aliquots of deionized water using the deionized water bottle. Allow filtrate to completely filter prior to next rinse.

14.1.14 After vacuum has been applied for at least an additional three minutes, turn the stopcock off and remove the filter paper from the filtration manifold using tweezers or forceps. Record the filtration start time for each sample in the corresponding spot on the bench sheet.

NOTE: If sample filtration takes longer than ten minutes and there is sample volume remaining, re-filter using remaining sample volume such that it can filter in ten minutes.

14.1.15 Check each filter paper for damage caused by the vacuum filtration. If damage is observed, record on the bench sheet and initiate the corrective action process.

14.2 First Drying Phase

14.2.1 Place all filter papers in the oven. Record the drying start time and the oven temperature in the corresponding spots on the bench sheet for each sample.

14.2.2 Allow filter papers to dry for, at least, one hour

14.2.3 Remove filter papers from oven, using dish tongs, and place in desiccator to cool. Record the drying stop time and the oven temperature in the corresponding spots on the bench sheet for each sample.

14.3 Initial Weigh Phase

NOTE: Be sure the verifications steps listed in P13.1 are completed prior to this step.

14.3.1 Per filter, remove from aluminum tray, using tweezers, and place in center of weighing tray of the analytical balance. Allow the weight to stabilize for at least three seconds and record the initial weight in the corresponding spot on the bench sheet.

14.3.2 After weighing, replace each filter in its dish right side up and place aside until the entire batch is weighed.

14.4 Second Drying Phase

14.4.1 Perform steps listed in P14.2.

14.5 Final Weigh

14.5.1 Perform steps listed in P14.3. Record reweigh weights in the corresponding spots on the bench sheet.

14.5.2 Ensure that the initial weight (P14.3) and the reweigh weight (P14.5.1) is less than 0.5 mg (0.0005 g). If the weight change is greater, re-perform P14.4 and P14.5, ensuring that the additional parameters required are documented in an organized manner on the bench sheet, until the two most recent weights meet this criteria.

NOTE: Weight stability criteria in SM 2540 D also states that filters papers weights shall not differ more than 4% between weighings. However, this criteria will only ever apply to filters originally weighing less than 0.0125 g.

14.6 Drying Phase - Volatiles Only

NOTE: The muffle furnace should be started at least an hour, to ensure that it has reached the target temperature, prior to attempting this step.

14.6.1 Place all filter papers requiring volatile solids analysis in the muffle furnace. Record the drying start time and the oven temperature in the corresponding spots on the bench sheet for each sample.

14.6.2 Allow filter papers to dry for, at least, 15 minutes.

14.6.3 Remove filter papers from oven, using crucible tongs, and place in dessicator to cool. Record the drying stop time and the furnace temperature in the corresponding spots on the bench sheet for each sample.

14.7 Volatile Weigh

14.7.1 Per filter, remove from aluminum tray, using tweezers, and place in center of weighing tray of the analytical balance. Allow the weight to stabilize for at least three seconds and record the volatile weight in the corresponding spot on the bench sheet.

14.7.2 After weighing, replace each filter in its dish and place aside until the entire batch is weighed.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Total Suspended Solids

15.1.1 Concentration of total suspended solids in sample, in mg/L.

15.1.2 Applicable Formulas

15.1.2.1 $$TSS\left(\frac{mg}{L}\right) = \frac{m_{reweigh}(grams) - m_{blank}(grams)}{V_{filtrate}\,(mL)} * 1000\,\frac{mL}{L} * 1000\,\frac{mg}{g}$$

$$where\ m\ indicates\ mass\ and\ V\ indicates\ volume$$

15.2 Total Volatile Suspended Solids

15.2.1 Concentration of total volatile suspended solids in sample, in mg/L

15.2.2 Applicable Formulas

15.2.2.1 $$VSS\left(\frac{mg}{L}\right) = \frac{m_{volatilization}(grams) - m_{reweigh}(grams)}{V_{filtrate}\,(mL)} * 1000\,\frac{mL}{L} * 1000\,\frac{mg}{g}$$

$$where\ m\ indicates\ mass\ and\ V\ indicates\ volume$$

15.3 Average

15.3.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $$Average(X) = \sum \frac{X_i}{n}$$

where X contains n values X_i

15.4 Relative Standard Deviation (RSD)

15.4.1 The relative standard deviation of all aliquots analyzed during an initial demonstration of capability.

15.4.2 Applicable Formulas

15.4.2.1 $$Standard\ Deviation(X) = \sqrt{\sum \frac{(X_i - \bar{X})^2}{n-1}}$$

where X contains n values X_i with average $\overline{X}$

15.4.2.2 $$RSD = \frac{Standard\ Deviation(X)}{Average(X)} * 100\%$$

15.5 Relative Percent Difference (RPD)

15.5.1 The relative percent difference between a sample and its duplicate.

15.5.2 Applicable Formulas

15.5.2.1 $$RPD(X_1, X_2) = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} * 100\%$$

where X_1 and X_2 are results of samples analyzed in duplicate

15.6 Normalized Weight Difference (NWD)

15.6.1 The deposited weight difference between a sample and its duplicate, normalized based on filtered volume.

15.6.2 Applicable Formulas

15.6.2.1 $$NWD(X_1, X_2) = \left| m_1 * \frac{\max(V_1, V_2)}{V_1} - m_2 * \frac{\max(V_1, V_2)}{V_2} \right|$$

$$where\ m\ indicates\ mass\ and\ V\ indicates\ volume$$

15.7 Significance

15.7.1 Values are generally significant to 3 significant figures or to two decimal points, whichever is less significant.

P16. METHOD PERFORMANCE

16.1 Limit of Quantitation: 1.0 mg/L (TSS), 10 mg/L (TVSS).

16.2 Precision: RPD ≤ 20% or NWD ≤ 0.6 mg

P17. POLLUTION PREVENTION

17.1 All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. Adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 The analyst should review all data for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Proficiency test results must fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Average must fall within the acceptance range specified by the provider.

18.3.2 RSD must be less than 20%.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 Results must fall within the range established by the provider.

18.5 P12.5: Duplicates

18.5.1 RPD must be less than 20% or NWD must be less than 0.6 mg (0.0006 g).

NOTE: A quicker empirical approach to determining if duplicates pass the Total Suspended Solids (NWD) parameter is to check if the TSS difference between the sample and duplicate is less than 0.6 mg/L for effluent matrices or 6 mg/L for influent matrices.

18.6 P12.6: Pro-Weigh® Filter LQC

18.6.1 Total Suspended Solids must be less than 0.5 mg/L.

18.7 The sample will be rejected if more than 7 days have elapsed between the time the sample was taken and the time it arrived at the lab, unless otherwise requested by the client.

18.8 The Quality Manager shall review all QC procedures and perform spot checks on routine analyses.

18.9 Duplicate results shall be monitored through the use of control charts.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 If adequate volume remains, samples can be reanalyzed.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

P21. WASTE MANAGEMENT

21.1 The wastes generated in this method are not hazardous. They can be discarded in the following manner: the water, filtrate, and raw sample can be discarded in the laboratory sink and filter papers can be discarded with the paper trash.

P22. REFERENCES

22.1 2009 TNI V1M4

22.2 EPA 160.4

22.3 SM 2540 D

22.4 SM 2540 E

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	1/12/04	Added Section P3. Revised Section P7.
B	1/24/08	Revised Sections 10.2 & 13.0 According to DEQ inspector, Steve Thomas, during an on- site inspection on 1/24/08: 1) Drying oven temperature must be recorded on TSS logbook. 2) The adequacy of drying time must be verified with a documented drying-cooling-weighing cycle once per year for each client. 3) A laboratory duplicate sample must be analyzed after every twenty samples tested.
C	02/28/11	Revised Sections 13.2 and 13.4 to reflect QC changes requiring duplicates at a rate of 10%; and drying, cooling, reweighing cycle repeated for all samples to confirm constant weight.
D	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
5	03/01/12	Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition. Calculations changed wording of aluminum dish to aluminum dish
	05/01/13	At least 10% of samples (or one per batch) analyzed in duplicate done everytime analysis is performed. Latest version 22nd ed.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	12/18/14	MSDS changed to SDS. Formatting revised.
	05/15/15	Carry out two replicates on the same sample matrix with each batch of 20 or fewer sample every time analysis is performed. On site vist 04/14-16/2015 DCL inspector stated that a blank does not have to measured with each batch of samples. One blank should be measured with eacn new lot of filters

6	01/31/2017	Add requirement for yearly RPD average to be less than 5% in addition to handling control charted values where values are below the limit of quantitation. Individual batches should have a reproducibility less than or equal to 20%. Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change. Changed file format.
7	01/31/2019	Incorporated modifications from working copy to increase accuracy of procedure. Reorganized for increased clarity. Incorporated normalized weight difference duplicate QC parameter to address increasing frequency of effluent duplicate failures. Added steps in procedure for analyst to check filter paper for damage.
8	06/05/2019	Added verbiage from SM 2540 D of 4% weight difference requirement for TSS to meet VELAP finding requirement. Added procedural steps for determining volatile TSS per EPA 160.4 to invalidate SOP-WI-20.

SOP-WI-17

Total Hardness as $CaCO_3$

Revision 2

Approval:

Marianne Sinko — 2-4-19

Environmental Programs Manager — Date

2-4-19

Laboratory Director — Date

2-4-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

LJT & ASSOCIATES, INC.

ANALYTICAL LABORATORY PROCEDURES AND GUIDELINES

SOP-WI-17

Title: Total Hardness as $CaCo_3$

P1. IDENTIFICATION OF THE TEST METHOD

Determination of Total Hardness by HACH Method.

P2. APPLICABLE MATRIX OR MATRICES

Hardness of a sample is determined by titrating a sample to a specific pH end-point value. Hardness is significant in many uses and treatment of natural waters and wastewaters.

P3. DETECTION LIMIT

The limit of quantitation (LOQ) is 5 mg/L unless using low-hardness method as specified in Standard Method 2340 C.

P4. SCOPE AND APPLICATION

This method is applicable to drinking, surface, saline, domestic and industrial waste water. The method is suitable for all concentration ranges of hardness; however, in order to avoid large titration volumes, use a sample aliquot containing not more than 25 mg $CaCO_3$.

P5. SUMMARY OF THE TEST METHOD

In this test, *total hardness* will be determined by titration with EDTA. Total hardness is defined as the *sum* of calcium and magnesium hardness, in mg/L as $CaCO_3$.

P6. DEFINITIONS

Duplicate (DUP): Two aliquots of the same sample treated identically throughout a laboratory analytical procedure. Analysis of duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

Hardness: Hardness refers to the measure of the total concentration of the calcium and magnesium ions expressed as calcium carbonate.

$$Hardness, [CaCO_3] = 2.497\,[Ca^{2+}] + 4.118\,[Mg^{2+}]$$

Laboratory Fortified Blank (LFB): Sometimes referred to as a standard, the LFB contains the analyte of interest at a known concentration. The LFB is treated identically to the other samples and is used to determine bias (accuracy) of a method.

Method Blank (MB): A blank of deionized water or extraction blank of the same matrix as the samples and standards used. The method blank is treated identically to the other samples and is used to assess contamination introduced during sample preparation.

Quality Control Sample (QCS): An externally generated sample containing analytes of interest at unknown concentrations. Sometimes referred to as a **Proficiency Test** (PT), the QCS is used to determine laboratory and method competence. It is obtained from an external lab semi-annually and results are compared to acceptance results provided by the external laboratory. If results do not meet acceptance criteria, investigate why, take corrective action, and run a new QCS.

Safety Data Sheets (SDS): Written information provided with a chemical by vendors about handling that particular chemical, including: toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7. INTERFERENCES

7.1 Highly colored or turbid samples may mask the color change at the endpoint—use a pH meter to determine the endpoint.

7.2 Chlorine may interfere with the indicators. Add one drop of Sodium Thiosulfate (0.1N) to eliminate this effect.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Wear Personal Protective Equipment (PPE) such as lab coat, safety glasses, and gloves when performing Hardness analysis.

8.3 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

9.1 Apparatus

- Burette clamp, double
- Burette, Class A, 25 mL
- Beakers
- Graduated Cylinder
- Support stand
- Magnetic stir plate and stir bar

P10. REAGENTS AND STANDARDS

- Buffer Solution, Hardness 1
- ManVer 2 Hardness Indicator Powder Pillows
- TitraVer Hardness Titrant, 0.020 N

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Collect the samples in a clean glass or HDPE plastic container that has been washed with detergent, tap water, and rinsed with 1:1 nitric acid and DI water. Place samples on ice. Avoid excessive agitation or prolonged exposure to air. Fill bottles completely and cap tightly.

11.2 If residual chlorine is present, dechlorinate sample. Record residual chlorine on chain of custody and add one drop of Sodium Thiosulfate (0.1N) to eliminate this effect.

11.3 Preserve samples by storing at 4^0C and analyze immediately. When immediate analysis is not possible, preserve by acidification with nitric acid to a pH of 2 or less and store at 4 degrees Celsius.

11.4 Holding time is 7 days. If samples are preserved and stored at 4^0C, samples need to be brought back to room temperature and pH of approximately 7 with 5.0 N NaOH prior to analysis.

P12. QUALITY CONTROL

12.1 Record the analysis date and time, as well as starting and ending titrations for this analysis in the hardness log book.

12.2 *Annually perform two Quality control samples (QCS)/Proficiency Test Studies (PT).* Follow the procedures for preparing the sample and obtaining test results. Proficiency testing will be performed semi-annually using samples from an outside source.

12.2.1 To demonstrate method performance, the laboratory analyzes a QCS twice per year or otherwise as required for each method where QC samples are available. Quality control samples (QCS) are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in. QCS are not analyzed multiple times unless routine environmental samples are analyzed multiple times.

12.3 *Perform Initial demonstration of capability* (for any new laboratory personnel)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

12.3.2 Carry out four analyses using 50 mL samples for each.

12.3.3 Calculate the average and standard deviation of the values.

12.3.4 The average should be within the acceptance range provided with the QCS.

12.3.5 The standard deviation should be less than or equal to 20% of the average value. If it is not, evaluate the procedures to identify sources of error.

12.4 *Method Blank*

12.4.1 Analyze a reagent water test blank with each batch.

12.5 *Laboratory Fortified Blank*

12.5.1 Quality control samples are used to document the validity of data and to control data quality within acceptance limits. QC samples are processed through the system in the same manner as any other sample, except when the analyst is aware of the source, concentration, and acceptance ranges of target analytes and calculates analyte recoveries to evaluate method performance in real time.

12.5.2 For each laboratory fortified blank (LFB) determine the percent recovery using Equation 1. The percent recovery should be within 10% of the true value.

$$\%\ Recovery = \frac{found\ value}{true\ value}\ x\ 100\% \qquad \text{Equation 1)}$$

12.6 *Duplicate (Dup)*

12.6.1 Carry out a duplicate analysis on a sample. At least 5% of samples or one per batch are analyzed in duplicate every time analysis is performed, whichever is more frequent. Calculate the precision (standard deviation) involved with each duplicate sample set.

12.6.2 The relative percent difference (RPD) between the two hardness values should be ≤ 20% of their average value and is calculated using Equation 2.

$$RPD = \frac{|(sample - dup)|}{[(sample + dup)/2]}\ x\ 100\% \qquad \text{Equation 2)}$$

12.7 *Accuracy Check*

12.7.1 *Standard Additions Method*
Use the standard additions method to find if the sample has an interference with each sample batch.

Procedure for use with the **0.020 N EDTA** titrant:

1. Open the standard solution ampule- Hardness Voluette Ampule Standard Solution, 10,000 mg/L as $CaCO_3$

2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.

3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point

4. Use the TenSette Pipet to add an additional 0.1 mL of standard to the titrated sample. Swirl to mix.

5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

6. Use the TenSette Pipet to add an additional 0.1 mL of standard to the titrated sample. Swirl to mix.

7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

Note: Each 0.1 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference or the concentration of the titrant has changed (see *Standard solution method 12.7.2*).

12.7.2 *Standard Solution Method*

Complete the following test to confirm the analytical technique and reagent performance with each sample batch.

Procedure for use with the **0.020 N EDTA** titrant:

1. Add 25.0 mL of a calcium chloride standard solution, 1000 mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.

2. Add the Hardness 1 Buffer Solution and ManVer 2 indicator. Swirl to mix.

3. Titrate the standard to the end point with the 0.020 N EDTA hardness titrant and calculate the result. The titration should use 25 (± 0.3) mL of titrant.

12.8 The Lab Supervisor shall review the results to ensure accuracy and precision. In addition, the lab supervisor shall initial the results to indicate that the calculations have been reviewed and are accurate.

P13. CALIBRATION AND STANDARDIZATION

13.1 Standards and reagents are commercially prepared. Reagents are re-standardized with each analysis by comparing the normality against the expected value and adjusted to fit the experimental value (see *Standard solution method 12.7.2*).

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13.2 Laboratory fortified blanks are run at the beginning of each batch to verify method.

13.3 Duplicates are run with each batch to ensure precision of procedure.

P14. PROCEDURE

14.1 Complete the following documents for each sample or set of samples submitted for analysis:

14.1.1 Chain-of-Custody sheet

14.1.2 Laboratory Bench sheet (See Attachment 1)

14.1.2.1 Sample information

14.1.2.2 Calculation

14.2 Titration Procedure

1. Check to ensure reagents are not expired. Do not perform analysis with expired reagents.

2. Prepare a blank. One blank must be run with each set of samples. With every batch perform a blank, LFB, dup, and CV.

3. For samples with an expected hardness of less than 1000 mg $CaCO_3$/L use 25 mL of sample and 0.020 N EDTA solution as the titrant. For samples with greater hardness (rarely) use 10 mL of sample instead. For samples with expected hardness of less than 5 mg $CaCO_3$/L use 100 mL of samples and see the low-hardness method 2340 C.3.c in Standard methods 22nd edition. Sample should require <15 mL EDTA titrant and titration should be completed within 5 minutes of buffer addition.

4. After sample is equilibrated to room temperature, measure appropriate volume (typically 25 mL) of sample as described above using a graduated cylinder. Dilute to 50 mL with deionized water if necessary. Transfer the sample into a 250-mL beaker equipped with stir bar and set sample on stir plate.

5. Fill a 50-mL buret to the zero mark with the appropriate TitraVer Hardness Titrant. Remove any air bubbles from tip of buret. Record the actual starting volume prior to starting titration.

6. Add 1 mL of Hardness 1 Buffer Solution using the 1-mL calibrated dropper and swirl to mix.

7. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow to make the prepared sample. Allow indicator to mix thoroughly.

8. While stirring, titrate slowly toward the end point with standard EDTA titrant until the color changes from red to pure blue. Record the amount of titrant added to this end point.

9. Calculate hardness results as per equation 3 found in section *P15 Calculations*. Record total hardness in the logbook. Add more titrant, as required and rinse stir bar before each subsequent sample.

P15. DATA ANALYSIS AND CALCULATIONS

$$Hardness, \frac{mg\ CaCO_3}{L} = \frac{mL\ titrant * 1000}{mL\ solution} \qquad \text{Equation 3)}$$

$$1\ mL\ EDTA * \frac{1\ L\ EDTA}{1000\ mL\ EDTA} * \frac{x\ mol\ EDTA}{1\ L\ EDTA} * \frac{1\ mol\ CaCO_3}{1\ mol\ EDTA} * \frac{100\ g\ CaCO_3}{1\ mol\ CaCO_3} * \frac{1000\ mg\ CaCO_3}{1\ g\ CaCO_3} =$$

1 mg $CaCO_3$ equivalent to 1.00 mL EDTA if using 0.020 N EDTA.

P16. METHOD PERFORMANCE

This method was validated through inter-laboratory studies. LJT & Associates, Inc. participates in Proficiency Testing (PT) Program for VELAP certification.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Method blanks (MB) should be less than the LOD.

18.2 Quality Control Samples (QCS) must meet the criteria of the lab where purchased.

18.3 Laboratory fortified blanks (LFB) should exhibit a percent recovery of less than a 10% variation from the known value.

18.4 Duplicate samples should exhibit a RPD $\leq$ 20% of the average value of the samples.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

If any of the quality control procedures indicate that the method is out of control or that a problem exists, corrective action must be taken. This includes rerunning QC measurements, ordering or preparing new standards, and cleaning out all equipment that may be contaminated.

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

20.1 If data is unacceptable for any reason, the analyst should review their analytical technique prior to conducting the analysis again.

20.1.1 Analyze a quality control sample from a PT provider to ensure that any deficiencies in test procedure have been resolved.

P21. WASTE MANAGEMENT

21.1 The samples may be neutralized by the addition of water in accumulation container (e.g., a large beaker or flask). Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- HACH 8226
- 22nd ed. Standard Methods for the Examination of Water and Wastewater 2340 C, 2011

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

23.1 See Attachment 1 for hardness bench sheet.

Attachment 1:

Hardness, Total - LABORATORY BENCH SHEET
Method Reference: SM 2340C (as HACH 8226)

	1	2	3	4	5	6	7	8
Sample ID								
Sample Date								
Sample Time								
Sample Site								
Sample Type								
Preservative								
Test Date								
Start Time								
Analyst								
Start Volume								
End Volume								
Difference								
Sample Volume								
Hardness (mg/L)								

$$Hardness, \frac{mg\ CaCO_3}{L} = \frac{mL\ titrant * 1000}{mL\ solution}$$

Titrant Lot #/Exp. Date: _____

Standard Lot #/Exp. Date: _____

Indicator Lot #/Exp. Date: _____

Buffer Lot#/ Exp. Date: _____

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
	05/30/13	Carry out two replicates on the same sample. At least 10% of samples or one per batch are analyzed in duplicate every time analysis is performed. With every batch perform a blank, duplicate, QC and spike.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	01/14/2015	MSDS changed to SDS. Formatting and Quality Control revised.
	05/15/2015	Quality control section revised: Re-standardize reagents with each sample batch. Interference section revised: remove Chlorine by sample pretreatment.
	06/01/2016	Changed bench sheet and made revisions for clerical errors.
	12/14/16	Fixed bench sheet to include sample type, preservation option, titrant lot #, and method reference. Editted standard addition description to more conform to HACH 8226.
2	01/31/2017	Grammatical changes. Changes to procedure to reflect internal audit findings. Accumulation of minor changes under a single revision warrants a revision change.

Title: Sample Handling

P1. INTRODUCTION

1.1 This standard operating procedure (SOP) applies to all samples received by Ingenicomm, LLC Analytical Laboratory Services. Processes described in this method are derived from the TNI 2016 and ISO/IEC 17025:2017 Standards.

1.2 Safety is a major consideration with sample handling. The laboratory handles many different sample matrices, including those with biological and chemical hazards, as well as physical hazards (i.e., high pressure and cryogenic fluids). Safety considerations for each aspect of sample handling are discussed in each section below. Generally, laboratory employees are responsible for following requirements of the Chemical Hygiene Plan and applicable laboratory SOPs at all times.

P2. DEFINITIONS AND ACRONYMS

2.1 Aliquot – A single portion of sample

2.2 Chain of Custody Form – The document that contains all of the information relevant to a sampling event and documents the sample receiving event, where the customer relinquishes ownership of the stated samples to a laboratory employee.

2.3 EPA – Environmental Protection Agency

2.4 Holding time – The maximum time that can elapse between two specified activities (2016 TNI). Typically, this is the maximum elapsed time from sample collection to first use of a sample in a test.

2.5 IEC – International Electrotechnical Commission

2.6 ISO – International Organization for Standardization

2.7 Job Hazard Analysis (JHA) – A standardized approach for assessing and mitigating all potential hazards of an operation.

2.8 Mishandle – Mistreatment of sample that invalidates sample analysis.

2.9 Personal Protective Equipment (PPE)

2.10 Preservation - Method(s) used to maintain the integrity of a sample for a given analysis. Preservation methods can be physical, such as refrigeration, or chemical, such as addition of an acid to lower the pH.

2.11 Safety Data Sheet (SDS) – A standardized document, provided by material vendors, concerning toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions specific to their product.

2.12 Sample – A single sample matrix collected from the same location at the same time utilizing the same sampling technique.

2.13 Sample matrix – A sample type classified by a common characteristic across a given industry standard.

2.14 Sample transport - The transfer of sample containers to and from the laboratory.

2.15 SOP – Standard Operating Procedure

2.16 Temperature blank – A sample container (of representative size to the samples received) containing deionized or reagent water transported with a sample batch throughout the entire sample collection and transport process. A temperature blank can be opened and analyzed for temperature without needing custody of the sample batch being received.

2.17 TNI – The NELAC (National Environmental Laboratory Accreditation Program) Institute

2.18 WFF – Wallops Flight Facility

P3. COLLECTION

3.1 Samples collected by laboratory personnel shall be done according to an approved sampling plan. This sampling plan shall include a sampling procedure, required equipment, sample locations, and safety considerations (including PPE) requirements.

3.2 A job hazard analysis (JHA) is required prior to performing any new type of sample collection. JHAs for all laboratory activities are reviewed annually or more frequently, if new concerns arise in the course of laboratory activities.

3.3 Sample collection is thoroughly documented using all applicable fields on a laboratory Chain of Custody Form. Deviations from the sampling plan shall be included as a note on the Chain of Custody Form.

P4. TRANSPORT

4.1 Sample transport to and from the laboratory shall only be done by a licensed driver following all federal, state, and local regulations.

4.2 Samples shall be transported to the laboratory in such a way to safeguard their integrity and maintain preservation conditions. This includes the use of iced coolers, specialized containers, or even containers with bubble wrap, as appropriate.

4.3 Samples shall be transported with their corresponding Chain of Custody Form.

P5. RECEIVING

5.1 Samples received the laboratory shall be accompanied by a complete Chain of Custody Form. This form shall include the sample location, date and time of collection, collector's name, preservation, sample matrix, customer contact information, customer sample ID (when

provided), required testing, and special remarks, for every sample received. All writing on the Chain of Custody Form and containers shall be made in indelible ink (pen).

5.2 The customer and the laboratory employee receiving the sample must sign the Chain of Custody Form upon receipt. Customers requiring expedited turnaround time (faster than 10 business days) or other special considerations must note these requests, in writing, on the Chain of Custody Form. Laboratory management may then initial behind the notation, if feasible, indicating the request shall be met. If a request is not feasible for the laboratory, laboratory management shall contact the customer to determine alternatives that the laboratory can meet.

5.3 Minor errors in received sample documentation may be resolved by contacting the customer. These conversations are documented according to the laboratory's customer consultation procedures and cited on the Chain of Custody Form. The laboratory shall reject samples received with major documentation errors or deficiencies.

5.4 Meeting Commercial Customers at the Gate

5.4.1 Given the access restrictions to the WFF Main Base facility, sample receiving of commercial samples occurs in the parking lot directly adjacent to the guard shack entrance, accessed by making a U-turn after the guard shack.

5.5 Sample integrity is verified by laboratory personnel upon receipt. This includes examination of integrity of sample aliquots (i.e., containers closed, undamaged), adequacy of received sample volume, holding times, and preservatives.

5.6 Samples that are found unsuitable by any of the above criteria are rejected, unless otherwise requested by the customer. Sample rejection is communicated to the customer verbally, or in writing, and documented on the received Chain of Custody Form and the sample receiving record. Results of samples meeting any of the above criteria are issued with qualifiers.

P6. PRESERVATION

6.1 Samples requiring thermal preservation may be verified by temperature blank using an infrared thermometer; if no temperature blank is submitted, laboratory personnel shall perform direct verification of the received samples.

NOTE: Temperature blanks measuring below freezing (0°C) shall be verified by direct sample measurement or comparative analysis using another laboratory thermometer. In either case, substantiating documentation for such a temperature measurement is required.

6.2 Samples requiring chemical preservatives shall be verified according to the requirements of the reference method. In most cases, the reference method requires these preservatives be verified at Sample Receiving; however, methods such as EPA 1664 (Oil and Grease) actually discourage this verification step, as it may compromise the sample.

6.3 Clearly label sample containers with preservatives that may be considered hazardous, including the name of the preservative and indicators like "CAUTION" or "DANGER", as appropriate.

P7. SAMPLE LOG-IN

7.1 Upon receipt, samples information provided in Section 5.1 is entered into the appropriate sample receiving record (either the analytical or environmental sample receiving record). The employee entering this information initials the record and assigns the unique laboratory sample ID number according to the following criteria.

7.1.1 Sample ID numbers for samples requiring environmental testing are assigned in the following format: YY-xxxx, where YY is the last two digits of the current year, and the xxxx is a 4-digit incremental counter.

7.1.2 Sample ID numbers for samples requiring analytical testing are assigned in the following format: S-YY-xxxx, where S denotes the sample was received at the South Wing sample receiving location, YY is the last two digits of the current year, and the xxxx is a 4-digit incremental counter.

7.2 Using indelible markings, the sample ID number is recorded or affixed onto each received sample aliquot.

P8. HANDLING

8.1 Laboratory personnel shall use appropriate PPE, as prescribed by the Chemical Hygiene Plan or the specific method SOP, paying particular attention to PPE requirements for samples containing known hazards. Disposable PPE (i.e., gloves) should be changed between tasks, on an established frequency, or when suspected contamination occurs.

8.2 Samples are handled carefully to prevent accidental mishandle. Movement of multiple sample containers should always be done with a pushcart or cooler.

8.3 Samples that emit volatile vapors or dusts are handled under an external-venting fume hood.

8.4 Sample mishandles shall be reported to laboratory management. If the mishandle creates a hazardous situation, work is stopped and the hazard is addressed according to the Chemical Hygiene Plan. Mishandles are documented on the Chain of Custody Form and handled according to the laboratory's corrective action process.

P9. STORAGE

9.1 Water samples are typically stored in a sample refrigerator, unless the sample is to be analyzed immediately upon receipt. The laboratory has separate refrigerators for storage of potable and non-potable water samples. Potable (drinking water) samples may be stored in the same refrigerator as standards, as long as they are appropriately separated (i.e., by shelf, secondary containment).

9.2 Laboratory refrigerators should be cleaned on a weekly basis to minimize potential for sample contamination.

9.3 Samples received for analytical testing are considered stable at room temperature and may be retained at the sample receiving location until testing begins. Cylinders and filter apparatuses should remain in secondary containment to minimize the risk of unintentional impact.

9.4 The laboratory shall periodically ensure that sample labels are intact and that sample retention areas are kept organized

P10. REFERENCES

10.1 2016 TNI – The NELAC Institute, EL-V1-2016-Rev.2.1., *Volume 1 – Management and Technical Requirements for Laboratories Performing Environmental Analysis*

10.2 ISO/IEC 17025:2017 – International Organization for Standardization, ISO/IEC 17025 (Third Edition, 2017-11), *General requirements for the competence of testing and calibration laboratories*

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	09/1992	
A	12/2010	Additional information was added to meet National Environmental Laboratory Accreditation Program Standards
2	12/23/2011	
	10/22/2014	• Contract change from URS/EG&G to LJT & Associates, Inc. • MSDS changed to SDS. • Formatting revised.
3	01/31/2017	• Added interferences formerly listed in WI 24 and sample transportation guidelines into WI. • Major grammatical and formatting overhaul.
4	03/27/2017	Section 14.3 revised to include procedure on handling samples whose temperature blanks read below freezing.
5	01/23/2019	Grammatical syntax changes to increase clarity and completeness.
6	03/06/2020	• Change to Ingenicomm SOP format. • Reorganized SOP, as many of the headers including in these types of documents are not applicable (i.e., method performance) • Reworded document with “shall”, “should”, and “may” statements to be more consistent with the Quality Manual. • Changed TNI reference to 2016 edition and ISO/IEC 17025 to 2017 edition.

SOP-WI-23

File Retention and Archiving

Revision 5

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3/12/2020
Date

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3-12-20
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3-12-2020
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Effective Date: 03/12/2020

Title: File Retention and Archiving

P1. INTRODUCTION

1.1 This Standard Operating Procedure (SOP) applies to all documents and records ("files") associated with analyses conducted by Ingenicomm, LLC. Analytical Laboratory Services, including those not created in-house by the laboratory.

1.2 This SOP does not apply to NASA-owned environmental compliance records and documents retained in the laboratory. NASA-owned records shall be controlled according to GPR 1440.8B (or more current) and NASA-owned documents are controlled according to GPR 1441.1H and GPR 1441.2E (or more current).

P2. DEFINITIONS AND ACRONYMS

2.1 Document – Reference material written by or maintained by a laboratory as a part of its quality system. Documents can include books, standard operating procedures, and templates.

2.2 File – A collection of related information collated for future ease of access.

2.3 LIMS – Laboratory Information Management System

2.4 Record – A laboratory file detailing a specific laboratory activity. Records include all raw data, field notes, interim reports, master copies of all final reports, and quality records (i.e., reports from audits and corrective/preventive actions) generated by the laboratory. Records also consist of correspondence, observations, and interpretations relevant to the final report.

2.5 Retention schedule – The required period for which an archived file must be maintained.

2.6 SOP – Standard Operating Procedure

2.7 WI – Work Instruction

P3. PHYSICAL FILE RETENTION

3.1 The laboratory maintains both loose and bound volumes of physical records, as well as physical copies of its documents.

3.2 Bound volumes are maintained in good condition by laboratory personnel until the end of the calendar year or the record has reached its capacity (i.e., a logbook).

3.3 Completed records, generated by laboratory personnel, are submitted to the Laboratory Director and filed until the end of the calendar year. At the beginning of the next calendar year, completed records from the previous calendar year shall be archived.

3.4 The Quality Assurance Manager assures that only current revisions of laboratory documents are kept in circulation. Out-of-date revisions of documents shall be removed from issue locations, marked out-of-use, and filed until practical to archive.

P4. ELECTRONIC FILE RETENTION

4.1 The laboratory retains digital records and documents on the Chem Lab shared drive (\\wff-wicc.ndc.nasa.gov\wiccII\sow 7) and within the X-LIMS software (http://wff-enviro/xlims), hosted on the Environmental shared drive (\\wff-enviro\).

4.2 Access to on-site computer assets is restricted to personnel possessing a NASA Personal Identity Verification Smartcard. Specific share drives are only accessible to those with a specific business justification for access.

4.2.1 The Chem Lab shared drive is only accessible by laboratory staff and its management.

4.2.2 The "envir" subdirectory of the Environmental shared drive is accessible to both civil servant and contractor personnel supporting the Medical and Environmental Management Division at Wallops Flight Facility. The "xlims" subdirectory is more stringently restricted, requiring specific authorization by the IT contractor to access the web address and a user account created by laboratory management to access the system itself.

4.3 All electronic file spaces undergo partial backup once a day with full backup occurring once a week. Backups are retained both on-site and mirrored to a data center off-site.

4.4 Electronic records stored on the Chem Lab shared drive are subdivided in folders based on the year the record was created.

4.5 Current document revisions are stored in the root directory of their designated retention location, while out-of-date revisions are stored in an Archive subfolder.

4.6 Electronic files are retained in modern file formats to ensure continued accessibility. Any necessary file conversions from antiquated formats shall be documented.

P5. ARCHIVES

5.1 Physical files not required for day-to-day laboratory operations are bound with a cover sheet and plastic protective sheets and stored in such a way as to prevent untimely degradation. Each file shall be assigned a unique identifier, which references its assigned file box or retention location.

5.2 Archiving is done only by laboratory management. Files shall be organized so that information is readily retrievable (i.e., sorting by date or sample ID number).

5.3 When access to archived information is required by laboratory personnel, the borrower shall sign out only the pertinent documents to their task. External requests for archived laboratory files are addressed on a case-by-case basis, at the discretion of the Laboratory Director.

5.4 Each time a file is borrowed, the Laboratory Director shall fill out the pertinent information in the Archive Check-Out Logbook, including the date of the request, unique identifier, and file name. Both the borrower and the Laboratory Director initial the request, guaranteeing that no modifications of any kind will be made to the file while in the borrower's possession.

5.5 The file shall be returned to the laboratory by the borrower directly in the same state in which it was borrowed. The borrowed file is then examined by the Laboratory Director to ensure its condition and initialed as returned by both the Laboratory Director and the borrower. The file is then returned to its assigned location in the archives.

P6. RETENTION SCHEDULE

6.1 The laboratory shall retain records and documents required for the historical reconstruction of data (as required by V1M2 4.13.3.f of the TNI Standard) for a minimum of five years.

6.2 Filed records (i.e., reports, chain of custody forms) are retained for at least five years following the last record entry.

6.3 Method validation records and analytical SOPs shall be retained for five years following the last date the method was in-use.

6.4 Most electronic files are retained indefinitely, but shall be retained for no less than five years following the last modified date.

6.5 Specific files may be subject to longer retention periods. These retention schedules and the reasons for which they are established shall be documented.

P7. DISPOSAL

7.1 Laboratory management shall only dispose of records or documents after their established retention schedule. The laboratory shall retain documentation for records and documents disposed.

P8. REFERENCES

8.1 2016 TNI – The NELAC Institute, EL-V1-2016-Rev.2.1., Volume 1 – Management and Technical Requirements for Laboratories Performing Environmental Analysis

8.2 ISO/IEC 17025:2017 – International Organization for Standardization, ISO/IEC 17025 (Third Edition, 2017-11), *General requirements for the competence of testing and calibration laboratories*

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	04/2011	
A	12/23/2011	URS/EG&G Guidelines, revised to include NELAC criteria.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
2	01/31/2017	Grammatical changes. Increased specificity of archiving requirements per file type. Accumulation of minor changes under a single revision warrants a revision change.
3	11/06/2017	Grammatical changes to specify the scope of this SOP as both records and documents.
4	01/23/2019	Syntax changes to improve clarity. Clarified the laboratory's policy for document file storage and retrieval. Added explicit policy statements regarding electronic documents.
5	03/12/2020	• Change to Ingenicomm SOP format. • Reorganized SOP, as many of the headers including in these types of documents are not applicable (i.e., method performance) • Changed document title from "File Storage and Retrieval" to more aptly describe the SOP • Reworded document with "shall", "should", and "may" statements to be more consistent with the Quality Manual. • Changed TNI reference to 2016 edition and ISO/IEC 17025 to 2017 edition. • Removed ambiguous verbiage referencing a "lab administrator" • Migrated language from QM, requiring analysts using logbooks to maintain them in good condition • Migrated language from SOP-WI-26 to consolidate electronic file retention requirements.

SOP-WI-24

Laboratory Process and Control Guidelines

Revision 6

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6/7/19
Date

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Effective Date: 06/05/2019

SOP-WI-24

Laboratory Process and Control Guidelines

Revision 6

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Jason Freischlag		6-7-19	new revision
Mark Cuba		6-7-19	new rev.

Title: Laboratory Process and Control Guidelines

P1. IDENTIFICATION OF THE TEST METHOD

1.1 Laboratory Process and Control Guidelines

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMIT

N/A

P4. SCOPE AND APPLICATION

4.1 This standard operating procedure (SOP) applies to the LJT & Associates, Inc. Analytical Laboratory personnel and details the basic workflow and process control of laboratory activities.

P5. SUMMARY OF METHOD

5.1 This procedure describes the processes for providing analytical laboratory support activities including, obtaining samples and performing analyses/testing for customers requiring analytical services.

5.2 The LJT & Associates, Inc. Analytical Laboratory is responsible for ensuring compliance with drinking water and wastewater permits through sample collection, wet chemistry, microbiological testing, data reporting and filing, tracking requirements, and data evaluation. The Analytical Laboratory is also responsible for performing tests on various matrices such as oils, fuels, and gas commodities. Requests for new services are evaluated and developed when feasible.

5.3 It is the responsibility of lab personnel to track incoming samples, purchase equipment and materials, maintain equipment in good working order (including equipment calibration) and to ensure proper disposal of samples and hazardous waste materials produced or remaining after analytical testing. The lab prepares permit applications for the drinking water and wastewater systems. The lab tracks permit requirements and reports data to state agencies.

P6. DEFINITIONS

6.1 **Designee**: A staff member designated by a technical manager to handle day-to-day supervisory tasks in their absence. The role of the designee is given to a member of the laboratory either as a blanket appointment or on a per-absence basis.

6.2 **Lot Quality Control** (LQC): Quality control procedure performed on incoming commercially-prepared reagents to ensure usability.

P7. INTERFERENCES

7.1 Some interferences or potential problems that can disrupt the laboratory workflow arise from improper sample handling, storage, and preservation. See P7 of SOP-WI-22, *Sample Handling*, for more information.

7.2 Inadequate sample volume may prohibit the requested analyses from being performed.

7.3 Additional interferences, pertinent to specific analyses, are listed in the corresponding SOP.

P8. SAFETY PRECAUTIONS

8.1 When working with potential hazardous materials, follow U.S. EPA, OSHA and corporate health and safety procedures.

8.2 Additional safety precautions, pertinent to specific analyses, are listed in the corresponding SOP and the laboratory's Chemical Hygiene Plan.

P9. TOOLS, EQUIPMENT AND SUPPLIES

9.1 Due to the wide variety of sampling equipment in use by the laboratory, refer to P9 of each SOP for sampling and analysis equipment requirements.

9.2 Laboratory glassware and sample containers:

9.2.1 Only use container types that are compatible with the chemicals being used. Most aqueous solutions and almost all organic chemicals are compatible with glass containers; solutions of hydroxides and carbonates, however, are not recommended for long-term storage as these chemicals slowly etch glass. Glass can be used for short-term storage of such chemicals, but plastic containers should be used for long-term storage. The lids for reagent bottles containing corrosive chemicals should be plastic.

9.2.2 Keep reusable sample containers clean. See SOP-WI-34, *Dishwasher*, describing the use of the laboratory dishwashers, for a more detailed description of cleaning procedures.

9.3 Additional tools, equipment, and supplies required for specific analyses are listed in the corresponding SOP.

P10. REAGENTS AND STANDARDS

10.1 Purchasing

10.1.1 Laboratory supplies shall not be purchased from vendors not on the vetted, approved vendor list. This list is maintained through the Maximo purchase management software.

10.1.2 A request for supplies is made by a laboratory member by the following process. This process is identical to the laboratory's procedure for requesting services.

10.1.2.1 The laboratory member contacts the sales representative of an approved vendor to ensure they have the supply of interest in stock and to the required specification of the laboratory and the analytical method being performed. If the product is acceptable, the laboratory member requests a formal sales quote through email.

10.1.2.2 The laboratory member submits the sales quote to the laboratory purchase requisitioner, copying the Laboratory Technical Director and any other concerned parties.

10.1.2.3 A purchase requisition is prepared and the product is purchased according to the procedures outlined in LJT-SOP-400, *Procurement Practices and Guidelines*.

10.2 Reagents and consumables are not used until deemed acceptable for their intended purpose.

10.3 All purchased reagents and solvents are dated upon receipt. Additional date annotations should be made on reagent containers upon: reagent in-use, reagent out-of-use, LQC. Reagents and consumables that are commonly used by laboratory staff for environmental testing are tracked by a spreadsheet located on the Chem Lab Drive (\\WFF-WICC.NDC.NASA.GOV\wiccII\SOW 7\Chem Lab\WW-DW\Supplies Receipt Record). When the non-expired stock for any item in the inventory dips below a pre-determined empirical threshold, it is the responsibility of laboratory personnel to order additional supplies. Additional documentation can be found in the relevant test SOPs.

10.4 All reagents used for AIHA-LAP, LLC accredited work are assigned a unique identifier and logged into the Standard Logbook. This unique identifier should be handwritten onto each container of the specified reagent or, if implemented, bar coded.

10.5 The laboratory shall store reagents and consumables according to the manufacturer's and/or distributor's instructions such that their storage environment and other pertinent conditions does not lead to the untimely degradation of the reagent/consumable and to mitigate events such as cross contamination or reaction with incompatible substances.

10.6 Expired reagents are discarded as waste (P14.10). Some reagents may be used past their stated expiration date. Check the pertinent SOP for these requirements.

NOTE: Some expired reagents may be retained for non-conformance use (i.e., student interns). Such materials shall be clearly designated and labeled as such.

10.7 Reagents required for specific analyses are listed in the corresponding SOP.

10.8 Reagents used for microbial analyses should be acquired from manufacturers capable of sterile packaging in single-use containers.

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Samples should be collected using equipment and procedures appropriate to the matrix, the parameters to be analyzed, and sampling objective. The volume of the sample collected must be

sufficient to perform the analysis requested, accounting for the quality assurance/quality control requirements. Samples must be stored in the proper types of containers and preserved in a manner appropriate to the analysis to be performed.

11.2 Samples to be subcontracted must be sent in compliance with all United States Department of Transportation requirements.

P12. QUALITY CONTROL

12.1 Quality control requirements differ based per analysis performed and can be found in the corresponding SOP.

P13. CALIBRATION AND STANDARDIZATION

13.1 Instruments

NOTE: In the context of this work instruction, the terms “instrument” and “equipment” are used interchangeably.

13.1.1 Lab equipment that requires calibration is calibrated by an external entity annually (or as otherwise indicated) to establish traceability back to an International Standard (namely, NIST) and in accordance with GPR 8730.1K. Equipment is maintained and used in accordance with manufacturer recommendations and the pertinent work instructions.

NOTE: Transportation of instrumentation (i.e., when changing workspaces or equipment to be sent to subcontractors) should be done safely to mitigate lifting hazards and should be packaged as to ensure its safe arrival to the subcontracting laboratory.

13.1.2 Specific instrument calibrations are described in method SOPs where the instruments are used.

13.1.3 All maintenance activities (i.e., troubleshooting, repair, and recurring maintenance) and calibration records are retained within an Equipment Logbook. Additionally, calibration certificates are posted alongside calibrated instrumentation currently in-use.

NOTE: Hard copy laboratory bench sheets, where required, have spaces for both the analyst measurement and corrected measurements as stated on the applicable calibration certificate.

13.2 Pipettors

13.2.1 Pipettors used for accredited work are gravimetrically checked for accuracy and precision at three volumes with five repetitions on a quarterly basis. To be considered acceptable for use, the pipettor exhibits a bias less than 2% and a relative standard deviation less than 2% at each known volume checked.

13.2.2 Pipettors failing to meet the specifications of the previous section are taken out of service. Maintenance activites are performed according to the manufacturer’s specifications and recorded as in P13.1.3.

13.3 Hoods

13.3.1 All exhaust or fume hoods are checked annually for average face velocity with the sash open to the indicated mark.

13.4 Reagent Standardization

13.4.1 Some reagents require LQC procedures prior to use. Check the pertinent SOP for these requirements.

13.4.2 Standards shall be prepared from diluting certified reference materials with method-sterile diluents (i.e., Type I deionized water prepared according to SOP-WI-35).

13.4.3 Titrants, standards, and other solutions frequently used for analytical purposes must often be standardized against certified or traceable standards upon preparation. Method SOPs specify if standardization is necessary.

P14. PROCEDURE

NOTE: For ease of use, this section is organized in reading order according to the flowchart pictured in P23 of this SOP. Additional procedural steps are listed afterwards.

14.1 Sample Delivery

14.1.1 Samples are delivered to the laboratory either by facility tenants or, by commercial clients at the main entrance gate. These procedures are outlined in SOP-WI-22, *Sample Handling*. The chain of custody form, signed by both parties, signifies the binding contract of work.

14.1.2 The laboratory shall complete the analyses stated on the completed chain of custody form using accredited methods. Analytical results and any applicable acceptability statement shall be reported to the client on a Certificate of Analysis.

14.1.3 The client is expected to provide an accurate chain of custody for the samples requiring analysis. The client is responsible for remission of payment for results and interpretation provided by the Certificate of Analysis. Accuracy of customer provided information pertaining to listed results and acceptability statements is the responsibility of the client.

14.2 Permits, Schedules & Sampling Plans

14.2.1 The LJT & Associates, Inc. Analytical Laboratory provides consulting services to its clients to assist them in ongoing compliance with federal and state environmental and health regulations by maintaining a working knowledge of their permits and sampling plans.

14.3 Collect Scheduled Samples

14.3.1 Laboratory personnel are sometimes contracted to perform sample collection activities in addition to their analytical work.

14.3.2 Samples for drinking water compliance are taken according to SOP-WI-21, *Drinking Water Sampling*.

14.3.3 While not frequent, the laboratory is sometimes contracted to perform sample collection of waste water and other matrices. Such samples are taken in accordance with an established sampling plan.

14.4 Label & Log in

14.4.1 Sample login and container labeling are discussed in detail in SOP-WI-22, *Sample Handling*.

14.5 Sample Storage & Handling

14.5.1 Sample storage and handling are discussed in detail in SOP-WI-22, *Sample Handling*. Special handling requirements can be found in analytical and sampling SOPs.

14.6 Store & maintain materials

14.6.1 The procedures for initial reagent handling, as well as their maintenance, are discussed in P13.4.

14.7 Test Methods

14.7.1 The laboratory performs both accredited and unaccredited work. Procedures to complete all accredited work performed by the laboratory is explained, in detail, in a standard operating procedure.

14.7.2 Review of the aforementioned SOPs is required prior to completing ongoing demonstrations of capability and is recorded on the second page of the SOP Signature Sheet.

14.7.3 When a new revision of an analytical SOP is issued, all analysts competent in that field of testing must sign the new SOP Signature Sheet for that document for their demonstration of capability to still be valid.

14.8 Sample Analysis

14.8.1 Accredited procedures are performed by competent analysts (e.g., those meeting the applicable demonstration of capability requirements) according to the laboratory's SOPs. Test bench sheets are designed to capture all parameters essential to traceability by historical reconstruction (2009 TNI V1M2 4.13.13.f).

14.8.2 In limited circumstances, not all analysis requested on the Chain of Custody Form may be performed. For example, criteria for sample acceptance (discussed further in SOP-WI-22, *Sample Handling*) or a client may request an analysis be canceled. In these cases, the reason(s) for not performing work are documented on the Chain of Custody Form and all other work is still performed.

14.9 QA/QC Records

14.9.1 QA and QC records are generated as a direct result of laboratory activities (namely, P14.8).

14.9.2 QA records include corrective and preventive actions. These record types are discussed in greater detail in the laboratory's VELAP and AIHA-LAP, LLC quality manuals.

14.9.3 QC records include direct results of QC parameters and derived contents (namely, control charts). Control charts are maintained for all routinely performed analyses with method-mandated QC sample requirements.

14.10 Waste Disposal

14.10.1 Requirements for pollution prevention and waste management can be found in P17 and P21 of every laboratory SOP. General requirements can be found in the laboratory's Chemical Hygiene Plan.

14.11 Report Analysis

14.11.1 All accredited analyses performed by the laboratory are reported to the client. Methods of reporting are discussed in the following two subsections.

14.12 Lab Reports

14.12.1 The laboratory reports data according to the policies discussed in SOP-WI-42, *Data Reporting*.

14.13 Permits & Other Reporting Media

14.13.1 The laboratory is also contracted to prepare reports with analytical and client-provided parameters to state agencies (i.e., discharge monitoring reports).

14.13.2 As a courtesy to its clients, the laboratory provides preliminary notification of exceedance or sample mishandling to its clients. This reporting may be done by phone or by electronic mail.

14.13.3 The laboratory also reports the results of compliance drinking water analysis of public works to the Virginia Department of Health Office of Drinking Water.

14.14 Protocol Review

14.14.1 Electronic documents, in their original state, are maintained by laboratory management on the WFF file servers, in accordance with SOP-WI-26, *Data Management and Data Security*, section 14.5.

14.14.2 Laboratory staff shall review all documents pertinent to their areas of responsibility on an annual basis. Changes recommended by the laboratory staff shall be submitted through physical modification of the document's working copy with their initials and the modification date or via electronic mail.

14.14.3 The document name (or truncated name) and revision number shall be clearly denoted on every laboratory generated document.

14.14.4 Between revisions, all changes to a document made by laboratory management shall be tracked using the built-in 'Track Changes" feature in Microsoft Word.

14.14.5 A new revision of a document is issued as soon as practical to reflect necessary modifications. New documents are signed by laboratory management to approve for use and by laboratory staff to signify intent to comply with all policies implemented by the document.

14.14.6 All current documents shall be accurately listed on the Master List of Documents, which contains the document title, revision number and date, and the authorized retention location(s) (electronic hyperlinks and/or hardcopy locations).

14.15 Calibrate Equipment

14.15.1 Equipment calibration is discussed, in detail, in P13.1 of this SOP.

14.16 Other: Laboratory Record Retention

14.16.1 The laboratory generates both physical and electronic records in the performance of its workflow.

14.16.2 Physical record retention is discussed in SOP-WI-23, *File Storage and Retrieval*.

14.16.3 Electronic record retention is discussed in SOP-WI-26, *Data Management and Data Security*.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Data analysis and calculation procedures are detailed in all pertinent SOPs.

P16. METHOD PERFORMANCE

16.1 Method performance goals are detailed in all pertinent SOPs.

P17. POLLUTION PREVENTION

17.1 Pollution prevention criteria are detailed in all pertinent SOPs.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Data assessment and acceptance criteria for quality control measures are detailed in all pertinent SOPs.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 Refer to pertinent SOPs for measures requiring corrective action. The laboratory's corrective action processes are detailed in SOP-WI-30, *Control of Non-Conforming Work.*

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 Refer to pertinent SOPs for contingencies for dealing with out-of-control data. The laboratory's corrective action processes are detailed in SOP-WI-30, *Control of Non-Conforming Work.*

P21. WASTE MANAGEMENT

21.1 Waste management steps are detailed in all pertinent SOPs.

21.2 Waste chemicals and samples designated as hazardous waste are placed in the satellite accumulation areas in appropriately labeled containers. The WFF Environmental (Ext. 1718) is contacted to remove the hazardous waste from the lab and a completed Hazardous Waste Disposal Inventory form is included with the waste.

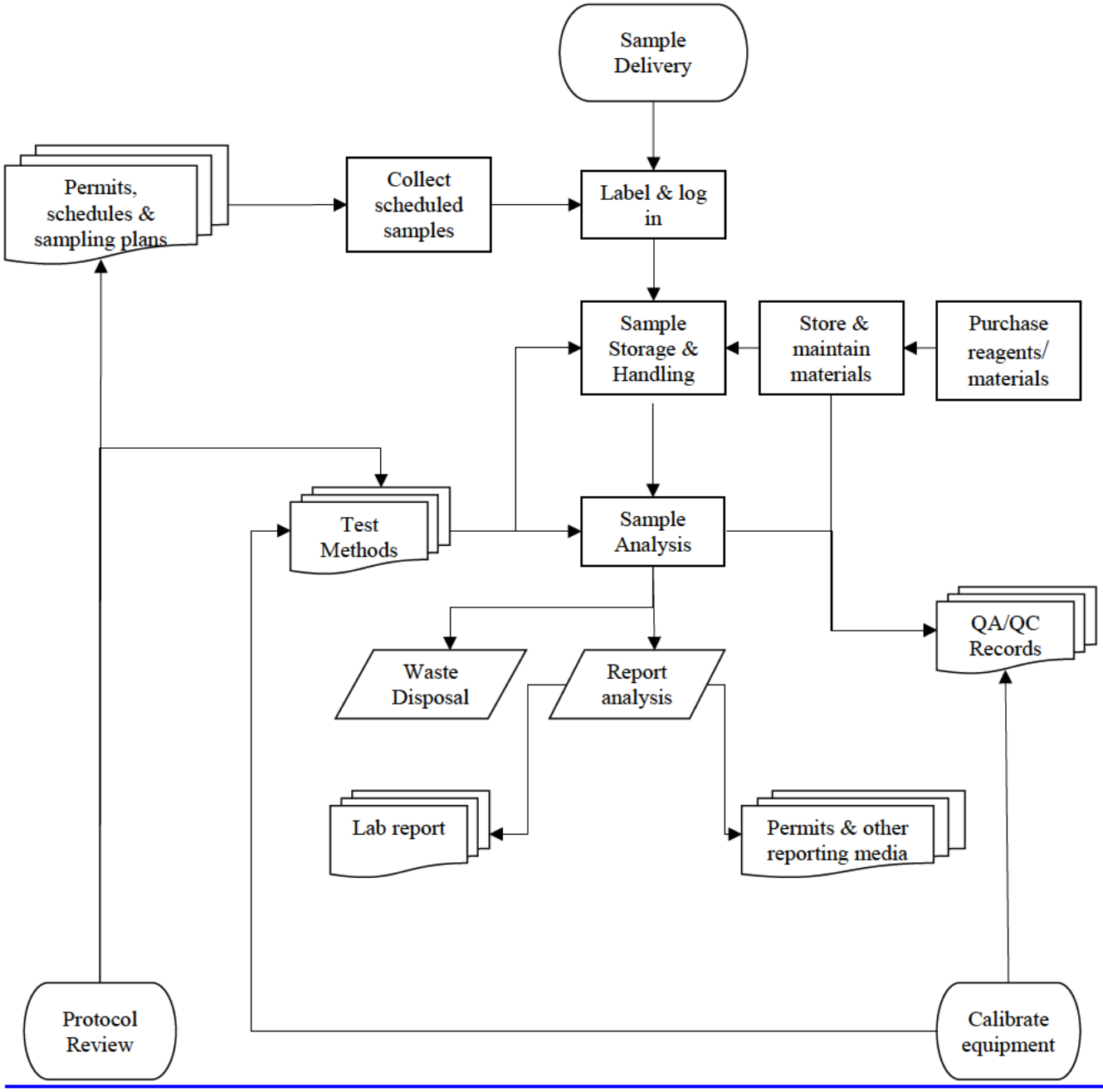
P22. REFERENCES

22.1 2009 TNI

22.2 AIHA-LAP, LLC Policy Modules

22.3 Standard Methods for the Examination of Water and Wastewater

P23. TABLES, DIAGRAMS AND FLOWCHARTS



CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	01/11	URS/EG&G Guidelines
2	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
	08/28/13	Sections lacked sufficient procedural detail. Sections were updated to reflect changes since the last revision.
	04/01/2014	Contract change from URS/EG&G to LJT & Associates, Inc.
	10/02/2014	Added method for pH test for alkaline detergent residue. Changed bromothymol blue to phenolphthalein.
3	01/31/2017	Updated file format. Moved sample handling interferences to SOP WI 22. Moved cleaning procedures to SOP WI 34. Updated flow chart to more accurately reflect process control. Accumulation of minor changes under a single revision warrants a revision change.
4	11/06/2017	Underwent AIHA-LAP, LLC audit review 10/13/2017. The SOP/WI lacked sufficient detail and sections were updated in 10.1, 14.1 and 14.2.
5	01/23/2019	Updated grammar and syntax to improve clarity. Further clarified laboratory reporting procedures in response to AIHA-LAP, LLC internal audit.
6	06/05/2019	Updated document review policies and procedures. Clarified laboratory policy on sample rejection and not performing work in response to a corrective action.

Title: Disposal of Materials as Hazardous Waste

P1. IDENTIFICATION OF THE TEST METHOD

To ensure proper and safe disposal of materials as hazardous waste.

P2. APPLICABLE MATRIX OR MATRICES

Not Applicable

P3. DETECTION LIMIT

Not Applicable

P4. SCOPE AND APPLICATION

This work instruction applies to laboratory personnel disposing of waste materials that are classified as hazardous waste.

P5. SUMMARY OF THE TEST METHOD

5.1 Chemical liquid wastes are generated and placed in a 5G Drum and stored in the F160 satellite accumulation areas: (E-113, E-114, E-115, E-116, E-108, and E-110).

5.2 Biological liquid wastes are generated in culture tubes. The media in these tubes are disinfected and thoroughly cleaned before disposing as trash.

P6. DEFINITIONS AND ACRONYMS

Analyst: the designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality. (NELAC)

Disinfection: To destroy most (but not necessarily all) of the harmful or objectionable microorganisms by means of chemicals, heat, ultraviolet light, etc.

Hazardous Waste: A waste that poses substantial or potential threats to public health or the environment.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Satellite Accumulation Area - An area for the storage of hazardous waste that is located at or near the point of waste generation.
Standard Operating Procedures (SOPs): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

P7. INTERFERENCES

7.1 Not Applicable

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory protocol for safety procedures.

8.3 Wear Personal Protective Equipment (PPE) such as lab coat, safety glasses, and gloves when performing hazardous waste disposal.

8.4 Refer to SDS sheets for all chemicals and reagents before commencing hazardous waste disposal for more safety information.

8.5 The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.6 Read the labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.7 Disinfect all inoculated tubes prior to disposal of bacteriological test waste materials. Be careful not to splash bleach on lab coat or clothing during disinfection.

8.8 Always put tops back on containers or drums when not in use.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- 5 gallon drum storage containers for Hazardous Waste accumulation

P10. REAGENTS AND STANDARDS

Not Applicable

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Chemical liquid wastes are generated and placed in a 5gallon drum and stored in the F160 satellite accumulation area.

P12. QUALITY CONTROL

12.1 All biological liquid wastes are disinfected before disposing as trash. The disinfection date is recorded on the appropriate laboratory bench sheet.

P13. CALIBRATION AND STANDARDIZATION

Not Applicable

P14. PROCEDURE

RECORDS, REPORTS AND FORMS

- Hazardous Waste Labels
- Hazardous waste disposal inventory form

INSTRUCTIONS**

14.1 Liquid hazardous waste will be placed in a container (typically 5-gallon) equipped with a lid or screw cap. (Liquid waste may include materials such as: samples, reagents, and used chemicals.) The container will remain closed at all times except during the transfer of waste to the container.

14.2 The container used to hold hazardous waste, if not in good condition (e.g., severe rusting, structural defects) or if it begins to leak, will be replaced with a container in good condition.

14.3 The container used to store hazardous waste will be compatible with the material to be stored so that the ability of the container to contain the waste is not impaired.

14.4 A “HAZARDOUS WASTE” label, with the constituents properly identified on the label, will be affixed to the container.

14.5 A funnel will be used to transfer liquid waste to the storage container when there is a potential for a spill to occur.

14.6 **NEVER COMBINE INCOMPATABLE MATERIALS/WASTES IN A CONTAINER.** Verify container content and compatibility by reading the label prior to placing any material in a waste container.

14.7 Hazardous waste will be stored in the Accumulation Area located in Building F-160, rooms E-113, E-114, E-115, E-116, E-108, and E-110 until containers are full.

14.8 When the hazardous waste container is full a hazardous waste disposal inventory form will be filled out (see attached example). The Environmental Office will be contacted (ext. 1718) and arrangements made to pick up the material.

14.9 When a solid hazardous waste is generated or a disposable gas cylinder is generated as waste, a hazardous waste disposal inventory form will be filled out. The Environmental Office will be contacted (ext. 1718) and arrangements made to pick up the material.

**In the event of an EMERGENCY (SPILL, FIRE, etc.) contact the FIRE DEPARTMENT at ext. 1333 or 911.

P15. DATA ANALYSIS AND CALCULATIONS

Not Applicable

P16. METHOD PERFORMANCE

Not Applicable

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. Dispose of the hazardous waste produced at the site where the work was performed, if the operating site has proper disposal available. If there is no disposal that meets regulatory requirements, the waste must be brought to the F-160 satellite accumulation area and transferred to the hazardous waste manager for disposal. The sample volume should be minimized during any analysis to reduce unnecessary waste.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

Not Applicable

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

Not Applicable

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Not Applicable

P21. WASTE MANAGEMENT

21.1 All waste must be handled and disposed of in accordance with state and federal regulations. After bottles and/or culture tubes have all been checked against logbooks for recording errors, dispose of bottles and/or culture tubes in accordance with Good Laboratory Practices. Check each Standard Operating Procedure which contains further instructions on hazardous waste disposal for each respective analysis.

22. REFERENCES

- 40 CFR 261-264: Hazardous Waste Identification; Storage, Handling, Disposal and Accumulation

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	01/11	URS/EG&G Guidelines
2	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	10/22/14	MSDS changed to SDS. Formatting revised.
3	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
	12/9/2019	Updated approvals. Change does not constitute updated revision number.

SOP-WI-26

Data Management and Data Security

Revision 6

Approval:

Marianne Smith — 1/23/2019

Environmental Programs Manager — Date

1-23-19

Laboratory Director — Date

1-23-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 01/23/2019

Title: Data Management and Data Security

P1. IDENTIFICATION OF THE TEST METHOD

Data Management and Data Security

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMIT

N/A

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the procedures employed by the LJT & Associates, Inc. Analytical Laboratory to ensure the integrity and security of laboratory data and the privacy of its customers.

P5. SUMMARY OF THE TEST METHOD

N/A

P6. DEFINITIONS

Designee: A staff member designated by a technical manager to handle day-to-day supervisory tasks in their absence. The role of the designee is given to a member of the laboratory either as a blanket appointment or on a per-absence basis.

P7. INTERFERENCES

N/A

P8. SAFETY PRECAUTIONS

N/A

P9. TOOLS, EQUIPMENT AND SUPPLIES

N/A

P10. REAGENTS AND STANDARDS

N/A

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

N/A

P12. QUALITY CONTROL

N/A

P13. CALIBRATION AND STANDARDIZATION

N/A

P14. PROCEDURE

14.1 Data Integrity

14.1.1 All employees of the LJT & Associates, Inc. Laboratory are held to high professional and ethical standards in the performance of their duties. All employees are required to read, understand and sign an 'Ethics Statement' attesting to their commitment to honesty and integrity in performance of their duties.

14.1.2 All employees are required to attend an annual ethics training and data integrity training class. The contents of this annual training include protocols for reporting ethics issues, examples of ethical violations, the consequences of unethical behavior, and additional resources to assist in clarifying the training content. The training is updated each year to ensure content relevance. Attendance of this training is verified by an attendance sheet.

14.1.3 Improper, unethical or illegal actions will be dealt with according to LJT & Associates, Inc. policies.

14.1.4 All employees are held accountable for the quality and integrity of the laboratory services they provide. This requires that employees accurately document all laboratory functions per the requirements of the laboratory's quality system documents to ensure data produced are of a known and reproducible quality.

14.1.5 All employees are required to obey all pertinent federal, state, and local laws and regulations in addition to LJT & Associates, Inc. company policies.

14.1.6 When a deviation from a documented policy occurs, including SOPs/WIs, analytical methods, QA/QC criteria, etc., the laboratory both notifies the client by phone or electronic mail and on the Certificate of Analysis under the Notes section or in a supplemental report indicating the deviation and the reasons for it.

14.1.7 All deviations from SOPs are reviewed by the QA Manager and any response actions required are delegated by the Technical Manager.

14.1.8 When mistakes occur in the course of physical recordkeeping (i.e., log sheets), each mistake is crossed out with one line, leaving it legible, and the correct value and initial of person making the correction and date are entered alongside.

NOTE: Obliteration in raw laboratory records undermines the goals of the laboratory's quality system, as it increases the ambiguity and decreases the defensibility of laboratory data.

14.1.9 When corrections are due to reason other than transcription errors, a notation justifying the correction is required.

14.1.10 Electronic bench sheets are protected to minimize accidental overwrite or deletion.

14.1.11 Where the identification of nonconformances or departures casts doubts on the laboratory's compliance with its own policies and procedures, or on its compliance with its accreditations, the laboratory shall audit the affected area(s) of activity in accordance with the applicable QM and document the investigation through its corrective action process (SOP-WI-30, *Control of Non-Conforming Work*).

14.1.12 With the transition of many laboratory services to electronic media, it is important to maintain accountability for electronic records. As such, it is essential that electronic work sheets used to record testing activities involving multiple analysts be digitally signed by all parties using NASA's Personal Identity Verification or notarized e-signature.

14.2 Data Retention on File Servers

14.2.1 Files and data are stored electronically on the NASA WFF servers associated with each work area that is password protected.

14.2.2 These documents are controlled in accordance with GPR 1410.1 and GPR 1410.2.

14.2.3 Obsolete records and documents shall be removed or clearly marked as obsolete and maintained as legacy content.

14.2.4 Laboratory Records shall be managed in accordance with relevant federal and state regulations, NPR 1441.1, NASA Records Retention Schedule and GPR 1441.8, Records Management.

14.2.5 Other records that relate to the operation of the Laboratory are identified as such on the NASA WFF computer server.

14.2.6 Backups of the database are performed Monday through Saturday evenings using a network backup system. One backup is conducted locally from the data center. After successful backups, the external disk array is stored locally and mirrored in an alternate

location. Detailed backup procedures can be requested through the ACES (Agency Consolidated End-User Services) program manager.

14.2.7 Direct access to laboratory-specific data on the NASA WFF computer server is restricted to laboratory staff and its management.

14.2.8 End-user access to the database is controlled through the compiled ACES web server (currently limited to read-only access of “public” data).

14.2.9 Users are required to login to the system using a NASA Personal Identity Verification Smartcard. Access is controlled by stratified access privileges assigned to each user. Appropriate access privileges are determined by laboratory management, based on job responsibilities, and assigned by the administrator of the respective file server.

14.2.10 Access to the NASA Virtual Private Network is required for data access of any kind.

14.3 Data Reporting

14.3.1 Reporting of final data is restricted to laboratory management or their designees.

14.3.2 These data are submitted solely to personnel authorized to receive laboratory data.

14.3.3 After data has been reported, it cannot be reported again in any modified state without reference to the original report.

14.4 The laboratory ensures confidential handling of its clients’ records by following the procedures outlined in the LJT & Associates, Inc. Confidentiality Policy (LJT Employee Handbook, Section 14).

P15. DATA ANALYSIS AND CALCULATIONS

N/A

P16. METHOD PERFORMANCE

N/A

P17. POLLUTION PREVENTION

N/A

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

N/A

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

N/A

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

N/A

P21. WASTE MANAGEMENT

N/A

P22. REFERENCES

2009 TNI
AIHA-LAP, LLC Policy Modules

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	04/11	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
2	10/24/12	Data Integrity Section added
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	03/24/15	Contract change from ODIN to ACES (Agency Consolidated End-User Services). Roles and responsibilities are changed to Program Manager and QA/QC Officer or Laboratory Technical Director.
3	01/31/2017	Minor grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
4	03/27/2017	Section 14.4.3 revised to emphasize the importance of cross out corrections in laboratory policy Section 14.4.5 revised to include protection of electronic spreadsheets as a good practice required for data integrity. Password protect formula cells in electronic benchsheets.
5	11/06/2017	Minor grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
6	03/15/2018	Restructured and reworded to improve clarity. Added requirements for electronic signature.

SOP-WI-27

Oxygen, Dissolved

Revision 2

Approval:

Marianne Sinha — 2-4-19

Environmental Programs Manager — Date

2-4-19

Laboratory Director — Date

2-4-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

Title: Oxygen, Dissolved

P1. IDENTIFICATION OF THE TEST METHOD

The DO determination measures the amount of dissolved (or free) oxygen present in water or wastewater.

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for determining the relative oxygen requirements of municipal and industrial wastewaters.

P3. DETECTION LIMIT

The method detection limit is 0.1 mg/L.

P4. SCOPE AND APPLICATION

This document will provide specific instructions for meter and probe preparation, probe reconditioning, calibration, dissolved oxygen measurement, and instrument storage.

P5. SUMMARY OF METHOD

The membrane electrode procedure utilizes a meter and electrode, and is based on the rate at which oxygen molecules diffuse (or pass through) a membrane covering a set of electrodes. The oxygen molecules react with an internal filling solution to develop a small electrical charge between the electrodes which can be read on a meter. The readings on the meter correspond directly to the amount of DO present in the sample.

P6. DEFINITIONS

Initial Demonstration of Capability (IDC): A procedure to establish the ability of the analyst to generate data of method acceptable accuracy and precision. (NELAC)

Duplicate (Dup): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7.0 INTERFERENCES

There are very few substances which will interfere with the DO method when utilizing the electrometric meter. Salinity caused by dissolved inorganic salts (such as sea water, estuaries, and industrial or manufacturing processes) can influence the probe's readings. Reactive compounds and gases (like hydrogen sulfide and other sulfur compounds) can interfere with the reading by reducing probe sensitivity. Chlorine residual can create a positive interference. Read the manufacturer's instructions for the care and handling of the DO electrode and meter for specific remedies, if any, for these interferences.

P8.0 SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follows all laboratory protocol for safety procedures.

8.3 Wear Personal Protective Equipment (PPE) such as lab coat, safety glasses, and gloves when performing dissolved oxygen analysis.

8.4 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- Dissolved Oxygen Meter
- Probe

P10. REAGENTS AND STANDARDS

- Internal membrane solutions (if required)
- Distilled Water

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Sample collection is done with clean glass or plastic bottles.

11.2 Measurement should be performed in the field at the time of collection. However, if measurement is performed in the laboratory, collect the samples in container with zero headspace. Avoid excessive agitation or prolonged exposure to air. Holding time is 15 minutes.

P12. QUALITY CONTROL

12.1 Initial Demonstration of Laboratory Capability

12.1.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

12.1.1.1 Prepare air-saturated water by bubbling air for at least 30 min. through 1500mL water that is at room temperature (± 2°C). **NOTE:** An in-line air filter must be used with the aeration tubing (i.e., cotton, glass wool, other suitable material).

12.1.1.2 Allow air-saturated water to equilibrate for 45 – 60 minutes.

12.1.1.3 Transfer aerated water to four clean BOD bottles, beakers or other suitable containers until overflowing, then sealed.

12.1.1.4 Analyze samples.

12.1.1.5 Use a D.O. table to calculate theoretical D.O. based on sample temperature and barometric pressure (or altitude correction factor applied). Results must be between 97-104% of calculated value.

12.1.2 Carry out four analyses using 100 mL samples for each.

12.1.3 Calculate the average and standard deviation of the values.

12.1.4 The standard deviation should be less than or equal to 20% of the average value. If it is not, evaluate the procedures to identify sources of error.

12.2 Duplicate Analysis

12.2.1 Carry out two replicates on the same sample matrix with each batch of 10 or fewer sample every time analysis is performed.

P13. CALIBRATION AND STANDARDIZATION

13.1 Follow the manufacturer's instruction for calibration of meters and probes.

P14. PROCEDURE

14.1 Condensation may collect on the membrane. This will interfere with the readings. Shake off excess water and gently blot dry with a laboratory tissue.

14.2 Record the dissolved oxygen reading from the display panel in mg/L.

14.3 Follow the manufacturer's instruction for further guidance on operating meters and probes.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 There are no calculations for this method. Read the concentration of dissolved oxygen in milligrams per liter directly from the meter of the instrument.

15.2 Report dissolved oxygen concentrations to the nearest 0.1 mg/L.

15.3 Reporting

15.3.1 Discharge Monthly Report (DMR)

15.3.2 If a duplicate sample is analyzed, the reported value for that sampling event is the average concentration of the sample and the duplicate for the reported value on the DMR.

P16. METHOD PERFORMANCE

16.1 The desired performance criteria for this measurement are:

16.1.1 Detection limit: 0.1 mg/L

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

Using most commercially available membrane electrode systems, an operator can obtain an accuracy of +/- 0.1 mg/L DO and a precision of +/- 0.05 mg/L DO.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 The samples can't be reanalyzed because of the 15 minute sample holding time.

P20. CONTINGENCIES FOR HANDLLING OUT-OF-CONTROL DATA

20.1 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

P21. WASTE MANAGEMENT

21.1 All wastes from these procedures shall be collected and disposed in accordance with state and federal regulations. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

Standard Methods for the Examination of Water and Wastewater, 22st Edition, 4500-OG

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	03/01/12	URS/EG&G Guidelines, revised to include NELAC criteria. Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	10/22/14	MSDS changed to SDS. Formatting revised.
2	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.

SOP-WI-28

Temperature

Revision 2

Approval:

Mariane Smith	2/4/19
Environmental Programs Manager	Date
	2-4-19
Laboratory Director	Date
	2-4-19
Quality Assurance Manager	Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

LJT & ASSOCIATES, INC. ANALYTICAL LABORATORY PROCEDURES AND GUIDELINES

SOP-WI-28

Title: Temperature

P1. IDENTIFICATION OF THE TEST METHOD

Temperature readings are performed during general laboratory operations and field methods.

P2. APPLICABLE MATRIX OR MATRICES

Not Applicable

P3. DETECTION LIMIT

The thermometer should have a scale marked for every 0.1^0 C, with markings etched on the capillary glass.

P4. SCOPE AND APPLICATION

Not Applicable

P5. SUMMARY OF THE TEST METHOD

Not Applicable

P6. DEFINITIONS

NIST = National Institute of Standards and Technology.

Correction Factor = the difference in the vendor-certified NIST traceable thermometer and the Observed Temperature measured by the thermometer

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

P7. INTERFERENCES

Allow the thermometer or equipment temperature sensor to equilibrate with the solutions before taking the temperature reading.

P8. SAFETY

8.1 If a mercury thermometer breaks, discard mercury thermometers appropriately.
8.2 Do not touch the liquid mercury with your hand.
8.3 Because of the properties of liquid mercury, it will tend to form "liquid beads" on a hard surface like a counter or floor. Use a mercury absorption sponge to collect the "beads."
8.4 Do not flush mercury down the drain.
8.5 Do not use a broom or vacuum to clean up mercury spills.
8.6 BE CAREFUL of broken glass fragments. After all the mercury is collected with the mercury absorbing sponge, a broom and dust pan can be used to clean up the broken glass. Dispose of the glass in an appropriate trash container.
8.4 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

P9. EQUIPMENT AND SUPPLIES

- Thermometers
- Instruments with temperature sensors

P10. REAGENTS AND STANDARDS

Not Applicable

P11. SAMPLE COLLECTION AND PRESERVATON

Thermometers can easily become damaged or out of calibration.
Take care to:

- Keep thermometers clean (follow manufacturer's recommendations).
- Carry thermometers in protective cases; thermometers and cases must be free of sand and debris.
- As an additional precaution on field trips, carry extra calibrated thermometers as spares, and a supply of batteries for instrument systems.

P12. QUALITY CONTROL

Thermometers are calibrated once a year by a company certified to perform calibration verification of thermometers. Once performed, thermometers may be used for one year.

P13. CALBIRATION AND STANDARIZATION

All thermometers are checked for accuracy using a NIST traceable thermometer.

Most instrument manuals state there is no calibration of the temperature sensor, but the temperature sensor must be checked to determine its accuracy. This accuracy check is performed at least once per

year and the accuracy check data/information is kept with the instrument. If the instrument contains multiple temperature sensors, each sensor must be checked.

All thermometers are calibrated annually.

P14. PROCEDURES

A label displaying the date of calibration and correction factor (even if it is zero), is placed around the top of the corresponding thermometer/equipment or attached with the thermometer/equipment.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 The Observed Temperature is the temperature reading of the thermometer.

15.2 The correction factor for the NIST thermometer is determined by the certified company that performs the calibration verification for this thermometer. The True Temperature measured by the NIST traceable thermometer = the Observed Temperature of the NIST traceable thermometer plus its correction factor.

15.3 The correction factor for the laboratory thermometer that is being calibrated against the NIST traceable thermometer is calculated by subtracting the Observed Temperature of the laboratory thermometer from the True temperature of the NIST traceable thermometer.

15.4 The True Temperature measured by the laboratory thermometer = the Observed Temperature plus the correction factor for that thermometer.

P16. METHOD PERFORMANCE

Not Applicable

P17. POLLUTION PREVENTION

Not Applicable

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

Not Applicable

P19. CORRECTIVE ACTION FOR OUT-OF-CONTROL DATA

19.1 When routinely recording temperatures for the laboratory, laboratory equipment, media etc., the observed temperature reading of the thermometer read plus the correction factor for that specific thermometer must be recorded.

19.2 On occasion, thermometers must be discarded (e.g., broken columns, unreasonably large correction factor). Contact the facility Safety, Health, and Environmental Management Program Manager for proper disposal procedures.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL FOR UNACCEPTABLE DATA

Not Applicable

P21. WASTE MANAGEMENT

Not Applicable

P22. REFERENCES

P23. TABLES, DIAGRAMS, FLOWCHARTS

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	03/01/12	URS/EG&G Guidelines, revised to include NELAC criteria. Latest version 21st ed. SM. No changes to the method. Updated reference - method title- to the latest Standard Method edition.
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
	10/22/14	MSDS changed to SDS. Formatting revised.
2	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.

SOP-WI-29

Chlorine – Pocket Colorimeter

Revision 4

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

11/6/2018
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

11-6-18
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

11-6-18
Date

LJT & Associates, Inc.

Effective Date: 11-6-18

Title: Chlorine – Pocket Colorimeter

P1. IDENTIFICATION OF THE TEST METHOD

Determination of free or total residual chlorine in drinking water and waste water by Pocket II Colorimeter by addition of DPD powder pillow.

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for the determination of free or total residual chlorine in a 10 mL water sample.

P3. DETECTION LIMIT

The desired detection limit for this method is 0.02 mg/L as Cl_2 with a limit of quantitation at 0.05 mg/L as Cl_2 and an upper limit of 2.00 mg/L as Cl_2. Dilution can be used to achieve results greater than this if necessary. If Actual limits of detection are required for accreditation, they will be determined through 40 CFR Part 136 Appendix B, Revision 2.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test method for the collection and analysis of water samples for the quantification of free or total residual chlorine in water or wastewater.

P5. SUMMARY OF THE TEST METHOD

The procedure described here meets the requirements of HACH 8021 for free chlorine and HACH 8167 for total residual chlorine, which are equivalent to the procedure of SM 4500 Cl - G, which describes the determination of chlorine using a DPD reagent, which causes a color change at 515 nm.

P6. DEFINITIONS

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Chlorine Residual: Chlorine remaining in a system after an allotted contact time.

Free Residual Chlorine: Chlorine residual dosed into a drinking water system as hypochlorite.

Laboratory Fortified Blank (LFB): A standard prepared from a certified reference material of a different lot than is used to for creation of the primary standard. The LFB is used as a second-source accuracy verification of the calibration curve.

Limit of Detection (LOD): The lowest analyte concentration that produces a signal significantly differentiable from the blank.

Limit of Quantitation (LOQ): The lowest analyte concentration that produces a signal significantly stronger than the blank, such that it can be detected with a specified level of reliability during routine operations.

Method Blank (MB): A sample of method appropriate pure water containing no target analyte that is taken through the entire sampling and analytical procedure.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Total Residual Chlorine: The sum of all free and unavailable (i.e., chloroamines) chlorine residuals.

P7 INTERFERENCES

7.1 Known interferences for these methods include extreme pH, high buffering capacity, manganese, chromium, and peroxide.

P8 SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

8.3 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.4 Always put tops back on containers when not in use.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- Pocket II Chlorine meter
- 10-mL sample cells

P10. REAGENTS AND STANDARDS

10.1 DPD Free or Total Chlorine Reagent Powder Pillows

10.2 Secondary Standard Kit, Chlorine DPD

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Analyze samples for chlorine immediately after collection. Chlorine is unstable in natural waters, as it is highly reactive.

11.2 Avoid plastic containers since these may have a large chlorine demand.

11.3 Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

11.4 If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark.

11.5 If analysis must be delayed, the maximum holding time for analysis is 15 minutes.

P12 QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for free and total chlorine obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.2.1 Sign in the proficiency test in the Sample Receiving Logbook. Be sure to record the chain of custody number on the sample container.

12.2.2 Follow the PT provider's included instructions for preparing the sample and analyze according to P14.

12.3 Initial Demonstration of Capability (IDC)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 The analyst should prepare four aliquots using the certified reference materials and sample containers provided by the qualified analyst according to the manufacturer's specifications.

12.3.4 The analyst then shall carry out the analysis of these four samples as per P14. The interpretation of the analyst's results is discussed in P18.

12.4 On-going Demonstration of Capability (ODC)

12.4.1 Analysts are required to re-verify their capability in this field of testing through the on-going demonstration of capability.

12.4.2 The analyst should prepare a single aliquot using a certified reference material and sample container according to the manufacturer's specifications.

12.4.3 The analyst shall carry out the analysis of this sample and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Method Blank (MB)

12.5.1 The laboratory shall perform analysis of a method blank from a "secondary chlorine standard kit" set once per day of analysis. The interpretation of the method blank is discussed in P18.

12.6 Laboratory Fortified Blank (LFB)

12.6.1 The laboratory shall perform analysis of three color standards from a "secondary chlorine standard kit" set once per day of analysis. The interpretation of the laboratory fortified blank samples is discussed in P18.

12.7 Duplicates

12.7.1 If analyzing for accredited compliance testing, the laboratory shall (and may otherwise) perform analysis of a duplicate sample aliquot once per day of analysis. The interpretation of duplicate samples is discussed in P18.

P13. CALIBRATION AND STANDARDIZATION

Pocket II colorimeters are factory calibrated and do not require recalibration unless recurring quality control failures occur.

P14. PROCEDURE

14.1 Turn on the Pocket II Colorimeter using the middle power button.

14.2 Analysis of Secondary Standard Set

14.2.1 Put the clear vial into the colorimeter and hold the lid perpendicular in the etched grooves to prevent ambient light interference.

14.2.2 Press the blue button to zero the colorimeter.

14.2.3 Remove the blank vial from the colorimeter and reset it into the colorimeter according to P14.2.1.

14.2.4 Press the green button to measure the standard. Record the measurement on the chlorine field log sheet.

14.2.5 Place each of the remaining three standards into the colorimeter according to P14.2.1 and analyze the standard by pressing the green button. Record each measurement on the chlorine field log sheet.

14.3 Collecting Field Sample (see P11.4)

14.4 Measuring Free Chlorine

14.4.1 After collecting sample aliquot, zero the colorimeter, as in P14.2.1 and P14.2.2, by pressing the blue button using the unreacted sample.

14.4.2 Add the free chlorine DPD powder pillow to the sample aliquot and swirl gently to dissolve the reagent.

14.4.3 Measure the free chlorine in the sample as in 14.2.3 and 14.2.4. Record the sample measurement on the chlorine field log sheet.

14.5 Measuring Total Chlorine

14.5.1 After collecting sample aliquot, zero the colorimeter, as in P14.2.1 and P14.2.2, by pressing the blue button using the unreacted sample.

14.5.2 Add the total chlorine DPD powder pillow to the sample aliquot and swirl gently to dissolve the reagent.

14.5.3 After three minutes, measure the total chlorine in the sample as in 14.2.3 and 14.2.4. Record the sample measurement on the chlorine field log sheet.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Chlorine (RPD)

15.1.1 The relative percent difference between a sample and its duplicate.

15.1.2 Applicable Formulas

15.1.2.1 $RPD(X_1, X_2) = \frac{200*|X_1 - X_2|}{X_1 + X_2}$,
where X_1 and X_2 are results of samples analyzed in duplicate

15.2 Chlorine (Average)

15.2.1 The arithmetic mean of all aliquots analyzed during an initial demonstration of capability.

15.2.2 Applicable Formulas

15.2.2.1 $\bar{x} = \sum \frac{x_i}{n}$,
where X contains n values x_i

15.3 Chlorine (LCL)

15.3.1 The arithmetic mean minus a standard deviation. Used to assess acceptability of an analyst's IDC.

15.3.2 Applicable Formulas

15.3.2.1 $s_x = \sqrt{\sum \frac{(x-\bar{x})^2}{n-1}}$,
where X contains n values x_i

15.3.2.2 $LCL = \bar{x} - s_x$

15.4 Chlorine (UCL)

15.4.1 The arithmetic mean plus a standard deviation. Used to assess acceptability of an analyst's IDC.

15.4.2 Applicable Formulas

15.4.2.1 $s_x = \sqrt{\sum \frac{(x-\bar{x})^2}{n-1}}$,
where X contains n values x_i

15.4.2.2 $UCL = \bar{x} + s_x$

P16. METHOD PERFORMANCE

16.1 Accuracy: See Secondary Standard Set Certificate of Analysis

16.2 Precision: ± 20% RPD

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Analysts should review all data and calculations for correctness.

18.2 P12.2: Proficiency Test

18.2.1 Proficiency test results should fall within the range established by the provider for the analyte of interest.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Chlorine (Average) should fall within the acceptance range specified by the provider for the analyte of interest.

18.3.2 Chlorine (LCL) should be greater than or equal to the lower acceptance limit specified by the provider for the analyte of interest.

18.3.3 Chlorine (UCL) should be less than or equal to the upper acceptance limit specified by the provider for the analyte of interest.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 Chlorine measurement should fall within the acceptance range specified by the provider for the analyte of interest.

18.5 P12.5: Method Blank

18.5.1 Chlorine method blank values should generally fall below half the method limit of quantitation.

18.5.2 Chlorine method blank values at or above the limit of quantitation require immediate corrective action prior to continuing analysis. Chlorine measurements associated with a method blank meeting these criteria should be invalidated.

18.6 P12.6: Duplicates

18.6.1 Chlorine (RPD) should be less than 20%. Exceedance of this limit may be deemed acceptable for those chlorine measurements below the method limit of quantitation.

18.7 The completed bench sheet will be reviewed by the QA/QC Officer

18.8 If the tests over ranges dilute the sample with a known volume of high quality, chlorine demand-free water and repeat the test. Some loss of chlorine may occur due to the dilution.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 The samples can't be reanalyzed because of the 15 minute sample holding time.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

P21. WASTE MANAGEMENT

21.1 All wastes from these procedures shall be collected and disposed in accordance with state and federal regulations. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste

21.2 Outdated chemicals are disposed in accordance with state and federal regulations.

P22. REFERENCES

- HACH 8021 and HACH 8167
- Standard Method 4500-CL G

P23. TABLES, DIAGRAMS AND FLOWCHARTS

Attachment 1: Black Pocket Colorimeter Sheet

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/91	
A	12/23/11	URS/EG&G Guidelines, revised to include NELAC criteria.
	04/01/14	Contract changed from URS/EG&G to LJT & Associates, Inc.
	10/22/14	MSDS changed to SDS. Formatting revised.
2	11/21/16	Revised to reflect changes to new instrument DR3900
3	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
4	11/6/2018	Overhaul from parent document SOP-WI-19 Rev 3 to remove focus from DR 3900 and emphasize the use of the Pocket II colorimeter. Grammatical changes for increased clarity and content changes to more accurately reflect the requirements of the parent HACH methods as well as Standard Methods. SOP now applicable to Free and Total Residual Chlorine.

SOP-WI-30

Control of Non-Conforming Work

Revision 5

Marianne Simko
Ingenicomm, LLC
Environmental Programs Manager

1-8-2020
Date

Jason Freischlag
Ingenicomm, LLC
Laboratory Director, Technical Manager

1-8-20
Date

Mark Culver
Ingenicomm, LLC
Quality Assurance Manager

1-8-2020
Date

Ingenicomm, LLC

Effective Date: 01/08/2020

SOP-WI-30

Control of Non-Conforming Work

Revision 5

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Mark Culver	[illegible]	1-8-2020	new
Marianne Simko	Marianne Simko	1-8-2020	new
Jason Freischlag	[illegible]	1-8-2020	new revision

Title: Control of Non-Conforming Work

P1. IDENTIFICAITON OF THE TEST METHOD

1.1 Control of Non-Conforming Work

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMIT

N/A

P4. SCOPE AND APPLICATION

4.1 This standard operating procedure (SOP) applies to all accredited processes used by Ingenicomm, LLC Analytical Laboratory Services to generate results.

P5. SUMMARY OF METHOD

5.1 This procedure described here establishes the authority and responsibility hierarchies used applicable to non-conforming work. Examples of the authorities described herein are those authorized to halt and resume analytical testing or procedures related to nonconformities and to invalidate test results.

P6. DEFINITIONS

6.1 **Nonconformance**: A departure of a quality characteristic from its intended level or state that occurs with a severity sufficient to cause a service not to meet a specification requirement.

P7. INTERFERENCES

N/A

P8. SAFETY PRECAUTIONS

N/A

P9. TOOLS, EQUIPMENT AND SUPPLIES

N/A

P10. REAGENTS AND STANDARDS

N/A

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

N/A

P12. QUALITY CONTROL

N/A

P13. CALIBRATION AND STANDARDIZATION

N/A

P14. PROCEDURE

14.1 Identification of nonconformities can occur at many levels within the quality system. Examples include customer complaints, failing quality control parameters, instrument problems, sample mishandles, environmental conditions, faulty reagents, observations, management reviews and audits. When a nonconformity is detected, laboratory data is held until the disposition of the data (P14.4) is determined through the corrective action process.

14.2 All nonconformities are documented as corrective action plans. Depending on the nonconformance, some corrective action plans may initiate the laboratory's corrective action process while others just document remediative actions already performed by the laboratory. All corrective action plans are assigned a unique identifier upon review by the Quality Assurance Manager in the following format: CAPYYZZZ, where "YY" is the last two digit of the calendar year, and "ZZZ" is a three digit numerical counter.

14.3 Corrective Action Process

14.3.1 Stop work in the affected area of work. All employees have the authority to initiate this step.

14.3.2 Identify the nonconformance and notify laboratory management.

14.3.3 Identify the immediate steps taken in response to the nonconformance.

14.3.4 Determine the root cause of the nonconformance.

14.3.5 Determine the corrective actions required to resolve the nonconformance and most likely to prevent recurrence.

14.3.6 Approval of the corrective action plan by the Quality Assurance Manager, Laboratory Director, and Environmental Program Manager.

14.3.7 Assign responsible personnel to perform corrective action steps (management).

14.3.8 Authorization to resume work by the Technical Manager or Laboratory Director.

14.3.9 Specify documents and records that demonstrate laboratory's compliance with the corrective action plan.

14.3.10 After time has passed, review corrective action plan to ensure implemented corrective actions have corrected the problem, identified in P14.3.3. If previously listed deadlines or actions are no longer reasonable (i.e., workload shift, equipment failure), corrective action plans can be updated, at the discretion of the Quality Assurance Manager, by either handwriting an amendment or by issuing a revised corrective action plan, entirely.

14.3.11 Corrective action plans that are reviewed and verified resolved after six months may be closed by the Quality Assurance Manager and the Environmental Programs Manager.

14.4 Non-releasable data is not approved by the Laboratory Director (or the responsible program's Technical Manager) until product disposition has been determined and documented. Possible dispositions for non-conforming work are: rework, redo, use as-is, and unusable.

14.4.1 "Rework" products have sufficient holding time and sample volume remaining to repeat the processes affected by the non-conformance.

14.4.2 "Redo" products do not have sufficient holding time and sample volume remaining to repeat the processes affected by the non-conformance; however, when the non-conformance was identified, the client specified that the sample analysis should be done on a newly procured sample aliquot.

14.4.3 "Use as-is" products do not have sufficient holding time and sample volume remaining to repeat the processes affected by the non-conformance and resampling is not an option. The results of these products are qualified on the final Certificate of Analysis, clearly explaining the non-conformance.

14.4.4 "Unusable" products do not have sufficient holding time and sample volume remaining to repeat the processes affected by the non-conformance and resampling is not an option. However, the severity of the nonconformance is such that the laboratory questions the quality of the data and will not report the result.

14.5 More thorough review processes should be performed on all analyses in affected areas of work immediately following nonconformities.

14.6 When necessary, the customer is notified within 72 hours of the nonconformance and specifications may be changed depending on the usage of the data, for example, informational purposes only (use as-is). The Laboratory Director may need to recall reported results, depending on the severity of the nonconformance.

14.7 A customer supplied sample which is lost, damaged or otherwise unsuitable will be annotated in the Chain of Custody and Certificate of Analysis and reported to the customer verbally or electronically (unusable/redo).

14.8 If properly executed, quality control parameters can monitor the various aspects of data quality on a routine basis. In instances where performance falls outside acceptable limits, the data

produced can be questioned and, after investigation, a determination made as to its validity. The laboratory's internal quality control program is the principal recourse available for ensuring that only a quality product is released. Quality control parameters and quality assurance elements are further defined in the laboratory's quality manual.

P15. DATA ANALYSIS AND CALCULATIONS

N/A

P16. METHOD PERFORMANCE

N/A

P17. POLLUTION PREVENTION

N/A

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

N/A

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 See 14.3.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

N/A

P21. WASTE MANAGEMENT

N/A

P22. REFERENCES

22.1 2009 TNI V1M2 4.9

22.2 AIHA-LAP, LLC Policy Modules

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	07/12	
	04/01/14	Contract change from URS/EG&G to LJT & Associates, Inc.
1	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.
2	03/27/2017	Section 14.5 added requirement for qualifiers to be used when reporting non-conforming work as is. Also updated reference to use the 2009 TNI standard.
3	03/15/2018	Grammatical and structural changes to improve clarity. Added numbering schema for CAPs.
4	06/05/2019	Added policy that laboratory shall revise corrective action plans when deadlines are deemed unreasonable due to changes in laboratory workflow.
5	01/08/2020	Lab ownership updated to Ingenicomm, LLC. Migrated language from QM to applicable sections. Integrated feedback from SOP working copies.

SOP-WI-31

Autoclave

Revision 1

Approval:

Marianne Sinh 2-4-19

Environmental Programs Manager Date

2-4-19

Laboratory Director Date

2-4-19

Quality Assurance Manager Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

SOP-WI-31
AUTOCLAVE OPERATION

Title: Autoclave

P1. IDENTIFICATION OF THE TEST METHOD

Proper protocol for autoclave disinfection of wastewater and drinking water samples after analysis has been completed, prior to disposal.

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for all bacteriological samples after analysis has been completed, prior to disposal.

P3. DETECTION LIMIT

Not Applicable

P4. SCOPE AND APPLICATION

Samples of drinking water and wastewater are tested by incubation. The result of these analyses is the cultivation of various bacteriological organisms. The resulting media and containers are unsafe to dispose of in their current state. This Work Instruction is provided to emphasize correct processes and procedures to be followed to assure consistent quality and safe handling of waste generated by these tests. In addition, this method will ensure proper autoclave procedures are followed to disinfect waste products in a safe and secure manner.

P5. SUMMARY OF THE TEST METHOD

5.1 Check chamber to ensure racks are empty and chamber is relatively clean.

5.2 Attach draining hose to nozzle on front of instrument.

5.3 Turn nozzle counterclockwise while keeping draining hose in position above waste bucket.

5.4 Empty reservoir into waste bucket.

5.5 Flush reservoir into waste bucket with Deionized Water (DI) water while nozzle is open.

5.6 Close nozzle clockwise and remove draining hose.

5.7 Fill reservoir with DI water to the fill line, denoted on reservoir lid.

5.8 Insert samples into red biohazard autoclave bags, then into autoclave chamber.

5.9 Close front door of autoclave and turn front locking mechanism arm clockwise to seal.

5.10 Press “start” button.

P6. DEFINITIONS AND ACRONYMS

Analyst: the designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality. (NELAC)

Disinfection: To destroy most (but not necessarily all) of the harmful or objectionable microorganisms by means of chemicals, heat, ultraviolet light, etc.

Standard Operating Procedures (SOPs): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

P7. INTERFERENCES

Only use deionized water when operating the autoclave. Other types of water/liquid may leave residue and deteriorate the interior of the autoclave chamber.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 All samples should be treated as hazardous. Safety glasses, gloves, and lab coats are to be worn.

8.2 Be careful not to open chamber door until pressure has been reduced. This could produce steam that may burn skin.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- Tuttnauer 3545E Autoclave Sterilizer
- Biohazard bags
- Autoclave tape
- Lab Coat
- Disposable Rubber Gloves
- Safety Glasses
- Incubator capable of maintaining a temperature of 60°C.
- Drain Bucket

P10 REAGENTS AND STANDARDS

- Deionized Water
- Biological Indicators

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Not Applicable

P12. QUALITY CONTROL

Quality Control using BTSure Biological Indicator (*Geobacillus stearothermophilus*)

12.1 Remove BTSure ampules from the box. It is recommended that two are run per QC check, along with a positive control.
Note: For the positive control, start this procedure at 12.7

12.2 Place the ampules in a horizontal orientation in with a load representative of normal usage in an area where lethality is least likely, if possible.

12.3 Run cycle, as in 14.2.

12.4 Remove load from autoclave, allowing it to cool for at least 10 minutes.

12.5 Retrieve test ampules from the load.

12.6 Observe autoclave tape on the ampule labels. A blue color indicates an unexposed unit, while a green color indicates proper exposure.

NOTE: If a black color is observed, the results of that ampule are not to be considered.

Incubation period

12.7 Set microbiological incubator to 60°C.

12.8 Activate the media by gently squeezing the ample with plyers to break the glass ample.

12.9 Incubate ampules that passed the prerequisite of 12.6 for 24 hours.

Positive Control

12.10 To ensure the BTSure Biological Indicator is not defective, a separate ampule should be incubated.

Interpretation of Results

12.11 Examine the ampule for a yellow color change. If no color change occurs within 24 hours, the sterilization procedure is considered adequate.

Positive Results

12.11.1 Yellow color ampule indicates a positive result and sterilization was not adequate. Refer to owner's manual and/or call the manufacture for troubleshooting.

P13. CALIBRATION AND STANDARDIZATION

Not Applicable

P14. PROCEDURE

RECORDS, REPORTS AND FORMS

14.1 This model is equipped with a printer. Every run will produce a printout with details of the run. These printouts are to be filed in the designated logbook labeled "Autoclave Printouts."

INSTRUCTIONS

14.2 Sterilization by Autoclave

14.2.1 Check chamber to ensure racks are empty and chamber is relatively clean. If there is visible rust, deterioration, or damage, maintenance will be required before proceeding.

14.2.2 Attach draining hose to front nozzle, located at the bottom left of the front of the instrument. Position hose to point directly into drainage bucket.

14.2.3 While retaining hose position, turn nozzle counterclockwise to open drain valve. Allow draining until reservoir is completely empty.

14.2.4 Flush reservoir with DI water for a few seconds. Once flush has completed, close drainage valve and remove draining hose.

14.2.5 Fill reservoir with DI water to fill line on reservoir cap. Be sure not to overfill or under fill reservoir. The fill line is roughly above the heating element coil inside the reservoir tank. Place reservoir cap on top of reservoir. Be sure it is positioned correctly so it rests level with the instrument.

14.2.6 Insert an appropriate amount of waste sample containers into red biohazard autoclave bags.

14.2.7 Place filled autoclave bags on the racks inside the autoclave chamber. Be sure to leave some space in the chamber for steam to enter and disinfect the waste product.

14.2.8 Close chamber door and place locking mechanism into forward position.

14.2.9 Turn sealing handle clockwise until hand tight. Be sure not to turn the handle too tightly as this could wear down the sealing gasket.

14.2.10 Make sure the “Door Closed” light is on and the “Add Water” light is off.

14.2.11 Press the “Start” button. There should be a loud “whirring” sound a few seconds after initializing.

14.2.12 Once the cycle is complete, a loud “beep” will ring for 4 seconds. Let the instrument settle for at least 30 minutes following the cycle to relieve pressure.

14.2.13 Once the pressure gauge has reduced significantly, relieve pressure by turning the sealing handle counterclockwise a few full turns. If gratuitous amounts of steam are emitted, back away from instrument.

14.2.14 After pressure is relieved, open chamber and remove bags. **CAUTION.** Bags will most likely be very hot and may cause burns if not handled carefully. Avoid this by pulling the trays out and allowing time for the samples to cool.

14.2.15 Place autoclaved bags into an appropriate disposal container.

14.2.16 Remove printout from the front of the instrument. File this accordingly in the designated “Autoclave Printouts” log book.

14.3 Cleaning the Autoclave system

14.3.1 Set sterilization temperature to 134°C and sterilization time to 5 minutes.

14.3.2 Pour the contents of one pack of Chamber Brite down the center of the Autoclave chamber.

14.3.3 Run Autoclave by following steps outlined above in 14.2.

14.3.4 After cycle is complete and system has cooled to a safe temperature, drain water basin into waste bucket.

14.3.5 Wipe inside of chamber with tech wipes.

14.3.6 Flush water basin and refill.

14.3.7 Run system again with just water at 134°C for 5 minutes again.

P15. DATA ANALYSIS AND CALCULATIONS

Not Applicable

P16. METHOD PERFORMANCE

Not Applicable

P17. POLLUTION PREVENTION

Appropriate ventilation during the instrument cycle is required to prevent any potential olfactory pollution. Proper disposal of disinfected media is required to prevent potential bacteriological pollution.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

Not Applicable

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

Not Applicable

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Not Applicable

P21. WASTE MANAGEMENT

The wastes generated in this method are not hazardous. They can be discarded in the following manner: the sample containers can be discarded in the laboratory trash. Likewise, all water waste can be dumped down the sink.

P22. REFERENCES

- BTSure Biological Indicator use of Steam Sterilization Test method in package insert.

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	01/17/13	
	04/01/14	Contract change for URS/EG&G to LJT & Associates Inc.
	10/13/15	New equipment. Procedural change.
1	01/31/2017	Grammatical changes. Accumulation of minor changes under a single revision warrants a revision change.

SOP-WI-34

Dishwasher

Baseline

Approval:

Marianne Smith 2-4-19

Environmental Programs Manager Date

2-4-19

Laboratory Director Date

2-4-19

Quality Assurance Manager Date

LJT & Associates, Inc.

Effective Date: 2/1/2019

Title: Dishwasher

P1. IDENTIFICATION OF THE TEST METHOD

Proper cleaning of plastic and glassware used for metals, microbiological, and fuels testing.
Instructions for operating an industrial dishwasher.

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for all plastic and glassware used in metals, microbiological, and fuels testing after analysis has been completed. To ensure effective cleaning of glassware and plastic sampling containers, use an automatic industrial dishwasher. Proper cleaning will help prevent cross-contamination of sampling containers re-used in field and laboratory studies.

P3. DETECTION LIMIT

Not Applicable

P4. SCOPE AND APPLICATION

Plastic and glassware are used for analyzing various metallic contents, microbiological contents, fuel quality, and sampling. The containers used for storage, measuring, sampling, and other quality controls must be cleaned specifically to remove any metals, microbiological matter, organic compounds, and fuel residue from their interior and exterior surfaces. This document will provide specific instructions for operation of the industrial dishwasher to clean glassware and sampling containers used in field and laboratory studies. It is important to clean the glassware and equipment after each use.

P5. SUMMARY OF THE TEST METHOD

Laboratory plastic and glassware are placed in the proper dishwasher, located in rooms E114 and E110. The dishwashers are run with deionized water and the appropriate detergent.

P6. DEFINITIONS AND ACRONYMS

Analyst: the designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality. (NELAC)

Standard Operating Procedures (SOPs): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

P7. INTERFERENCES

Only use deionized water when operating the dishwasher. Only use the appropriate detergent based on the type of analysis the plastic and glassware was used to perform. Other types of water/liquid and detergents may leave residue or may not remove all metallic, biological, organic, or fuel residue from the containers.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 All sample containers and detergents should be treated as hazardous. Safety glasses, gloves, and lab coats are to be worn.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- Labconco FlaskScrubber dishwasher (located in E114)
- VWR dishwasher (located in E110)
- Carboy for holding deionized water, attached to each dishwasher unit

P10 REAGENTS AND STANDARDS

- Deionized Water
- Citrajet® low foaming acid detergent
- Tergajet® detergent
- RBS Solid detergent

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Not Applicable

P12. QUALITY CONTROL

12.1 Quality control is performed with a pH indicator on glassware after each run is complete to check for detergent residue. The type of indicator used is based on the type of detergent used in the cleaning process.

12.1.1 Use phenolphthalein as pH indicator for dishwasher loads washed with Tergajet® or RBS solid detergent.

12.1.2 Use bromothymol blue as pH indicator for dishwasher loads washed with Citrajet®.

12.2 Procedure for quality control on glassware

12.2.1 Remove a piece of glassware from the dishwasher after the cycle has been completed.

12.2.2 Place 3-5 drops of indicator in glassware in different locations. If indicator changes color, detergent residue is still present and there may be an issue with the dishwasher unit. See user manual or contact manufacturer to determine issue and solution.

12.2.3 Record results in dishwasher quality control logbook.

P13. CALIBRATION AND STANDARDIZATION

Not Applicable

P14. PROCEDURE

14.1 Be sure to empty containers into respective waste containers or down the drain, based on contents (see appropriate SDS for waste disposal). Rinse with water or solvent before loading, if visible residue is present. If the items to be washed were used to collect water with a high percentage of sediment and particulate matter or were used for soil, it may be helpful to pre-soak the equipment/containers in a tub of warm soapy water to loosen the dirt. Some pieces may also require scrubbing with a bottle brush.

14.2 Check and empty drain filter trap for loose paper and stickers that may have washed off containers on previous runs.

14.3 Make sure the carboy on top of the lab counter has a water level that is greater than 2/3 the maximum volume before running the dishwasher to ensure that it will not empty out during the run.

14.4 For dishes used in metals, fuels, or oils analyses, use only the dishwasher located in room E114. For dishes used in biological analyses, use only the dishwasher located in E110.

14.5 Load dishes into the dishwasher ensuring that the front facing door will have room to close, the spinning water jets are not impeded by tall glassware, and the dishes are arranged in a way that will allow an adequate amount of water and detergent to wash over and through them.

14.6 Put the specific detergent into the detergent holding area.

14.6.1 **FOR PLASTIC AND GLASSWARE INVOLVED IN ANY <u>METALS</u> ANALYSIS ONLY USE <u>Citrajet®</u> low foaming acid detergent**

14.6.2 **FOR PLASTIC AND GLASSWARE INVOLVED IN ANY <u>BIOLOGICAL</u> ANALYSIS ONLY USE <u>Tergajet®</u> detergent.**

14.6.3 **FOR PLASTIC AND GLASSWARE INVOLVED IN ANY <u>FUELS OR OILS</u> ANALYSIS ONLY USE <u>RBS Solid</u> detergent.**

14.7 Consult the individual dishwasher user manual for instructions regarding running the specific machine.

14.7.1 The automatic timed cycle contains a pre-wash, wash, rinse and final rinse.

14.7.2 Once all cycles have finished, open the door and remove the items. If they do not look clean, leave them in and repeat steps. Place dishes on drying racks.

14.7.3 Once dry, plastic and glassware should be stored in appropriate location.

P15. DATA ANALYSIS AND CALCULATIONS

Not Applicable

P16. METHOD PERFORMANCE

Not Applicable

P17. POLLUTION PREVENTION

Regard appropriate Safety Data Sheet (SDS) to determine proper disposal of any wastes. All wastes from these procedures shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

Not Applicable

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

Not Applicable

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Not Applicable

P21. WASTE MANAGEMENT

The wastes generated in this method are not hazardous. Regard appropriate SDS to determine proper disposal of any wastes.

P22. REFERENCES

Not Applicable

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	02-25-16	
	01-31-2017	Annual Review. No changes.

SOP-WI-35

Deionized Water System

Revision 1

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

12/9/19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

12-19-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

12-9-19
Date

LJT & Associates, Inc.

Effective Date: 12/09/2019

Title: Millipore DI System

P1. IDENTIFICATION OF THE TEST METHOD

Using the Milli-Q® Direct 8 Millipore deionized water system for general purposes.

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for using the Milli-Q® Direct 8 Millipore deionized water systems located in the laboratory rooms E108 and E114.

P3. DETECTION LIMIT

Not Applicable

P4. SCOPE AND APPLICATION

Deionized water is used in this laboratory for making quality control solutions, dilution of samples, and cleaning. This water must be acquired from the Milli-Q® Direct 8 Millipore deionized water systems located in rooms E108 and E114. The purpose of this work instruction is to describe the procedure to retrieve deionized water from these systems.

P5. SUMMARY OF THE TEST METHOD

Water is taken from the system spout and certain parameters are recorded in the Milli-Q® Direct 8 Millipore deionized water system Log Book, located next to the system.

P6. DEFINITIONS AND ACRONYMS

Analyst: the designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality. (NELAC)

Standard Operating Procedures (SOPs): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

P7. INTERFERENCES

Not Applicable

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Consult user manual, located next to the system, for safety precautions and warning notes.

P9. TOOLS, EQUIPMENT, AND SUPPLIES

- Milli-Q Direct 8 Millipore deionized water system
- Replacement parts and filters for Milli-Q Direct 8 Millipore deionized water system
- HACH Ammonia, Mid Range Test Kit Model NI-8
- See WI-14 Colilert® Presence/Absence

P10 REAGENTS AND STANDARDS

Not Applicable

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Not Applicable

P12. QUALITY CONTROL

12.1 Once daily, when system is used, record readings displayed on the screen of the system for total organic compounds (TOC), conductivity, and temperature in the Quality Control Log Book.

12.2 Once monthly, perform a quality control check for ammonia on the deionized water from the system. Follow the manufacturer's instructions for the HACH Ammonia Mid Range test kit. Treat the quality control deionized water as a laboratory sample. Record the results in the Quality Control Log Book.

12.3 Once monthly, perform a quality control check for presence/absence of total coliform and E. coli on the deionized water from the system. The procedure is located in the Work Instructions manual under the title "WI-14 Colilert® Presence / Absence." Treat the quality control deionized water as a laboratory sample. Record the results in the Quality Control Log Book.

12.4 Once annually, perform a quality control check for chlorine on the deionized water from the system. This analysis can be done as stated in "WI-19 TR Chlorine" or "WI-29 Chlorine – Pocket Colorimeter". Record the results of this analysis on both the laboratory chlorine benchsheet and the date of the analysis on the laminated sheet located near the deionized water system.

12.5 Once annually, perform a quality control check for copper on the deionized water from the system. This analysis should be sent out to a certified subcontractor to be performed. Retain a copy of the analytical report in the Quality Control Log Book. Record the date of the analysis on the laminated sheet located near the deionized water system.

P13. CALIBRATION AND STANDARDIZATION

Not Applicable

P14. PROCEDURE

INSTRUCTIONS

14.1 ACQUIRING A SPECIFIC VOLUME OF DEIONIZED WATER

14.1.1 Press the down arrow key on the keypad until the selection “Volume” is highlighted. Press the right arrow key.

14.1.2 Use the up and down arrow keys until the desired volume is displayed on the screen. Press the “√” (check mark) symbol button and the volume of deionized water will be dispensed.

14.2 ACQUIRING A NONSPECIFIC VOLUME OF WATER

14.2.1 To acquire a nonspecific volume of water automatically dispensed, quickly click and release the blue plunger located on the dispensing arm. This will cause water to flow from the hose attached to the system spout until the analyst quickly clicks and releases the plunger again.

14.2.2 To acquire a nonspecific volume of water manually dispensed, click and hold the plunger until the desired volume of deionized water has been dispensed. Once the plunger has been released, the water will cease to dispense.

14.3 GENERAL MAINTENANCE AND REPLACING PARTS

14.3.1 When the system light is glowing yellow or red, some routine maintenance is required for the analyst to perform on the system. On the main menu screen, press the down arrow key until the bottom line is highlighted. Press the right arrow key for a description of the error. Follow the instructions on the system. Consult the user manual, located next to the system for detailed instructions on how to remedy the specific error.

P15. DATA ANALYSIS AND CALCULATIONS

Not Applicable

P16. METHOD PERFORMANCE

Not Applicable

P17. POLLUTION PREVENTION

Not Applicable

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Temperature of water used for making standards should be between 17°C and 23°C.

18.2 TOC should be less than 5ppb.

18.3 Ammonia should be less than the limit of quantitation for the laboratory.

18.4 Conductivity should be 200µS/cm.

18.5 The results of the Colilert® Presence/Absence test should be negative for both total coliform and E. coli.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 If temperature, TOC, ammonia, presence/absence, or conductivity results are out of acceptance range, sample and test again.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 After retesting, if the results remain out of acceptance range, consult user manual and/or contact manufacturer.

P21. WASTE MANAGEMENT

The wastes generated in this method are not hazardous. They can be discarded in the following manner: the used parts can be discarded in the laboratory trash. Likewise, all water waste can be dumped down the sink.

P22. REFERENCES

- "Milli-Q® Direct 8/16 System User Manual" Millipore Corporation. 2010. http://pdfs.wolflabs.co.uk/service/Millipore_Water-Purification_Milli-Q-Direct_Manual.pdf

P23. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

Not Applicable

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	02/29/16	
1	03/27/2017	Section 12.0 updated to include annual copper and chlorine checks.
	12/09/2019	Updating authorizing signatures. Does not constitute revision change.

SOP-WI-39

Use of Discrete and Composite Autosamplers

Baseline

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

12/9/19
Date

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12-19-19
Date

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12-9-19
Date

LJT & Associates, Inc.

Effective Date: 12/09/2019

Title: Use of Discrete and Composite Autosamplers

P1. IDENTIFICATION OF THE TEST METHOD

Standardized instruction for the operation of the HACH AS950 and the HACH SD900 portable autosamplers when collecting surface water.

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMITS

N/A

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes how to collect grab samples at a specified interval using a discrete autosampler and time based composite samples using a composite autosampler.

P5. SUMMARY OF METHOD

This SOP describes the procedural use of the HACH AS950 and HACH SD900 portable autosamplers, which includes sample container, sample tubing, power, calibration, and programming setup as well as diagnostic steps and sample handling tips for performing time weighted composite and discrete sampling.

P6. DEFINITIONS

Equipment Blank: A field quality control sample prepared by passing deionized or method-appropriate sterile water through field equipment. Equipment blanks are submitted to the laboratory and are processed as a regular sample. Analysis of an equipment blank is done when equipment sterility and effectiveness of decontamination procedures are called into question.

Trip Blank: A sample prepared with deionized or method-appropriate sterile water to accompany the field samples at all times. Trip blanks are processed as regular samples. Analysis of a trip blank is done when sample integrity in transit to the laboratory is called into question.

Field Sample Duplicate: A sample collected from the same setting as the field sample; that is, at the same time and location. A field duplicate is processed as a regular sample. Field duplicates are taken to access bias in sampling technique and analytical procedures and are processed periodically as a proper laboratory quality assurance program.

Automated Sampler (**"Autosampler"**): A portable unit that can be programmed to collect discrete samples or composite samples based on time or flow.

Base flow: The portion of water flow not associated with a rain event.
Grab Sample: A sample taken as the result of a single sampling event.

Composite Sample: An amalgamation of grab samples taken over a longer period of time as a function of flow or time. Composite samples are used to determine typical loadings or concentrations of analytes of interest. Composite samples taken with the SD900 autosampler are taken as a constant volume/constant time.

P7. INTERFERENCES

7.1 Large suspended solids can block the strainer; it is advisable to cover the strainer with a fine mesh or cloth that won't interfere with any analyses performed on collected samples.

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures.

8.2 Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory protocol for safety procedures.

8.3 Wear Personal Protective Equipment (PPE) such as hip waders, disposable nitrile gloves, and steel toed boots when performing sampler setup.

8.4 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information.

8.5 The setup of these autosamplers can involve exposure to stormwater that may contain live microorganisms and therefore pose some threat of infection. Personnel are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands as soon as possible and before consuming any food or beverages.

8.6 Field work can incur hazards such as exposure to sample preservative (see 8.4), falling, and heavy lifting. Use good judgement in the field and do not work alone.

P9. EQUIPMENT AND SUPPLIES

- Auto-sampler
- 2.5 gallon sample jug (composite sampler)
- 24 1 liter HACH AS950 bottles (discrete sampler)
- Sample preservatives (HNO_3 ,H_2SO_4, NaS_2O_3, etc. as needed)
- Plastic storage bags for sample jug lids
- Field Notebook
- Chain-of-Custody (COC) Forms
- Zip ties
- Water quality instrument and probe (pH/conductivity/temperature meter and probe).
- Stainless steel inlet strainer.

- Power source (deep cycle RV/Marine battery, rechargeable battery, or solar-powered batteries) \
- Connector cables (not necessary for rechargeable battery)
- Pre-cleaned Teflon suction intake tubing
- Steel Toed Boots
- Nitrile Gloves

P10. REAGENTS AND STANDARDS

- 2% HNO_3 (Nitric Acid) for use in clean techniques bottle/jug cleaning
- Tergajet
- DI Water (or equivalent reagent water)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Refer to P14 for sampling procedures.

11.2 Holding time: review the holding time for each parameter from the relevant SOP.

11.3 Preservation: review the preservation techniques for each parameter from the relevant SOP.

P12. QUALITY CONTROL

12.1 Trip Blank

12.1.1 If the transportation of samples to the laboratory is suspected in the causation of poor analytical results, a trip blank should be prepared.

12.1.2 Prepare a trip blank by sampling deionized or equivalent reagent grade water in the same type of container as the autosampler takes, including secondary containment.

12.1.3 Upon retrieval of the autosampler, use the same transportation procedures for both the field samples and the trip blank.

12.1.4 Submit trip blank along with field samples for analysis of analytes of concern.

12.2 Equipment Blank

12.2.1 If the sterility of the sampler is suspected in the causation of poor analytical results, an equipment blank should be prepared.

12.2.2 Prepare an equipment blank by sampling the same volume of deionized or equivalent reagent grade water that will be sampled in this field sampling event with the same autosampler and tube setup.

12.2.3 Submit equipment blank along with field samples for analysis of analytes of concern.

12.3 Field Sample Duplicate

12.3.1 Take additional sample from the same sampling event into a different sample container and submit to laboratory for analysis.

P13. CALIBRATION AND STANDARDIZATION

13.1 HACH AS950 Portable Sampler - The ideal volume calibration settings have been pre-determined through a calibration series.

13.1.1 Navigate to “Calibration” using the arrow keys on the controller and press “Select” (the top right button).

13.1.2 Press “Select” again on the “Volume” option.

13.1.3 Scroll down to “User Adjust”.

13.1.4 The screen will now display a scroll bar. The number below the scroll bar should be between 30 and 32%. If it isn’t, use the left and right arrow keys to make the proper adjustment.

13.1.5 To navigate to the main menu, press the top right button to save the calibration settings and press the top left button twice to go back.

13.2 HACH SD900 Portable Sampler - The volume calibration of this sampler must be done periodically to ensure accurate sample retrieval volume. The sampler should be calibrated to the volume used for the sampling event.

13.2.1 Navigate to “Program Setup” using the arrow keys on the controller and press “Enter” on the bottom right of the controller.

13.2.2 Scroll down to “Modify Selected” and press “Enter”.

13.2.3 Scroll down to “Liquid Sensor”, press “Enter”, scroll to “Enable”, and press “Enter”. This will now allow the sampler to undergo volume calibration.

13.2.4 Press the “Volume Calibration” button on the sampler controller. It is on the top line of buttons and is the 3rd button from the left.

13.2.5 Remove the tube leading from the pump to the fitting on the front of the sampler controller. Place the tube in a graduated cylinder with the appropriate volume of sample matrix water (or equivalent).

13.2.6 Select “Start” and press “Enter”.

13.2.7 Once the sampler has collected the sample, select “Done” and press “Enter”.

13.2.8 If the volume collected is different than the volume measured in the graduated cylinder, specify the actual volume collected using the arrow keys to navigate and press “Enter”. The sampler should now be calibrated to accurately sample the specified volume to a reasonable accuracy and precision level.

P14. PROCEDURE

14.1 Bottle Setup

14.1.1 To access the bottle setup inside the autosampler, pull the top component of all three of the lower latches down towards the lower metal fastening on the case.

14.1.2 The connection should be loosened such that the hook falls out of the lower metal fastening; if this does not happen, a light pull should easily break the connection.

14.1.3 Lift the top controller piece up by using the two handles positioned oppositely on the top half of the autosampler. The bottom stage should remain on the work surface.

14.1.4 HACH AS950 portable sampler

14.1.4.1 There should be 24 bottles in a circular arrangement inside the now exposed bottom portion of the autosampler. If there are bottles set up already, skip 14.1.6.

14.1.4.2 Position the provided 24 1 Liter bottles in the grooves on the inside of the autosampler; the tall cylindrical edge with the container mouth should be placed against the interior wall of the bottom stage.

NOTE: On a sticker inside the autosampler, the position of “Bottle #1” is marked. The distributor arm will start placing sample in Bottle #1 and move in a clockwise motion. This is useful when sampling during an event where chronological order is important, like monitoring changes during and after the course of an event such as a rocket launch.

14.1.4.3 Place the circular protective piece over the inner edge of the bottles and secure it using the 3 elastic loops on the bottom of the stage.

14.1.5 HACH SD900 portable sampler

14.1.5.1 There should be a single 2.5 gallon container inside the now exposed bottom portion of the autosampler. If the bottle is already present, skip to step 14.1.6.

14.1.5.2 Position the provided 2.5 gallon bottle in the center of the autosampler.

14.1.6 Place the top controller back on top of the stage. The orientation of this is important; the widest metal fastening on the bottom stage and the screen of the controller should be aligned.

14.1.7 Refasten the latches by hooking the metal fastening to the bottom component of the latch and push the top component of the latch up.

NOTE: The springs in the latches are not worn on the AS950 discrete sampler and may hurt to latch if your fingers are positioned incorrectly.

14.2 Tubing Setup

14.2.1 General Setup Guidelines

14.2.1.1 Before use, determine the diameter and length of the tubing to be used. This is essential for accurate sampling.

14.2.1.2 Attach one end of the tube to the node on the front of the controller and route it through the plastic guarder on the left side of the controller.

14.2.1.3 Attach a strainer to the free end of the tubing, if not already done. This will ensure that a minimal amount of large solid material makes it into the pumping apparatus.

14.2.1.4 Suspend the free end of the tubing into the source water you wish to sample. Ensure that the remaining tubing is not convoluted extensively or tangled as this will affect the precision of sample volume collected. Also be sure that the tubing is not on the bottom of the source water unless the strainer is mostly unobstructed.

14.2.2 Outfall Setup

14.2.2.1 Follow the steps provided in 14.2.1.1 through 14.2.1.3.

14.2.2.2 Attach a piece of cheesecloth to the exterior of the strainer using a zip tie. This will guarantee the absence of large suspended particles, which can interfere with tests such as Total Suspended Solids.

14.2.2.3 Attach a strainer to the free end of the tubing, if not already done. This will ensure that a minimal amount of large solid material makes it into the pumping apparatus.

14.2.2.4 Attach the strainer end of the tube to the neck of a buoyant, narrow-necked bottle about 2 inches before the previous zip tie; this will prevent tidal fluctuations from lodging the strainer into the floor of the outfall.

14.2.2.5 Using a zip tie, loosely attach the bottle/strainer setup to a long PVC pipe about an inch upstream from the last zip tie. The bottle should be able to move up and down on the PVC pipe.

14.2.2.6 Lodge the PVC/bottle/strainer setup under the surface of the outfall floor with the strainer just below the surface of the water.

14.2.2.7 Ensure that the remaining tubing is not convoluted extensively or tangled as this will affect the precision of sample volume collected.

14.3 Power Setup

14.3.1 Check to make sure the controller is plugged into a battery. On the left side of the controller, there is a charging port labeled “Power” with a diagram of a battery. If there is an attachment already, skip to the next step.

14.3.1.1 Find a viable power source. It will be a 12 volt battery or a solar power controller and a large battery. They both will have the same connector used to connect to the autosampler: a circular cord with three receivers on the inside labeled A, B, and C.

14.3.1.2 To connect the power source to the autosampler, align the three receiving ports in the cord with the three prongs on the controller’s power port ensuring the B connection is on bottom and simply plug it in. In order to secure the connection, it is advised to use the screw fasten provided on the cord, tightened hand tight only. Do not overtighten.

14.3.1.3 If using a small 12 volt battery, there are two clips on the back of the controller that are designed with the battery to fasten it to the case. To do this, simply pull the rubber fastenings up and slide them into the wedges in the battery case.

14.3.2 To power on the autosampler, press the power button on the bottom middle of the controller's screen. The screen should illuminate with a menu.

14.3.3 Before going out into the field, ensure that the battery is fully charged by plugging the battery into the HACH wall charger until the charge light is no longer yellow, in the case of a HACH battery.

14.4 Calibration Verification

See Section 13.

14.5 Programming

The autosamplers have many potential functions but the ones in this operating procedure are designed around perchlorate sampling of an outfall up until a day after a rocket launch. In order to accomplish this, some preset settings have been determined; however, depending on the application, some settings are changeable in this procedure without affecting the base functionality. In the following lists, the ideal settings for each sampler are listed with the customizable options in italics.

HACH AS950 Portable Sampler:

Template: **Custom Single**
Total Bottles: **24**
Bottle Volume: **1050 mL**
Tubing: 15.0 ft, 9.5 mm (3/8") (Depends on the specifications of the tubing actually used)
Pacing: **Time Weighted,** *1 hr*
Sample Volume: *1000 mL*. **Fixed**
Distribution: **Time Based,** Duration: *1 hr*, First Bottle: *1*
Program Start: *Immediately, on run*
Program End: **Time Duration**: *23 hrs*

HACH SD900 Portable Sampler:

Bottles: **1,** *9500 mL*
Intake Tubing: 25 ft, 3/8" Vinyl (Depends on the specifications of the tubing actually used)
Program Delay: *Enable, Time/Date, hh:nn mm/dd/yy*
Sample Pacing: **Time Paced**, *1 hour, Immediately,* **End After Last**, *24*
Liquid Sensor: **Enable**
Sample Volume: **350 mL**
Intake Rinses: **0**
Sample Retries: **0**
Site ID: *0000…1*
Adv Sampling: **All disabled**

14.5.1 HACH AS950 Programming – Menu Navigation

14.5.1.1 Navigate to “Programming” on the main menu and press the top right button to select it.

14.5.1.2 Scroll down to “Sample Programming” using the up and down arrow keys and select it using the top right button.

14.5.1.3 Scroll through the various options (listed above) using the up and down arrow keys to scroll through menu options and adjust numerical values such as times and measurements, the left and right arrow keys to scroll horizontally through numbers, the top left button to go back, and the top right button to confirm your selections.

14.5.2 HACH SD900 Programming – Menu Navigation

14.5.2.1 Navigate to “Program Setup” on the main menu and press the “Enter” button to select it.

14.5.2.2 Scroll down to “Modify Selected” using the up and down arrow keys and select it using the “Enter” button.

14.5.2.3 Scroll through the various options (listed above) using the up and down arrow keys to scroll through menu options and adjust numerical values such as times and measurements, the left and right arrow keys to scroll horizontally through numbers, the bottom left button (“Back”) to go back, and the bottom right button (“Enter”) to confirm your selections.

14.5.3 Program Execution

14.5.3.1 Now that the programming is set up, running the program is quite simple. Press the Run/Halt Program button on the left side of the controller.

14.5.3.2 Install the top cover of the autosampler on top using the top set of latches, similarly to 14.1. The cover is directional, however: for the AS950, the HACH insignia on the cover should be readable in the same direction of the controller. The SD900 has black arrows on both the top cover and the controller piece showing the proper lid orientation.

14.6 Review Sampling Program (Only necessary for the HACH AS950 Portable Sampler)

14.6.1 Using the same navigational instructions outlined in 14.5.1, navigate to Review Data > Sample History > All Samples. A self-explanatory log will appear, showing how the distributor arm allocated the sample at each given time (assuming the system clock is properly set).

14.6.2 Discard any samples that did not collect as expected.

14.7 Sample Removal and Laboratory Submittal

14.7.1 Access the bottom stage of the autosampler as in 14.1.

14.7.2 Cap all bottles without removing them from the stage with the provided caps.

14.7.3 Dry sample containers before labeling as the distributor arm setup of the autosamplers sprays the containers with a small volume of sample

14.7.4 Label every bottle with the pertinent information required by the laboratory for testing. (For the AS950, the order of the bottles is clearly labeled inside the base; review the sample history to determine exact sampling times). Be sure to consult the laboratory on the information they request to have about each sample.

14.7.5 If a preservative is required for laboratory analyses, distribute sample into bottle (provided by the laboratory) rated for use of that preservative. Do not overfill.

14.7.6 If using a composite sampler, it can be more feasible to distribute the sample to separate sample containers on a reasonably clean work surface in the field. Be sure to follow the guidance provided by 14.7.4 with new sample containers.

14.7.7 Send bottles back to the laboratory practicing the proper preservation techniques specified by the laboratory.

14.7.8 Record sample event in Field Notebook, noting date, time, facility and site identifier and field readings.

14.8 Laboratory Submission

14.8.1 Samples must be handled and processed following the laboratory's chain of custody procedures as is outlined in the laboratory quality assurance manual..

14.9 Cleaning

14.9.1 Sample Containers

14.9.1.1 Empty sample container contents, if any, and thoroughly rinse with hot water and Tergajet.

14.9.1.2 Rinse sample containers with deionized or equivalent reagent grade water.

14.9.1.3 Place sample containers and lids in laboratory dishwater on “Heavy Load” cycle and allow to dry.

14.9.2 Autosampler Purge

14.9.2.1 Prior to long term storage of the autosampler, the remnants of the sampling event must be purged from the autosampler.

14.9.2.2 Clean the exterior of the autosampler by rinsing and removing all solid contaminants.

14.9.2.3 Clean the inside of the bottom stage and the underside of the controller components as stated in 14.9.2.2.

14.9.2.4 Run identical volume of 2% Nitric Acid with a mild detergent as was done in the sampling event through the tube setup directly into the bottom stage; this will not only clean the external tube but also the internal components.

14.9.2.5 Soak the external tube setup in a cleansing solution overnight.

14.9.2.6 Remove any excess water from the lines by running the line.

14.9.2.7 The autosampler is now prepared to be stored indefinitely.

P15. CALCULATIONS

N/A

P16. METHOD PERFORMANCE

See P12 for quality controls used for the evaluation of method performance.

P17. POLLUTION PREVENTION

All wastes from this procedure shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

N/A

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

N/A

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

N/A

P21. WASTE MANAGEMENT

Excess, unpreserved sample may be discarded down the laboratory sink. If preserved, neutralize the preservative before disposing the sample. The samples may be neutralized by the addition of water in an accumulation container. Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

P22. REFERENCES

Sampler Manufacturer's Operating Manual

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes

Baseline	01/05/17	
	12/6/2019	Updated authorizing signatures. Does not constitute a revision change.

SOP-WI-41

Subcontract Work

Baseline

Approval:

Marianne Linker — 1/31/19

Environmental Programs Manager — Date

1-31-19

Laboratory Director — Date

1-31-19

Quality Assurance Manager — Date

LJT & Associates, Inc.

Effective Date: 1/31/2019

Title: Subcontract Work

P1. IDENTIFICATION OF THE TEST METHOD

Standardized instruction for subcontracting sample aliquots received for ISO 17025-compliant analyses (i.e., VELAP, AIHA-LAP).

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMITS

N/A

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the laboratory's procedure for subcontracting samples ensuring traceability per its quality system.

P5. SUMMARY OF METHOD

The procedure described here expands on the laboratory's policies for subcontracting sample analysis given in its Quality Assurance Manuals and those incorporated by reference from ISO 17025 Section 4.5, *Subcontracting of tests and calibrations.*

P6. DEFINITIONS

Laboratory Information Management System (LIMS): An information management system designed for the consistent entry of laboratory data with built-in flexibility to accommodate for ever-changing laboratory operations.

P7. INTERFERENCES

N/A

P8. SAFETY PRECAUTIONS AND WARNING NOTES

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory protocol for safety procedures including all PPE.

8.2 Refer to SDS sheets for all chemicals and reagents, particularly preservatives, which may be applicable to the subcontracted sample matrices.

8.3 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.4 Always put tops back on containers when not in use.

P9. EQUIPMENT AND SUPPLIES

- Shipping container (i.e., cooler)
- Padding material (i.e., bubble wrap, ice bags)
- Shipping tape
- Secondary containment (i.e., trash bag in cooler to contain melted ice)
- Adhesive shipping sleeves
- Sample containers (see applicable analytical method)
- Chain of Custody Forms (current revision of LJT form and form from subcontract lab)

P10. REAGENTS AND STANDARDS

- Preservatives (i.e., concentrated acids)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 Refer to an approved sampling plan for sampling procedures.

11.2 Refer to the reference method or the laboratory's analytical SOP for holding times and preservation requirements for each analyte of interest.

P12. QUALITY CONTROL

12.1 Temperature Blank

12.1.1 A temperature blank is required by most laboratories for all samples requiring thermal preservation.

12.1.2 The laboratory retains all of its temperature blanks in the refrigerator to mirror sample holding conditions.

12.1.3 Size of temperature blank chosen should be representative of the sample batch being shipped (i.e., if 20 mL sample containers are being shipped on ice to a subcontract lab, then a 20 mL temperature blank would be the most appropriate to send with it).

12.2 Trip Blank

12.2.1 If the transportation of samples to the laboratory is suspected in the causation of poor analytical results, a trip blank should be prepared.

12.2.2 If a trip blank is appropriate to the analyte of interest, the subcontract laboratory usually provides them as part of their sampling kit. These can easily be prepared in-house by using the laboratory's deionized water machines.

12.2.3 Trip blanks undergo the same transportation procedures as field samples and are submitted for analysis to the subcontract laboratory.

12.3 Duplicates

12.3.1 Certain analytes require multiple sample containers from the same sampling event to be submitted per analyte (i.e., TTHM, Oil and Grease). Consult with the subcontract laboratory if unsure about duplicate requirements.

P13. CALIBRATION AND STANDARDIZATION

N/A

P14. PROCEDURE

14.1 General Subcontracting Policies

14.1.1 Be sure to read and follow all of the laboratory's general policies on subcontracting in its Quality Assurance Manuals.

14.2 Subcontracted Samples Shipping Log (Use only if LIMS is not in use)

14.2.1 Using the information provided on each LJT Chain of Custody Form, complete a Chain of Custody Form provided by the subcontract laboratory.

NOTE: If shipping samples for both NASA and commercial customers, be sure to consult with the receiving laboratory prior to sending samples to see if NASA and commercial samples can be billed separately. The laboratory may require the samples to be listed separately on two different Chain of Custody Forms.

14.2.2 For each completed form, fill out the required number of lines in the Subcontracted Samples Shipping Log and assign each form a shipment batch ID (YY-XXXL, where YY is the two digit year, XXX is a 3 digit counter, and L is a single letter denoting the receiving laboratory, which are located on bottom of the Subcontracted Samples Shipping Log).

14.2.3 Transcribe this shipment batch ID on the subcontract laboratory's Chain of Custody Form.

14.2.4 Scan the completed Chain of Custody Form(s) and save them to the Subcontract Work folder.

14.3 Subcontract Work Batch in LIMS

14.3.1 Samples must be logged into Login by COC and assigned to a test template appended with a "Subcontracted" tag.

14.3.2 Complete chain of custody forms according to P14.2.1 and its note.

14.3.3 In Batching, assign all samples to be shipped to a "Subcontract Work" batch.

14.3.4 In the batch parameters, fill in all requested information and ensure the correctness of all pre-filled data.

NOTE: For items chosen by select list, inform the LIMS Administrator if a new parameter is required.

14.3.5 Transcribe the batch ID on all applicable subcontract laboratory Chain of Custody Forms.

14.3.6 Scan the completed forms and attach them to the "Subcontract Work" batch in Batching.

14.4 Packaging Samples

14.4.1 Ensure that samples can be shipped without DOT signage.

14.4.2 Choose an appropriate courier and delivery time for the samples being transported. (i.e., for samples requiring thermal preservation with a relatively short holding time, FedEx first overnight delivery is likely the way to go)

14.4.3 Ensure that shipping container holds sample container securely to minimize the risk of break or leak in transit and is appropriate to ensure holding conditions (i.e., use a cooler for samples requiring refrigeration). This can be accomplished by filling with bags of ice, in the case shipping samples requiring thermal preservation, or padding such as bubble wrap, for those that do not.

14.4.4 Ensure that the all applicable Chain of Custody Forms are transported with the samples to the subcontract laboratory in such a way to prevent damage to the forms (i.e., in an adhesive shipping sleeve stuck to the inner side of a cooler lid).

14.4.5 Securely seal the shipping container to ensure preservation conditions until received by the subcontract laboratory (i.e., ensuring the gasket on the cooler lid is properly sealed) using packing tape and affix all shipping documentation to the outside using an adhesive sleeve.

14.4.6 Drop off container to be shipped at appropriate courier pickup location. For FedEx Express deliveries, this is the drop box located near the Main Base front gate, which is picked up at 1630 every day. In the event that this pickup time is missed and

sample holding times are short, these can be taken to the FedEx Office in Salisbury until 1930.

14.5 Reporting of Subcontract Results

14.5.1 The laboratory team member that receives sample results from the subcontract laboratory saves the Report of Analysis in the Subcontract Work folder. The Quality Assurance Manager and the Laboratory Director should then be made aware of the received Report of Analysis by email so that the results may be scrutinized and reported.

14.5.2 Subcontract results still warrant the creation of a report of analysis. This report references the report of analysis from the subcontract laboratory, which is attached, prior to the original Chain of Custody Form.

P15. CALCULATIONS

N/A

P16. METHOD PERFORMANCE

N/A

P17. POLLUTION PREVENTION

All wastes from this procedure shall be collected and disposed of in accordance with state and federal regulations.

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

N/A

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

N/A

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

N/A

P21. WASTE MANAGEMENT

Excess, unpreserved sample may be discarded down the laboratory sink. If preserved, slowly discard sample down the sink with the faucet running and triple rinse the sample container to remove residual caustic compounds.

P22. REFERENCES

ISO:IEC 17025;2005

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline	01/31/2019	Established procedure to replace old subcontracting through Sample Receiving Logbook procedure, previously written into SOP-WI-22.

SOP-WI-42

Data Reporting

Baseline

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

3-20-19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

3-20-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

3-20-19
Date

LJT & Associates, Inc.

Effective Date: 03/20/2019

SOP-WI-42

Data Reporting

Baseline

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Jason Freischlag		3-20-19	new SOP
Marianne Simko	Marianne Simko	3-20-19	new SOP
Marga Morris		03.20.19	new SOP
Brian Cornwell		03-20-19	New SOP
Mark Culver		3-20-19	new SOP

Title: Data Reporting

P1. IDENTIFICATION OF THE TEST METHOD

Data Reporting

P2. APPLICABLE MATRIX OR MATRICES

N/A

P3. DETECTION LIMITS

N/A

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the laboratory's procedure for reporting data to its customers.

P5. SUMMARY OF METHOD

The procedure described here describes the laboratory's policies and procedures for generating final reports of analysis and supplemental data packages, as requested by customers.

P6. DEFINITIONS

N/A

P7. INTERFERENCES

N/A

P8. SAFETY PRECAUTIONS AND WARNING NOTES

N/A

P9. EQUIPMENT AND SUPPLIES

N/A

P10. REAGENTS AND STANDARDS

N/A

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

N/A

P12. QUALITY CONTROL

N/A

P13. CALIBRATION AND STANDARDIZATION

N/A

P14. PROCEDURE

14.1 Before generating a report of analysis (i.e., in 14.2, 14.3, and 14.4), get the most up-to-date report of analysis template applicable to the data being reported from the Master List of Documents or the QA Manager. The QA Manager is responsible for ensuring that templates meet accreditation and customer requirements (e.g., those listed in P22).

14.2 With the exception of data reported according to 14.3, data are peer-reviewed and approved by laboratory management prior to issuance.

14.3 Preliminary Data Reporting

14.3.1 For some applications, it may be appropriate to inform customers of laboratory results prior to issuing the final report of analysis. Examples where this may be applicable include a total coliform positive bacteriological sample, an in-house commodity test yields results outside of the client's required specifications, or where nonconformities in the test procedure (i.e., sample mishandles, QC failures) may nullify the test result in question. Preliminary data shall be qualified as such and not be issued where additional interpretation may be necessary.

14.3.2 Preliminary data reporting is done in one of two ways. The customer is informed by phone call, which is then documented in the laboratory's current Phone Log, or by email, which is printed and filed in the Customer Consultations binder.

14.3.3 Preliminary data are only released to the client's authorized points of contact.

14.4 Reports of Analysis – VELAP

14.4.1 Individual reports of analysis are created per Chain of Custody form, technical section (e.g,, microbiological and wet chemistry), matrix, and other customer requirements. Release of subcontract test results may be grouped with any section's data or reported separately.

14.4.2 The laboratory retains its populated templates for VELAP reporting in the Completed Word Docs folder on the WICC drive. For the laboratory's recurring

customers, using one of these as a template can make data reporting more consistent, as the person generating the report of analysis will not need to modify many parameters, such as the Client Name, Parameters, or Methods. Be sure to consult regularly with the QA Manager if using this approach to data reporting, as reporting methods can and likely will change over time.

14.4.3 Starting from a blank reporting template, fill in the Client, Point of Contact, Address, Phone, Fax, and Email parameters using customer-provided information on the Chain of Custody form or other customer information documentation.

14.4.4 If sample was collected by a laboratory representative, list their initials in the Sample Collected By field. If this is not the case and unless the customer requires otherwise, the Sample Collected By parameter is listed as "Client".

14.4.5 The Sample Receipt Date and Time should be transcribed as the date and time on the chain of custody where the customer representative relinquished custody of the samples to the laboratory. In the event that the template does not enforce formatting on the dates and times listed, format the dates as m/d/yyyy (no leading zeroes on month or day and a four digit year) and the times as HHnn (four digit time without a colon).

14.4.6 A single "block" on a report of analysis is created per Sample ID. This block includes a horizontal rule on top, the Site, Collection Date and Time, Sample ID, a PWSID number (for public water works only), and a data table. This data table has headers for Parameter, Method, Result, Unit, Limit of Detection/Limit of Quantitation, Completion Date and Time, and Analyst.

14.4.7 For each block, fill in the Site, Collection Date and Time, and Sample ID, as listed on the Chain of Custody form. If the sample matrix is drinking water, check the Virginia Department of Health monitoring schedule for the PWSID number associated with the site.

14.4.8 For each new line of the data table, list the Parameter and Method according to the laboratory's Scope of Accreditation, the Result and Completion Date and Time according to the bench sheet, and the Unit and Limit of Detection/Limit of Quantitation according to the applicable standard operating procedure. The analyst is listed as the initials all responsible analysts associated with the batch in order of participation, each separated by a slash and a line break (i.e., if Analyst A sets up a batch of microbiological samples and Analyst B performs the interpretation the next day, the analyst should be listed as A/B).

14.4.9 In the Notes section, any data qualifiers for affected data should be included, which clearly identify nonconformities that could affect test data (i.e., sample run out of hold, method blank failure). This can be done using asterisks next to the affected data with the notation making reference to the asterisk or by prefacing the qualifier with the test name.

14.4.10 Any non-accredited parameters listed on the final report of analysis should be listed below data qualifiers, prefaced with a statement, such as "Non-accredited parameters – ".

14.4.11 Authorized final reports of analysis are scanned and filed in the Electronic Copies folder on the WICC drive.

14.5 Reports of Analysis – AIHA-LAP, LLC

14.5.1 Individual reports of analysis are created per Sample ID.

14.5.2 The laboratory retains its populated templates for AIHA-LAP, LLC reporting in the Reporting Forms/Results folder on the WICC drive. For the laboratory's recurring customers, using one of these as a template can make data reporting more consistent, as the person generating the report of analysis will not need to modify many parameters, such as the Client Name, Parameters, or Methods. Be sure to consult regularly with the QA Manager if using this approach to data reporting, as reporting methods can and likely will change over time.

14.5.3 Starting from a blank reporting template, fill in the Client, Point of Contact, Address, Phone, Fax, and Email parameters, as applicable, using customer-provided information on the Chain of Custody form or other customer information documentation.

14.5.4 The Sample Date and Received Date should be transcribed as the date and time on the chain of custody where the customer representative relinquished custody of the samples to the laboratory. In the event that the template does not enforce formatting on the dates listed, format the dates as m/d/yyyy (no leading zeroes on month or day and a four digit year).

14.5.5 A single "block" on a report of analysis is created. This block includes a horizontal rule on top, the Site, Collection Date, Sample ID, Client Sample ID. commodity specification, and a data table. This data table has headers for Parameter, Result, Reporting Limit, Uncertainty, Unit, Allowable Limits, Method, and Detection Instrument, and Analyst.

14.5.6 For each block, fill in the Site, Collection Date, Sample ID, Client Sample ID, and commodity specification (if applicable) as listed on the Chain of Custody form.

14.5.7 For each new line of the data table, list the Parameter, Reporting Limit, Unit, Method, and Detection Instrument according to the applicable standard operating procedure. The result is reported as on the bench sheet which contains the bias-corrected analytical results to the correct significance. Uncertainty is reported according to the method standard operating procedure, where it is relevant to the validity of the result, it affects conformity to a specification limit, or where required by the customer The analyst is listed as the initials all responsible analysts associated with the batch, each separated with a slash followed by a line break.

14.5.8 Under the data table, include any non-accredited results, prefaced with a statement such as "Non-accredited parameters:", along with relevant instrumentation and acceptability criteria required for the commodity specification.

14.5.9 In the Comments section, any data qualifiers for affected data should be included, which clearly identify nonconformities that could affect test data (i.e., sample run out of hold, method blank failure). This can be done using asterisks next to the affected data with the notation making reference to the asterisk or by prefacing the qualifier with the test name.

14.5.10 Below any required data qualifiers, include a statement of conformity to the customer-provided specification based on analytical data and customer-provided data.

14.5.11 Authorized final reports of analysis are scanned and filed in the Reporting Forms/Results folder on the WICC drive.

14.6 Reports of Analysis – Commodities

14.6.1 Reports of analysis for the laboratory's non-accredited commodities testing are generated by templates adapted to historical customer requirements. While no accreditation applies to these reports, data should still be reported in a clear, accurate, and unambiguous manner.

14.7 When a corrected report of analysis is to be issued, the Notes section shall contain a statement regarding the change made and reference to the original report of analysis (i.e., its timestamp).

14.8 Summary Reports

14.8.1 Summary reports are compiled by the laboratory mostly to fulfill the requirement of an environmental compliance entity. These include discharge monitoring reports for the Virginia Department of Environmental Quality and the monthly drinking water report to the Virginia Department of Health LabAdmin group.

14.8.2 Discharge Monitoring Reports are prepared according to applicable regulatory requirements and submitted, along with the reports of analysis described in 14.4, to the customer for review and ultimate submission to the Virginia Department of Environmental Quality.

14.8.3 Following the instructions laid out by the Virginia Department of Health, the laboratory prepares two CSV templates of its public works drinking water sample analysis to the labadmin@vdh.virginia.gov contact group at the end of every month.

14.9 Data Packages

14.9.1 The laboratory is able to provide supplemental data packages to its customers and its accrediting bodies. These include, but are not limited to, QC data packages (i.e., a "Level 2" data package) and data packages including all of the laboratory's raw data (i.e., a "Level 4" data package).

P15. CALCULATIONS

N/A

P16. METHOD PERFORMANCE

N/A

P17. POLLUTION PREVENTION

N/A

P18. DATA ASSESSMENT AND ACCEPTABLE CRITERIA FOR QUALITY CONTROL MEASURES

N/A

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

N/A

P20. CONTINGENCIES FOR HANDLING OUT-OF –CONTROL OR UNAACCEPATABLE DATA

N/A

P21. WASTE MANAGEMENT

N/A

P22. REFERENCES

2009 TNI V1M2 5.10 (VELAP Reporting Requirements)
ISO/IEC 17025:2017(E) 7.8 (AIHA-LAP, LLC Reporting Requirements)
AIHA-LAP, LLC Policy Module 2A.7.8 (AIHA-LAP, LLC Reporting Requirements)
Virginia Department of Health Electronic Reporting/Template Instructions

P23. TABLES, DIAGRAMS AND FLOWCHARTS

N/A

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline		Established procedure to clarify laboratory requirements for data reporting.

SOP-WI-43

Wastewater MPN

Baseline

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

3/20/19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

3-20-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

3-20-19
Date

LJT & Associates, Inc.

Effective Date: 03/20/2019

SOP-WI-43

Wastewater MPN

Baseline

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Marianne Simko	Marianne Simko	3/20/19	New
Marga Morris	[illegible]	03.20.19	New
Brian Cornwell	[illegible]	03-20-19	New SOP
Mark Culver	[illegible]	3-20-19	New
Jason Fleischlag	[illegible]	3-20-19	New SOP
Thera Lombardi	[illegible]	20 Mar 19	New

Title: Wastewater MPN

P1. IDENTIFICATION OF THE TEST METHODS

Enterococci by Enterolert®, *E. coli* by Colilert® MPN, Fecal Coliform by Colilert®-18

P2. APPLICABLE MATRIX OR MATRICES

These methods are suitable for the determination of enterococci, *E. coli*, or fecal coliforms in an 100 mL wastewater sample.

P3. DETECTION LIMIT

The limit of quantitation does not apply for MPN testing, as there is no viable procedure for its calculation. The limit of detection is set at one most probable number (MPN) per 100 mL. The MPN is statistically equivalent to the microbiological term colony forming unit.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test methods for the collection and analysis of waste water samples for the enumeration of enterococcus, *E. coli*, or fecal coliform bacteria. These methods are intended for use in the detection and confirmation of enterococci, *E. coli*, or fecal coliforms in waste water.

P5. SUMMARY OF METHOD

5.1 The enterococcus procedure described herein is certified under the Enterolert method and largely complies with 23 SM 9230 D, which describes the determination of enterococci by metabolism of the Enterolert® reagent, which causes sample fluorescence. Definitive identification of Enterococci spp. is demonstrated by blue fluorescence under a 6 watt, 365 nm UV light after incubation at 41.5 ± 0.5°C for 24 to 28 hours.

5.2 The *E. coli* procedure described herein is certified under the Colilert method and largely complies with SM 9223 B, which describes the determination of total coliform and *E. coli* by metabolism of the Colilert® reagent, which causes sample yellowing and/or fluorescence, respectively. Positive identification of *E. coli* is demonstrated by blue fluorescence under a 6 watt, 365 nm UV light after incubation at 35.0 ± 0.5°C for 24 to 28 hours.

5.3 The fecal coliform procedure described here is certified under the Colilert-18 MPN procedure, which describes the determination of fecal coliform by metabolism of the Colilert®-18 reagent, which causes sample yellowing. Positive identification of fecal coliform bacteria is demonstrated by yellow color change after incubation at 44.5 ± 0.2°C for 18 to 22 hours.

P6. DEFINITIONS

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Colilert®: Based on IDEXX's Defined Substrate Technology, is used for the detection of total coliform and E. *coli* in water. Colilert® uses ONPG and MUG as the defined substrate nutrient-indicators for total coliform and E. *coli*, respectively. These compounds, when hydrolyzed, create compounds that cause solution yellowing and fluorescence observable with a UV_{365nm} lamp, respectively. This reagent system is specifically formulated to achieve optimum sensitivity and specificity in the detection and identification of total coliform and *E. coli*. The test is capable of detecting one colony forming unit of total coliform and *E. coli* in 100 mL of water within 24 hours.

Colilert®-18: Based on IDEXX's Defined Substrate Technology, is used for the detection of total and fecal coliform in water. Colilert®-18 uses ONPG as its defined substrate nutrient indicator. This compound, when hydrolyzed, creates compounds that cause solution yellowing. This reagent system is specifically formulated to achieve optimum sensitivity and specificity in the detection and identification of total coliform and fecal coliform. The test is capable of detecting one colony forming unit of total coliform, when incubated at ~35°C, or fecal coliform, when incubated at ~44.5°C, in 100 mL of water within 18 hours.

Disinfection: To destroy most (but not necessarily all) harmful or objectionable microorganisms by various treatment methods, such as chemical and thermal assault and ultraviolet light.

Enterococcus: A genus of facultative anaerobic bacteria of the phylum Firmicutes commonly found in soil, water and plants. While *Enterococcus* spp. are also commonly present in the gastrointestinal tract of most eukaryotic organisms, their presence in potable and non-potable water sources is highly regulated due to their correlation with human pathogenesis.

Enterolert®: Based on IDEXX's Defined Substrate Technology, is used for the detection of enterococci in water. Enterolert® uses 4-MUG as the defined substrate nutrient-indicator. This compound, when hydrolyzed, creates a compound that causes fluorescence observable under a UV_{365nm} lamp. This reagent system is specifically formulated to achieve optimum sensitivity and specificity in the detection and identification of enterococcus. The test is capable of detecting one colony forming unit of enterococcus in 100 mL of water within 24 hours.

Escherichia coli: Commonly abbreviated as *E. coli*, this species of coliform bacteria is ubiquitous in the gastrointestinal tracts of most eukaryotic organisms. While mostly harmless, some strains are known to cause illness in humans and the presence of any strain in water is indicative of fecal contamination, which could suggest the presence of organisms more significant to human pathogenesis.

Fecal coliforms: Rod-shaped thermo-tolerant bacteria prevalent in the gastrointestinal tract of most eukaryotic organisms. These bacteria are notable in their fermentation of lactose and, while not necessary pathogenic, are indicative of fecal contamination.

Limit of Detection (LOD): The lowest analyte concentration that produces a signal sigificantly differentiable from a matrix not containing the analyte of interest.

Lot Quality Control (LQC): Quality control procedure performed on incoming commercially-prepared reagents to ensure usability.

Most Probable Number (MPN): The number of organisms which are *most likely* to have produced the laboratory results noted in a particular test.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Sterilization: Destruction or removal of all viable organisms.

P7. INTERFERENCES

The presence of large amounts of heterotrophic bacteria in the sample may prohibit the use of these methods. High salinity,algal populations, and color are also positively interfering in these analyses such that dilution may be required for mitigation.

P8. SAFETY PRECAUTIONS

A lab coat and nitrile gloves should be worn at all times. Always use the UV light in a darkened room that others cannot access with UV protective glasses or contain UV light in a light box setup with a protective viewing screen. Do not make eye contact with the UV light. Always point the light towards the object only. Testing is performed by individuals with bacteriological training since live microorganisms are used for quality control testing. Properly disinfect any spills and sterilize all used containers before disposal according to appropriate regulations.

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of wastewater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples should protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter and clean bench surfaces with disinfectant before and after bacteriological testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

P9. TOOLS, EQUIPMENT AND SUPPLIES

- Sterile Transfer Pipettes (to remove excess volume)
- Quanti-Tray Heat Sealer
- 97-Well Rubber Insert
- Quanti-Tray® 2000 MPN Table
- Incubator capable of maintaining a temperature of 44.5°C ± 0.2°C (Fecal coliform)
- Incubator capable of maintaining a temperature of 41.0°C ± 0.5°C (Enterococcus)
- Incubator capable of maintaining a temperature of 35.0°C ± 0.5°C (*E. coli*)
- Sample storage refrigerator maintained at a temperature of 1 – 6°C
- Long wave (365-nm) ultra violet lamp with 6-watt bulb
- UV lamp light box

P10. REAGENTS AND STANDARDS

10.1 Enterolert® snap-pack reagent

10.2 Colilert® snap-pack reagent

10.3 Colilert®-18 snap-pack reagent

10.4 Quanti-Tray® 2000 (purchased from IDEXX Laboratories)

10.5 Sterile vessels, plastic containing sodium thiosulfate to neutralize up to 15 mg/L of residual chlorine (SM 9060 A2). 120 mL vessels are used for sample collection, while 150 mL vessels are used by the laboratory for mixing.

10.6 ATCC-traceable positive control and Gram-positive and Gram-negative negative control cultures (i.e., IDEXX-QC *Escherichia coli*, *Enterococcus faecium* or *Enterococcus faecalis* and *Streptococcus bovis* cultures) [Enterolert® LQC]

10.7 ATCC-traceable positive, total coliform positive, and negative control cultures (i.e., IDEXX-QC *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* cultures) [Colilert® LQC]

10.8 ATCC-traceable positive and negative control cultures (i.e., IDEXX-QC *Escherichia coli* and *Pseudomonas aeruginosa* cultures) [Colilert®-18 LQC]

10.9 Single-strength tryptic soy broth (Sterile vessel and Quanti-Tray LQC)

10.10 Residual chlorine standard, 1.5 mg at 100 mL (Sterile vessel LQC)

10.11 Quanti-Tray® 2000 Comparator for Colilert® and Colilert®-18 (purchased from IDEXX Laboratories)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Proper technique, equipment and sample preservation are critical to obtaining valid test results which can be used in the evaluation of process efficiency or water quality. These factors are especially critical in bacteriological sampling. The sample containers, sampling procedure, and handling after sampling can all be sources of errors long before testing actually begins. To ensure proper samples are collected, follow these instructions:

11.1 Bottles provided by the laboratory (as in P10.5) have a marked 100 mL fill line, a security seal around the lid, and solid sodium thiosulfate for residual chlorine removal.

11.2 To obtain a method-sterile, representative sample for bacteriological examination:

11.2.1 Do not touch or contaminate the cap or neck of the bottle.

11.2.2 If manual sampling is performed, hold the bottles near the base during sampling.

11.2.3 Partially submerge the sample bottle in the water (or water stream) to be sampled, taking care not to wash out the preservative.

11.2.4 Fill the sample bottle approximately ¾ full, but not less than 100 mL. Leave about an inch of head space to allow the sample to be mixed by shaking prior to testing.

11.2.5 Replace the cap on the bottle, ensuring minimal contact to the interior-facing bottle surfaces.

11.3 Examination of bacteriological water samples should be performed immediately after collection, as practical. If testing cannot be started within 15 minutes of sampling, the sample must be iced or refrigerated at 10°C or less (but not be frozen). If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.4 The maximum allowable holding time for wastewater microbiological samples is 8 hours. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis. Document LQCs using the template document(s) listed in the relevant section.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for enterococci, *E. coli*, and fecal coliforms obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.2.1 Sign in the proficiency test samples in the Sample Receiving Logbook. Be sure to record the chain of custody numbers on the sample containers.

12.2.2 Follow the PT provider's included instructions for preparing the samples and analyze according to P14.

12.3 Initial Demonstration of Capability (IDC)

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration per qualifying analyte.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 The analyst should prepare four aliquots using the certified reference materials and sample containers provided by the qualified analyst according to the manufacturer's specifications.

12.3.4 The analyst then shall carry out the analysis of these four samples as per P14. The interpretation of the analyst's results is discussed in P18.

12.4 On-going Demonstration of Capability (ODC)

12.4.1 Analysts are required to re-verify their capability in these fields of testing through the on-going demonstration of capability. Prior to completing the on-going

demonstration of capability, the analyst must review this SOP and sign off on the SOP Signature Sheet.

12.4.2 The analyst should prepare a single aliquot per analyte using certified reference materials and sample containers according to the manufacturer's specifications.

12.4.3 The analyst shall carry out the analysis of these samples and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Duplicates (QCD)

12.5.1 While no method requirement exists for performing duplicates, the laboratory requests its recurring clients to submit duplicate sample aliquots with the intent of performing duplicate analyses on a weekly basis, per analyte. Interpretation of duplicate analyses is discussed in P18.

12.6 Sample Bottle LQC

12.6.1 The following procedure is applicable to 120 mL and 150 mL sterile vessels with pre-added sodium thiosulfate. If a lot of sample vessels does not meet its criteria, they should either be re-tested (in the case of sterility only) or returned to the manufacturer.

12.6.2 Sample bottle QC is documented as a single line item on the IDEXX Sample Bottle QC Log.

12.6.3 Each lot of sample bottles is to be checked for blue fluorescence prior to use with a 6 watt, 365 – nm ultraviolet light. Record as a "Yes/No" in the appropriate column.

12.6.4 The 100 mL mark on each lot of sample bottles must be checked for accuracy.

12.6.4.1 Fill a single vessel from the lot to the 100 mL fill line with deionized water.

12.6.4.2 Transfer the water to an 100 mL graduated cylinder. The recovered volume should be between 98 and 102 mL, or within the tolerance certified by the manufacturer.

12.6.4.3 Record the recovered volume in the "Fill Line Accurate Against a Class A Graduated Cylinder" column.

12.6.5 Check the chlorine resiliency of each lot of sample bottles.

12.6.5.1 Inoculate a sample container from the lot with 1.5 mg of chlorine (equivalent to 15 mg/L chlorine at 100 mL). This can be done by addition of 100 mL of a 15 mg/L chlorine standard or a smaller volume from a concentrate (i.e., 10 mL of a 150 mg/L chlorine standard).

12.6.5.2 Gently swirl the sample container to allow for neutralization.

12.6.5.3 Check the residual chlorine of the water in the sample container using a chlorine strip or analyze for free residual chlorine according to SOP-WI-29. Record "Yes" in the "Chlorine Neutralization of 15 mg/L" column if resulting residual is 0 mg/L or the actual value, if not.

12.6.6 Check sterility of each lot of sample bottles.

12.6.6.1 Label a sample container from the lot, minimally, with the test intention (i.e., "Sterility Check"), the lot number of the container, and the incubation start date.

12.6.6.2 Aseptically pour, at least, 30 mL of commercially prepared 1X Tryptic Soy Broth into a sample container from the lot.

12.6.6.3 Incubate the container at 35.0°C ± 0.5°C for 48 ± 2 hours. Record the start and end times as well as the initial and final incubator temperatures in the appropriate fields.

12.6.6.4 The broth should be free of turbidity and/or a visible white pellet at the bottom of the vessel. Record the result using a "+" or "-" according to the directions on the bench sheet.

12.6.6.5 Each lot of 1X Tryptic Soy Broth will be subjected to the following QC tests: *E. coli* and *P. aeruginosa* will be inoculated into separate vials of TSB and used as positive controls to exhibit growth at 35.0°C ± 0.5°C after 24 + 4 hours and an empty tube as a negative control, exhibiting no growth under the same conditions but triple the time (72+ hours). Record results using Tryptic Soy Broth QC Log.

NOTE: Single-strength Tryptic Soy Broth can be used past its expiration date by a single year if and only if the procedure in 12.6.6.5 is performed to ensure its continued usability. The aforementioned check extends the media's expiration date by two calendar months after the completion of the analysis.

12.7 Enterolert® LQC

12.7.1 This procedure is applicable only to the Enterolert® reagent. If a lot does not meet its criteria, they should be returned to the manufacturer. All applicable parameters should be recorded on the IDEXX Enterolert Media QC Log.

12.7.2 Check for blue fluorescence under a 6 watt, 365 nm ultraviolet lamp. If the lot exhibits fluorescence, the rest of this procedure can be disregarded.

12.7.3 Prepare four aliquots of 100 mL deionized water in sterile 120 mL vessels.

12.7.4 One of these aliquots will be the negative control, which shall demonstrate method sterility. This vessel should be labeled, minimally, with the test intention (i.e., “Enterolert QC”), the reagent lot number, the QC type (i.e., negative control, blank), and the incubation start date.

12.7.5 The rest of these aliquots shall be used for specificity testing via inoculation with species from an Enterolert IDEXX QC pack (containing a method-positive species and two method-negative species). These vessels should be labeled, minimally, with the test intention (i.e., “Enterolert QC”), the reagent lot number, the name (or recognized abbreviation) of the inoculated specimen, its lot number, and the incubation start date.

12.7.6 Add a snap pack of the Enterolert reagent to each of the aforementioned aliquots and allow to dissolve.

12.7.7 Incubate all four aliquots at 41.0°C ± 0.5°C for 24 -28 hours.

12.7.8 The aliquots should be examined with a 6 watt, 365 nm UV light. The interpretation of this QC is discussed in P18.

12.8 Colilert® LQC

12.8.1 This procedure is applicable only to the Colilert® reagent. If a lot does not meet its criteria, they should be returned to the manufacturer. All applicable parameters should be recorded on the IDEXX Colilert Media QC Log.

12.8.2 Check for blue fluorescence under a 6 watt, 365 nm ultraviolet lamp. If the lot exhibits fluorescence, the rest of this procedure can be disregarded.

12.8.3 Prepare four aliquots of 100 mL deionized water in sterile 120 mL vessels.

12.8.4 One of these aliquots will be the negative control, which shall demonstrate method sterility. This vessel should be labeled, minimally, with the test intention (i.e., “Colilert QC”), the reagent lot number, the QC type (i.e., negative control, blank), and the incubation start date.

12.8.5 The rest of these aliquots shall be used for specificity testing via inoculation with species from a Colilert IDEXX QC pack (containing a method-positive species and two method-negative species). These vessels should be labeled, minimally, with the test intention (i.e., “Colilert QC”), the reagent lot number, the name (or recognized abbreviation) of the inoculated specimen, its lot number, and the incubation start date.

12.8.6 Add a snap pack of the Colilert reagent to each of the aforementioned aliquots and allow to dissolve.

12.8.7 Incubate all four aliquots at 35.0°C ± 0.5°C for 24 -28 hours.

12.8.8 The aliquots should be examined for yellow color change and fluoresence with a 6 watt, 365 nm UV light. The interpretation of this QC is discussed in P18.

12.9 Colilert®-18 LQC

12.9.1 This procedure is applicable only to the Colilert®-18 reagent. If a lot does not meet its criteria, they should be returned to the manufacturer. All applicable parameters should be recorded on the IDEXX Colilert-18 Media QC Log.

12.9.2 Prepare three aliquots of 100 mL deionized water in sterile 120 mL vessels.

12.9.3 One of these aliquots will be the negative control, which shall demonstrate method sterility. This vessel should be labeled, minimally, with the test intention (i.e., “Colilert-18 QC”), the reagent lot number, the QC type (i.e., negative control, blank), and the incubation start date.

12.9.4 The rest of these aliquots shall be used for specificity testing via inoculation with species from a Colilert-18 IDEXX QC pack (containing a method-positive species and one method-negative species). These vessels should be labeled, minimally, with the test intention (i.e., “Colilert-18 QC”), the reagent lot number, the name (or recognized abbreviation) of the inoculated specimen, its lot number, and the incubation start date.

12.9.5 Add a snap pack of the Colilert-18 reagent to each of the aforementioned aliquots and allow to dissolve.

12.9.6 Incubate all four aliquots at 44.5°C ± 0.2°C for 18-22 hours.

12.9.7 The aliquots should be examined for yellow color change. The interpretation of this QC is discussed in P18.

12.10 Quanti-Tray Sealer and UV Unit

12.10.1 Every month, ensure adequate sealing by adding Bromocresol Purple dye to 100 mL of water, pour into a Quanti-Tray 2000, and run through sealer. If dye is observed outside the wells, perform maintenance or use another sealer.

12.10.2 Every month, disconnect the UV unit from power and clean using a Kimwipe and ethanol.

12.10.3 Record completion on the UV Light and Sealer QC Log taped to the sealer.

12.11 Quanti-Tray 2000 LQC

12.11.1 The following procedure is applicable to Quanti-Tray 2000. If a lot of trays does not meet its criteria, they should either be re-tested or returned to the manufacturer. All applicable parameters should be recorded on the IDEXX Quanti-Tray 2000 QC Log.

12.11.2 Carefully pour 100 mL of commercially prepared 1X Tryptic Soy Broth into a tray from the lot.

12.11.3 Run the prepared tray through the sealer.

12.11.4 Label the tray, minimally, with the test intention (i.e., “Sterility Check”), the lot number of the container, and the incubation start date/time.

12.11.5 Incubate at 35.0°C ± 0.5°C for 48 ± 2 hours.

12.11.6 All wells should be free of turbidity and/or a visible white pellet.

12.12 Reagent Water Quality Control

12.12.1 The following procedure is required monthly when diluting client samples using laboratory-prepared reagent water (deionized water).

12.12.2 Allow deionized water system to dispense at least 1 L. During this time, record the withdrawal time, specific conductance, total organic carbon, and temperature on the Deionized Water QC Log.

12.12.3 Collect 100 mL of deionized water according to P11 of this SOP.

12.12.4 Analyze collected sample for heterotrophic bacteria (preferably following the IDEXX HPC for Quanti-Tray procedure) within 24 hours of collection. Interpretation of this QC is discussed in P18.

12.12.5 An aliquot of this reagent water should also be tested for chlorine residual (SOP-WI-29, *Chlorine by Pocket Colorimeter*) and ammonia (using test kit or method blank result from SOP-WI-2, *Ammonia*). Record all applicable parameters on the Reagent Water QC Log.

P13. CALIBRATION AND STANDARDIZATION

13.1 Thermometers

13.1.1 Calibrated, NIST-traceable thermometers with the bulb immersed in liquid (glycerin, water, or mineral oil) are kept in the wastewater incubators and the sample refrigerator. Thermometers are externally calibrated yearly and the Certificates of Calibration are retained in the laboratory's records.

13.1.2 There must be one thermometer per shelf in-use in each of the wastewater incubators. Alternatively, one thermometer may be placed on the top and bottom shelf of each incubator to bracket the incubator temperature conditions.

13.1.3 Position of thermometers in incubators shall be periodically adjusted to demonstrate uniform incubator temperature. Make notation of thermometer movements in Temperature Log or Equipment Maintenance Log, as space is available.

13.1.4 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.1.5 The Enterococci incubator is maintained at ~41°C. The temperature is checked twice daily, during each business day, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this incubator are 41.0°C ± 0.5°C.

13.1.6 The E. coli incubator is maintained at ~35°C. The temperature is checked twice daily, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this incubator are 35.0°C ± 0.5°C.

13.1.7 The Fecal incubator is maintained at ~44.5°C. The temperature is checked twice daily, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this incubator are 44.5°C ± 0.2°C.

13.1.8 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, during each business day, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

P14. PROCEDURE

14.1 Turn on Quanti-Tray 2000® sealer prior to analysis to allow for its warm-up. The sealer is ready to use when the indicator light turns green.

14.2 Preparing the method-sterile workspace

14.2.1 Spray and wipe down the work surface with disinfectant.

14.2.2 Place Kimwipe(s) over the sprayed area.

14.2.3 Place all samples to be analyzed on the Kimwipe(s) and allow to equilibrate to ambient temperature.

14.3 Sample Prep

14.3.1 Record all sample initial conditions in the applicable columns of the Enterococcus, Coliform and *E. coli*, and Fecal Coliform Bench Sheets.

NOTE: Ensure lot numbers are correct on the bottom of the page. If they are not, add an additional entry on the bottom of the page and use a concise symbol (i.e., an asterisk) to denote where that lot information is applicable.

14.3.2 Check to ensure sample has at least 1 inch of headspace to allow for adequate mixing. If headspace is adequate, shake the sample, at a minimum of 25 times at a 45 degree angle, to mix. Remove the excess volume utilizing a sterile pipette, discarding sample into waste disposal beaker until total sample volume in the sample container is 100 mL. If headspace is inadequate, transfer to 150mL sterile container to mix, as above, and remove the excess volume utilizing a sterile pipette.

NOTE: A visual comparator can be made for this headspace requirement by filling a 120 mL sterile vessel with ~107 mL of deionized water.

14.3.3 If sample does not come from a known UV effluent, check chlorine residual by pipetting two drops of sample excess onto a chlorine strip using a sterile pipette. If exactly 100 mL of sample is received, this step still needs to be performed. The removed drops should not significantly impact the analysis. Note the result of this on the sample chain of custody form. If chlorine is present, inform the Laboratory Director and do not proceed with the analysis.

NOTE: If sample is suspected to be highly contaminated, it may be pertinent to perform a dilution (recommended starting dilution is 1:10). Record the dilution factor used on the bench sheet. Be sure to shake the sample prior to dilution and use a sterile pipettor to retrieve the required amount of sample. The reagent water incurs an additional quality control procedure (P12.12).

14.3.4 Open a snap-pack of the correct reagent (Enterolert® for enterococci, Colilert® for *E. coli*, Colilert®-18 for fecal coliform) and pour into 100 mL of sample. Mix to dissolve reagent by gentle inversion as to prevent foaming. If foaming does occur, do not continue analysis until foam dissipates.

NOTE: If a blue flash is observed upon introduction of Colilert® or Colilert®-18 media, inform the Laboratory Director and do not proceed with the analysis. This should never need to be observed, as the step in 14.3.3 is indicative of the same property.

14.4 Traying Samples for Enumeration

14.4.1 Open a Quanti-Tray by pinching it to form a U-shape and pulling the foil tab. Pour the sample into the tray and gently tap the bottom of the tray to release entrapped air.

14.4.2 Align Quanti-Tray well side down on the rubber insert. Slide the Quanti-Tray into the sealer until the motor begins to draw it. When done, remove Quanti-Tray from the back of sealer.

14.4.3 Label tray, minimally, with chain of custody number and/or standard number, site, analyte identifier, date/time, and analyst initials. Do not pre-label trays, as this may damage the sealer.

14.4.4 Immediately transfer all tests to the corresponding incubator well side down. Do not stack too many tests on top of each other because it will block air flow in the incubator.

NOTE: Be sure to turn the sealer off when finished.

14.5 Incubation Stop and Reading Quanti-Trays

14.5.1 Allow tests to incubate for 24 + 4 hours (*E. coli* and enterococci) or 18 + 4 hours (fecal coliform). The upper time limits allow for re-incubation, if wells are ambiguous. Incubation times may be truncated in the event that the test is completely positive for the analyte of interest (i.e., for E. coli, all 97 wells are yellow <u>and</u> fluorescing blue after 14 hours). While not standard practice, negative well readings obtained after the upper time limit are also considered valid negative results.

NOTE: Test results may be read prior to their incubation windows solely for the purposes of preliminary reporting to the client. In correspondence with the client, these data should be qualified as a minimum possible result. Tests must then be returned to the incubator, if removed, and allowed to incubate for their full incubation time.

14.5.2 Check fecal coliform and *E. coli* tests for yellow color change. If yellow color changes observed are equal to or more prominent than the comparator, then the

sample is positive for fecal or total coliform, respectively. Record the large and small positive well counts.

14.5.3 Check *E. coli* tests under a 6 watt, 365 nm UV light for blue fluorescence within 5 inches of the sample in a dark room or a light box. If fluorescence observed is equal to or more prominent than the comparator and the well was considered positive in 14.5.2, the well is considered positive for *E. coli*. Record the large and small positive well counts.

14.5.4 Check enterococci tests under a 6 watt, 365 nm UV light for blue fluorescence within 5 inches of the sample in a dark room or a light box. If <u>any</u> blue fluorescence is observed, the well is considered positive for enterococci. Record the large and small positive well counts.

NOTE: If having trouble with distinguishing positive and negative wells, analysis of a 100 mL aliquot of deionized water can serve as a negative comparator.

14.5.5 Use the large and small well counts found in the previous step and find their intersection on the IDEXX Quanti-Tray 2000 MPN table posted in the Micro Room. This table can also be found in P23 of this SOP. That value is the analyte concentration in MPN/100 mL. Transcribe these value on the bench sheets.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Most Probable Number (MPN)

15.1.1 Concentration of analyte most likely to have caused yellow coloration and/or fluorescence observed by Quanti-Tray.

15.1.2 MPN values are calculated using the IDEXX Quanti-Tray 2000 MPN Table, multiplied by any applicable dilution factor.

15.2 Relative Percent Difference (RPD)

15.2.1 The relative percent difference between a sample and its duplicate.

15.2.2 Applicable Formulas

15.2.2.1 $$RPD(X_1, X_2) = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} * 100\%,$$

where X_1 and X_2 are results of samples analyzed in duplicate

NOTE: If either value is below the limit of quantitation, add one to both results before performing this calculation.

15.3 Average

15.3.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $Geomean(X) = e^{\frac{\sum \ln(X_i)}{n}}$,
where X contains n values X_i

15.4 Lower Control Limit (LCL)

15.4.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability divided by the geometric standard deviation. Conceptually similar to an arithmetic mean minus a standard deviation, the lower control limit (LCL) is used to assess acceptability of an analyst's IDC.

15.4.2 Applicable Formulas

15.4.2.1 $Geometric\ Standard\ Deviation(X) = e^{\sqrt{\frac{(\sum \ln(X_i) - \overline{\ln(X)})^2}{n-1}}}$,
where X contains n values X_i and has logarithmic average $\overline{\ln(X)}$

15.4.2.2 $LCL = \frac{Geomean(X)}{Geometric\ Standard\ Deviation(X)}$

15.5 Upper Control Limit (UCL)

15.5.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability multiplied by the geometric standard deviation. Conceptually similar to an arithmetic mean plus a standard deviation, the UCL is used to assess acceptability of an analyst's IDC.

15.5.2 Applicable Formulas

15.5.2.1 $Geometric\ Standard\ Deviation(X) = e^{\sqrt{\frac{(\sum \ln(X_i) - \overline{\ln(X)})^2}{n-1}}}$,
where X contains n values X_i and has logarithmic average $\overline{\ln(X)}$

15.5.2.2 $UCL = Geomean(X) * Geometric\ Standard\ Deviation(X)$

P16. METHOD PERFORMANCE

16.1 Limit of Detection: 1.0 MPN/100 mL.

16.2 Precision: RPD ≤ 20% (for high MPN values)

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed in accordance with state and federal regulations. Adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Analysts should review all data and calculations for correctness.

18.2 P12.2: Proficiency Test

18.2.1 MPN: Proficiency test results should fall within the range established by the provider.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 Average should fall within the acceptance range specified by the provider.

18.3.2 LCL should be greater than or equal to the lower acceptance limit specified by the provider.

18.3.3 UCL should be less than or equal to the upper acceptance limit specified by the provider.

18.4 P12.4: Ongoing Demonstration of Capability

18.4.1 MPN should fall within the acceptance range specified by the provider.

18.5 P12.5: Duplicates

18.5.1 RPD should be less than 20%. Exceedance of this limit may be deemed acceptable by referencing the well counts of the lowest non-zero value of the pair on the Micro MPN RPD Table.

18.6 P12.6: Sample Bottle LQC

18.6.1 Sample bottles must not auto-fluoresce.

18.6.2 Measured volume should be between 98 and 102 mL

18.6.3 Chlorine resiliency must be demonstrated at 15 mg/L for an 100 mL sample.

18.6.4 Bottles must exhibit sterility after 48 hours incubated at 35°C.

18.7 P12.7: Enterolert® LQC

18.7.1 *Enterococcus* spp. (namely *Enterococcus faecalis* and *Enterococcus faecium*) are the only ATCC-traceable organisms that should exhibit blue fluorescence.

18.8 P12.8: Colilert® LQC

18.8.1 *E. coli* subspp. are the only ATCC-traceable organisms that should exhibit blue fluorescence.

18.8.2 ATCC-traceable *E. coli* and *Klebsiella* spp. should exhibit a yellow color change darker than or equal to the Colilert/Colilert-18 comparator.

18.9 P12.9: Colilert®-18 LQC

18.9.1 *E. coli* subspp. are the ATCC-traceable organisms that should exhibit a yellow color change more equal to or more prominent than the Colilert/Colilert-18 Comparator.

18.10 P.12.12: Reagent Water Quality Control

18.10.1 Conductivity should be below 2 uS/cm.

18.10.2 Total organic carbon should be below 1 ppm.

18.10.3 Copper should be below 0.05 ppm.

18.10.4 Heterotrophic plate count should be less than 500 CFU/mL (MPN/mL).

18.10.5 Ammonia should not be detected (see reference method).

18.10.6 Total residual chlorine should be less than 0.1 mg/L.

18.11 The Quality Assurance Manager shall review all QC procedures and perform spot checks on routine analyses.

18.12 Duplicate recoveries of wastewater microbiological analyses are monitored by control charting.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 The samples cannot be reanalyzed because of the 8 hour sample holding time.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, data are taken through the corrective action process outlined by SOP-WI-30. The analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

P21. WASTE MANAGEMENT

Sample bottles, empty snap packs, and pipettes are not considered infectious and may be thrown in the trash. Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal. Place all used trays in an autoclavable biohazard bag and autoclave before disposal into municipal trash.

P22. REFERENCES

- 2009 TNI V1M5
- IDEXX Colilert®
- IDEXX Colilert-18®
- IDEXX Enterolert®
- 22 SM 9020 B
- 22 SM 9223 B
- 23 SM 9230 D

P23. TABLES, DIAGRAMS AND FLOWCHART

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

09-63235-01

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline		Combined information from SOP-WI-6, SOP-WI-7, and SOP-WI-8 into a single document. Incorporated 2019 VELAP audit comments about headspace and incubator thermometer placement.

SOP-WI-44

Drinking Water Coliforms

Baseline

Marianne Simko
LJT & Associates, Inc.
Environmental Programs Manager

3-20-19
Date

Jason Freischlag
LJT & Associates, Inc.
Laboratory Director, Technical Manager

3-20-19
Date

Mark Culver
LJT & Associates, Inc.
Quality Assurance Manager

3-20-19
Date

LJT & Associates, Inc.

Effective Date: 03/20/2019

SOP-WI-44

Drinking Water Coliforms

Baseline

Review Signatures			
Printed Name	Signature	Date	Purpose (new revision, DoC)
Jason Fleischlag		3-20-19	New Sop
Marianne Simko	Marianne Simko	3-20-19	New
Thera Lombardi		3-20-19	New
Marga Morris		03/20.19	New
Brian Cornwell		03-20-19	New SOP
Mark Culver		3-20-19	New

Title: Drinking Water Coliforms

P1. IDENTIFICATION OF THE TEST METHODS

Total Coliform and *E. coli* by SM 9223 Colilert (P/A or MPN)

P2. APPLICABLE MATRIX OR MATRICES

This method is suitable for the determination of total coliform and *E. coli* in an 100 mL potable water sample.

P3. DETECTION LIMIT

The limit of quantitation does not apply for MPN testing, as there is no viable procedure for its calculation. The limit of detection is set at one most probable number (MPN) per 100 mL for MPN testing but is not applicable for presence/absence testing. The MPN is statistically equivalent to the microbiological term colony forming unit.

P4. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the test method for the collection and analysis of potable water samples for the enumeration or qualification of total coliform bacteria and *E. coli*. This method is intended for use in the detection and confirmation of total coliforms and *E. coli* in potable water. Any positive sample for total coliform and/or *E. coli* is an indication of contamination.

P5. SUMMARY OF METHOD

The procedures described here is certified under SM 9223 B, which describes the determination of total coliform and *E. coli* by metabolism of the Colilert reagent, which causes sample yellowing and/or fluorescence, respectively. Positive identification of *E. coli* is demonstrated by blue fluorescence under a 6 watt, 365 nm UV light after incubation at 35.0 ± 0.5°C for 24 to 28 hours.

P6. DEFINITIONS

Certified Reference Material: A standard obtained from a third-party provider with a known analyte concentration, which may or may not be known by the laboratory and/or the analyst tasked with sample analysis.

Coliform bacteria: Rod-shaped bacteria prevalent in the environment and the gastrointestinal tract of most eukaryotic organisms. These bacteria are notable in their fermentation of lactose and are generally nonpathogenic but are indicative of contamination in potable water supplies.

Colilert®: Based on IDEXX's Defined Substrate Technology, is used for the detection of total coliform and E. *coli* in water. Colilert® uses ONPG and MUG as the defined substrate nutrient-indicators for total coliform and E. *coli*, respectively. These compounds, when hydrolyzed, create compounds that cause solution yellowing and fluorescence observable with a UV_{365nm} lamp, respectively. This reagent system is specifically formulated to achieve optimum sensitivity and specificity in the detection and identification of total coliform and *E. coli*. The test is capable of detecting one colony forming unit of total coliform and *E. coli* in 100 mL of water within 24 hours.

Disinfection: To destroy most (but not necessarily all) harmful or objectionable microorganisms by various treatment methods, such as chemical and thermal assault and ultraviolet light.

Escherichia coli: Commonly abbreviated as *E. coli*, this species of coliform bacteria is ubiquitous in the gastrointestinal tracts of most eukaryotic organisms. While mostly harmless, some strains are known to cause illness in humans and the presence of any strain in water is indicative of fecal contamination, which could suggest the presence of organisms more significant to human pathogenesis.

Limit of Detection (LOD): The lowest analyte concentration that produces a signal significantly differentiable from a matrix not containing the analyte of interest.

Lot Quality Control (LQC): Quality control procedure performed on incoming commercially-prepared reagents to ensure usability.

Most Probable Number (MPN): The number of organisms which are *most likely* to have produced the laboratory results noted in a particular test.

Safety Data Sheets (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill and handling precautions.

Sterilization: Destruction or removal of all viable organisms.

P7. INTERFERENCES

The presence of large amounts of non-coliform group bacteria in the sample may prohibit the use of this method. High salinity,algal populations, and color are also positively interfering for this analysis such that dilution may be required for mitigation.

P8. SAFETY PRECAUTIONS

A lab coat and nitrile gloves should be worn at all times. When analyzing samples with UV, UV protective glasses should be worn. Always use the UV light in a darkened room that others cannot access or contain UV light in a light box setup. Do not make eye contact with the UV light. Always point the light towards the object only. Testing is performed by individuals with bacteriological training since live microorganisms are used for quality control testing. Properly

disinfect any spills and sterilize all used containers before disposal according to appropriate regulations.

8.1 Adhere to standard laboratory safety procedures. Review the Laboratory Section's Chemical Hygiene Plan (CHP) and follow all laboratory safety procedures (including use of all prescribed PPE).

8.2 Refer to SDS sheets for all chemicals and reagents before beginning analysis for more safety information. The analysis involves handling of water samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples should protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.3 Keep bench areas free of clutter and clean bench surfaces with disinfectant before and after bacteriological testing.

8.4 Read labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels.

8.5 Always put tops back on containers when not in use.

P9. TOOLS, EQUIPMENT AND SUPPLIES

- Sterile Transfer Pipettes (to remove excess volume)
- Quanti-Tray Heat Sealer
- 97-Well Rubber Insert
- Quanti-Tray® 2000 MPN Table
- Incubator capable of maintaining a temperature of 35.0°C ± 0.5°C
- Storage refrigerator maintained at a temperature of 1 – 6°C
- Long wave (365-nm) ultra violet lamp with 6-watt bulb
- UV lamp light box

P10. REAGENTS AND STANDARDS

10.1 Colilert® snap-pack reagent

10.2 Quanti-Tray® 2000 (purchased from IDEXX Laboratories)

10.3 Sterile vessels, plastic containing sodium thiosulfate to neutralize up to 15 mg/L of residual chlorine (SM 9060 A2). 120 mL vessels are used for sample collection, while 150 mL vessels are used by the laboratory for mixing.

10.4 ATCC-traceable positive, total coliform positive, and negative control cultures (i.e., IDEXX-QC *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* cultures) [Colilert® LQC]

10.5 Single-strength tryptic soy broth (Sterile vessel QC)

10.6 Residual chlorine standard, 1.5 mg at 100 mL (Sterile vessel QC)

10.7 Quanti-Tray® 2000 Comparator for Colilert and Colilert-18 (purchased from IDEXX Laboratories)

P11. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Proper technique, equipment and sample preservation are critical to obtaining valid test results which can be used in the evaluation of process efficiency or water quality. These factors are especially critical in bacteriological sampling. The sample containers, sampling procedure, and handling after sampling can all be sources of errors long before testing actually begins. To ensure proper samples are collected, follow these instructions:

11.1 Bottles provided by the laboratory (as in P10.3) have a marked 100 mL fill line, a security seal around the lid, and solid sodium thiosulfate for residual chlorine removal.

11.2 To obtain a method-sterile, representative sample for bacteriological examination, collect samples as described in SOP-WI-21, *DW Sampling*.

11.3 Examination of bacteriological water samples should be performed immediately after collection. If testing cannot be started within 15 minutes of sampling, it is highly recommended, but not required, to ice or refrigerate, but not freeze, the sample. If samples are iced during transport or storage, use only enough ice to maintain the required preservation temperature. Excess ice can submerge the sample bottles after melting and potentially contaminate the sample.

11.4 The maximum allowable holding time for drinking water coliform samples is 30 hours. The storage temperature and holding time should be recorded as a part of the test data.

P12. QUALITY CONTROL

12.1 Record all lot and sample data on bench sheet before beginning analysis and at the completion of analysis. Document LQCs using the template document(s) listed in the relevant section.

12.2 Proficiency Testing

To demonstrate method performance, the laboratory annually participates in two rounds of proficiency testing for drinking water total coliform and *E. coli* for each method obtained from a certified third-party PT provider. PT samples are treated as typical samples in the normal production process where possible, including the same preparation, calibration, quality control and acceptance criteria, sequence of analytical steps, number of replicates, and sample log-in although an additional step is performed with PT sample login to establish traceability and record any preparation steps taken by the laboratory (i.e., those mandated by the PT provider).

12.2.1 Sign in the proficiency test samples in the Sample Receiving Logbook. Be sure to record the chain of custody number on the sample container.

12.2.2 Follow the PT provider's included instructions for preparing the sample and analyze according to P14.

12.3 Initial Demonstration of Capability

12.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analysts who have not completed this analysis in the previous twelve (12) months must complete a new initial demonstration per qualifying analyte.

12.3.2 A qualified analyst, designated by the Laboratory Director, must provide supervision to the unqualified analyst during the demonstration of capability process.

12.3.3 Colilert® MPN

12.3.3.1 The analyst should prepare four aliquots using the certified reference materials and sample containers provided by the qualified analyst according to the manufacturer's specifications.

12.3.4 Colilert® Presence/Absence

12.3.4.1 The analyst should prepare ten aliquots using the certified reference materials and sample containers provided by the qualified analyst according to the manufacturer's specifications.

NOTE: These aliquots are different samples so, when distinguishing in the paperwork, be sure to append the sample number (on the sample container) to the laboratory-assigned identifier to ensure differentiability.

12.3.5 The analyst then shall carry out the analysis of these samples as per P14. The interpretation of the analyst's results is discussed in P18.

12.4 On-going Demonstration of Capability

12.4.1 Analysts are required to re-verify their capability in these fields of testing through the on-going demonstration of capability. Prior to completing the on-going demonstration of capability, the analyst must review this SOP and sign off on the SOP Signature Sheet.

12.4.2 Colilert® MPN

12.4.2.1 The analyst should prepare a single aliquot using a certified reference material and sample container according to the manufacturer's specifications.

12.4.3 Colilert® Presence/Absence

12.4.3.1 Follow the procedural steps, outlined in P12.3.4, to complete this on-going demonstration of capability. For on-going demonstration of capability, the analyst is only required to analyze four single-blind aliquots.

12.4.4 The analyst shall carry out analysis and the rest of the batched samples (if present) as per P14. The interpretation of the analyst's results is discussed in P18.

12.5 Duplicates (QCD)

12.5.1 Per method requirements, samples are to be analyzed in duplicate at a 10% frequency. Interpretation of duplicate results is discussed in P18.

12.6 Sample Bottle LQC

12.6.1 The following procedure is applicable to 120 mL and 150 mL sterile vessels with pre-added sodium thiosulfate. If a lot of sample vessels does not meet its criteria, they should either be re-tested (in the case of sterility only) or returned to the manufacturer.

12.6.2 Sample bottle QC is documented as a single line item on the IDEXX Sample Bottle QC Log.

12.6.3 Each lot of sample bottles is to be checked for blue fluorescence prior to use with a 6 watt, 365 – nm ultraviolet light. Record as a "Yes/No" in the appropriate column.

12.6.4 The 100 mL mark on each lot of sample bottles must be checked for accuracy.

12.6.4.1 Fill a single vessel from the lot to the 100 mL fill line with deionized water.

12.6.4.2 Transfer the water to a 100 mL graduated cylinder. The recovered volume should be between 98 and 102 mL, or as otherwise certified by the manufacturer.

12.6.4.3 Record the recovered volume in the "Fill Line Accurate Against a Class A Graduated Cylinder" column.

12.6.5 Check the chlorine resiliency of each lot of sample bottles.

12.6.5.1 Inoculate a sample container from the lot with 1.5 mg of chlorine (equivalent to 15 mg/L chlorine at 100 mL). This can be done by addition of 100 mL of a 15 mg/L chlorine standard or a smaller volume from a concentrate (i.e., 10 mL of a 150 mg/L chlorine standard).

12.6.5.2 Gently swirl the sample container to allow for neutralization.

12.6.5.3 Check the residual chlorine of the water in the sample container using a chlorine strip or analyze for free residual chlorine according to SOP-WI-29. Record "Yes" in the "Chlorine Neutralization of 15 mg/L" if resulting residual is 0 mg/L or the actual value, if not.

12.6.6 Check sterility of each lot of sample bottles.

12.6.6.1 Label a sample container from the lot, minimally, with the test intention (i.e., "Sterility Check"), the lot number of the container, and the incubation start date.

12.6.6.2 Aseptically pour, at least, 30 mL of commercially prepared 1X Tryptic Soy Broth into a sample container from the lot.

12.6.6.3 Incubate the container at 35.0°C ± 0.5°C for 48 ± 2 hours. Record the start and end times as well as the initial and final incubator temperatures in the appropriate fields.

12.6.6.4 The broth should be free of turbidity and/or a visible white pellet at the bottom of the vessel. Record the result using a "+" or "-" according to the directions on the bench sheet.

12.6.6.5 Each lot of 1X Tryptic Soy Broth will be subjected to the following QC tests: *E. coli* and *P. aeruginosa* will be inoculated into separate vials of TSB and used as positive controls to exhibit growth at 35.0°C ± 0.5°C after 24 + 4 hours and an empty tube as a negative control, exhibiting no growth under the same conditions but triple the time (72+ hours). Record results using Tryptic Soy Broth QC Log.

NOTE: Single-strength Tryptic Soy Broth can be used past its expiration date by a single year if and only if the procedure in 12.6.6.5 is performed to ensure its continued usability. The aforementioned check extends the media's expiration date by two calendar months after the completion of the analysis.

12.7 Colilert® LQC

12.7.1 This procedure is applicable only to the Colilert® reagent. If a lot does not meet its criteria, they should be returned to the manufacturer. All applicable parameters should be recorded on the IDEXX Colilert Media QC Log.

12.7.2 Check for blue fluorescence under a 6 watt, 365 nm ultraviolet lamp. If the lot exhibits fluorescence, the rest of this procedure can be disregarded.

12.7.3 Prepare four aliquots of 100 mL deionized water in sterile 120 mL vessels.

12.7.4 One of these aliquots will be the negative control, which shall demonstrate method sterility. This vessel should be labeled, minimally, with the test intention (i.e., "Colilert QC"), the reagent lot number, the QC type (i.e., negative control, blank), and the incubation start date.

12.7.5 The rest of these aliquots shall be used for specificity testing via inoculation with species from a Colilert IDEXX QC pack (containing a method-positive species and two method-negative species). These vessels should be labeled, minimally, with the test intention (i.e., "Colilert QC"), the reagent lot number, the name (or recognized abbreviation) of the inoculated specimen, its lot number, and the incubation start date.

12.7.6 Add a snap pack of the Colilert reagent to each of the aforementioned aliquots and allow to dissolve.

12.7.7 Incubate all four aliquots at 35.0°C ± 0.5°C for 24-28 hours.

12.7.8 The aliquots should be examined for yellow color change and fluoresence with a 6 watt, 365 nm UV light. The interpretation of this QC is discussed in P18.

12.8 Quanti-Tray Sealer and UV Unit

12.8.1 Every month, ensure adequate sealing by add Bromocresol Purple dye to 100 mL of water and pour into a Quanti-Tray 2000 and run through sealer. If dye is observed outside the wells, perform maintenance or use another sealer.

12.8.2 Every month, disconnect the UV unit from power and clean using a Kimwipe and ethanol.

12.8.3 Record completion on the UV Light and Sealer QC Log taped to the sealer.

12.9 Quanti-Tray 2000 LQC

12.9.1 The following procedure is applicable to Quanti-Tray 2000. If a lot of trays does not meet its criteria, they should either be re-tested or returned to the

manufacturer. All applicable parameters should be recorded on the IDEXX Quanti-Tray 2000 QC Log.

12.9.2 Carefully pour 100 mL of commercially prepared 1X Tryptic Soy Broth into a tray from the lot.

12.9.3 Run the prepared tray through the sealer.

12.9.4 Label the tray, minimally, with the test intention (i.e., "Sterility Check"), the lot number of the container, and the incubation start date/time.

12.9.5 Incubate at 35.0°C ± 0.5°C for 48 ± 2 hours.

12.9.6 All wells should be free of turbidity and/or a visible white pellet.

12.10 Reagent Water Quality Control

12.10.1 The following procedure is required monthly when diluting client samples using laboratory-prepared reagent water (deionized water).

12.10.2 Allow deionized water system to dispense at least 1 L. During this time, record the withdrawal time, specific conductance, total organic carbon, and temperature on the Deionized Water QC Log.

12.10.3 Collect 100 mL of deionized water according to P11 of this SOP.

12.10.4 Analyze collected sample for heterotrophic bacteria (preferably following the IDEXX HPC for Quanti-Tray procedure) within 24 hours of collection. Interpretation of this QC is discussed in P18.

12.10.5 An aliquot of this reagent water should also be tested for chlorine residual (SOP-WI-29, *Chlorine by Pocket Colorimeter*) and ammonia (using test kit or method blank result from SOP-WI-2, *Ammonia*). Record all applicable parameters on the Reagent Water QC Log.

P13. CALIBRATION AND STANDARDIZATION

13.1 Thermometers

13.1.1 Calibrated, NIST-traceable thermometers with the bulb immersed in liquid (glycerin, water, or mineral oil) are kept in the Drinking Water E. coli incubator and the sample refrigerator. Thermometers are externally calibrated yearly and the Certificate of Calibration is retained in the laboratory's records.

13.1.2 There must be one thermometer per shelf in-use in the incubator. Alternatively, one thermometer may be placed on the top and bottom shelf of each incubator to bracket the incubator temperature conditions.

13.1.3 Position of thermometers shall be periodically adjusted to demonstrate uniform incubator temperature. Make notation of thermometer movements in Temperature Log or Equipment Maintenance Log, as space is available.

13.1.4 "In use" and "out of use" date/times are handwritten on each Certificate of Calibration, which is retained on the front of each incubator while the thermometer is in use.

13.1.5 The Drinking Water E. coli incubator is maintained at ~35°C. The temperature is checked twice daily, four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this incubator are 35.0°C ± 0.5°C.

13.1.6 The sample refrigerator is maintained at ~4°C. The temperature is checked twice daily, at least four hours apart, by reading the NIST-traceable thermometer. The temperature is transcribed onto the Temperature Log. The acceptable temperatures of this refrigerator are from 1.0°C to 6.0°C.

P14. PROCEDURE

NOTE: Steps required specifically for MPN analysis are prefixed as such.

14.1 MPN ONLY: Turn on Quanti-Tray 2000® sealer prior to analysis to allow for its warm-up. The sealer is ready to use when the indicator light turns green.

14.2 Preparing the method-sterile workspace

14.2.1 Spray and wipe down the work surface with disinfectant.

14.2.2 Place Kimwipe(s) over the sprayed area.

14.2.3 Place all samples to be analyzed on the Kimwipe(s) and allow to equilibrate to ambient temperature.

NOTE: Set up analyses for drinking water matrices separately, as cross-contamination may occur if set up with wastewater matrices.

14.3 Sample Prep

14.3.1 Record all sample initial conditions in the applicable columns of the Coliform and *E. coli* and Presence/Absence Bench Sheets.

NOTE: Ensure lot numbers are correct on the bottom of the page. If they are not, add an additional entry on the bottom of the page and use a concise symbol (i.e., an asterisk) to denote where that lot information is applicable.

14.3.2 Check to ensure sample has at least 1 inch of headspace to allow for adequate mixing. If headspace is adequate, shake the sample, at a minimum of 25 times at a 45 degree angle, to mix. Remove the excess volume utilizing a sterile pipette, discarding sample into waste disposal beaker until total sample volume in the sample container is 100 mL. If headspace is inadequate, transfer to 150mL sterile container to mix, as above, and remove the excess volume utilizing a sterile pipette. If sample color is prominent, preserve a small amount of sample in a 10 mL vial for comparison during sample analysis.

NOTE: A visual comparator can be made for this headspace requirement by filling a 120 mL sterile vessel with ~107 mL of deionized water.

14.3.3 All samples should be checked for chlorine residual by pipetting two drops of sample excess onto a chlorine strip using a sterile pipette. If exactly 100 mL of sample is received, this step still needs to be performed. The removed drops should not significantly impact the analysis. Note the result of this on the sample chain of custody form. If chlorine is present, inform the Laboratory Director and do not proceed with the analysis.

NOTE: If sample is suspected to be highly contaminated, it may be pertinent to perform a dilution (recommended starting dilution is 1:10). Record the dilution factor used on the bench sheet. Be sure to shake the sample prior to dilution and use a sterile pipettor to retrieve the required amount of sample. The reagent water incurs an additional quality control procedure (P12.11).

14.3.4 Open a snap-pack of the Colilert reagent and pour into 100 mL of sample. Mix to dissolve reagent by gentle inversion as to prevent foaming. If foaming does occur, do not continue analysis until foam dissipates.

NOTE: If a blue flash is observed upon introduction of the media, inform the Laboratory Director and do not proceed with the analysis. This should never need to be observed, as the step in 14.3.3 is indicative of the same property.

14.4 MPN ONLY: Traying Samples for Enumeration

14.4.1 Open a Quanti-Tray by pinching it to form a U-shape and pulling the foil tab. Pour the sample into the tray and gently tap the bottom of the tray to release entrapped air.

14.4.2 Align Quanti-Tray well side down on the rubber insert. Slide the Quanti-Tray into the sealer until the motor begins to draw it. When done, remove Quanti-Tray from the back of sealer.

14.4.3 Label tray, minimally, with chain of custody number and/or standard number, site, analyte identifier, date, time, and analyst initials. Do not pre-label trays, as this may damage the sealer.

14.4.4 Immediately transfer all tests to the Drinking Water E. coli incubator well side down. Do not stack too many tests on top of each other because it will block air flow in the incubator.

NOTE: Be sure to turn the sealer off when finished.

14.5 Incubation Stop and Reading Tests

14.5.1 Allow tests to incubate 24 + 4 hours. The 28 hour upper time limit allows for re-incubation, if color changes are ambiguous. Incubation times may be truncated in the event that the test is completely positive for *E. coli* (i.e., all 97 wells of an MPN test are yellow and fluorescing after 14 hours). While not standard practice, negative readings obtained after the upper time limit are also considered valid negative results.

NOTE: Test results may be read prior to their incubation windows solely for the purposes of preliminary reporting to the client. In correspondence with the client, these data should be qualified as a minimum possible result. Tests must then be returned to the incubator, if removed, and allowed to incubate for their full incubation time.

14.5.2 Check the test for yellow color change. If yellow color changes observed are equal to or more prominent than the comparator, then the sample is positive for total coliform.

14.5.3 Record total coliform results in applicable logbook. For presence/absence, record the presence/absence of total coliform as a “+” or “-“, respectively. For MPN testing, record the large and small positive well counts.

14.5.4 Check the test under a 6 watt, 365 nm UV light for blue fluorescence within 5 inches of the sample in a dark room or a light box. If fluorescence observed is equal to or more prominent than the comparator and the well was considered positive in 14.5.2, the well is considered positive for *E. coli*.

14.5.5 Record *E. coli* results in applicable logbook. For presence/absence, record the presence/absence of total coliform as a “+” or “-“, respectively. For MPN testing, record the large and small positive well counts.

14.5.6 MPN ONLY: Use the paired well counts found in the previous steps and find their intersection on the IDEXX Quanti-Tray 2000 MPN table posted in the Micro Room. This table can also be found in P23 of this SOP. Those values are the total coliform and *E. coli* concentrations, respectively, in MPN/100 mL. Transcribe these values on the bench sheet.

P15. DATA ANALYSIS AND CALCULATIONS

15.1 Most Probable Number (MPN)

15.1.1 Concentration of analyte most likely to have caused the yellow coloration and/or fluorescence observed by Quanti-Tray.

15.1.2 MPN values are calculated using the IDEXX Quanti-Tray 2000 MPN Table, multiplied by any applicable dilution factor.

15.2 Relative Percent Difference (RPD)

15.2.1 The relative percent difference between a sample and its duplicate.

15.2.2 Applicable Formulas

15.2.2.1 $$RPD(X_1, X_2) = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} * 100\%,$$

where X_1 and X_2 are results of samples analyzed in duplicate

NOTE: If either value is below the limit of quantitation, add one to both results before performing this calculation.

15.3 Average

15.3.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability.

15.3.2 Applicable Formulas

15.3.2.1 $$Geomean(X) = e^{\frac{\sum \ln(X_i)}{n}},$$

where X contains n values X_i

15.4 Lower Control Limit (LCL)

15.4.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability divided by the geometric standard deviation. Conceptually similar to an arithmetic mean minus a standard deviation, the lower control limit (LCL) is used to assess acceptability of an analyst's IDC.

15.4.2 Applicable Formulas

15.4.2.1 $Geometric\ Standard\ Deviation(X) = e^{\sqrt{\frac{(\sum \ln(X_i) - \overline{\ln(X)})^2}{n-1}}}$,
where X contains n values X_i and has logarithmic average $\overline{\ln(X)}$

15.4.2.2 $LCL = \frac{Geomean(X)}{Geometric\ Standard\ Deviation(X)}$

15.5 Upper Control Limit (UCL)

15.5.1 The geometric mean of all aliquots analyzed during an initial demonstration of capability divided by the geometric standard deviation. Conceptually similar to an arithmetic mean minus a standard deviation, the upper control limit (UCL) is used to assess acceptability of an analyst's IDC.

15.5.2 Applicable Formulas

15.5.2.1 $Geometric\ Standard\ Deviation(X) = e^{\sqrt{\frac{(\sum \ln(X_i) - \overline{\ln(X)})^2}{n-1}}}$,
where X contains n values X_i and has logarithmic average $\overline{\ln(X)}$

15.5.2.2 $UCL = Geomean(X) * Geometric\ Standard\ Deviation(X)$

15.6 Precision

15.6.1 Precision is to be established, as described in SM 9020B, by results from 15 duplicate analysis with detectable results in at least one sample.

15.6.2 For each duplicate pair meeting this criteria for total coliform and/or E. coli, the Quality Manager shall input the required data into the Drinking Water Precision Calculation control chart.

15.6.3 For each sample/duplicate pair, the base-10 log difference, R, is calculated in Excel, as follows: =IFERROR(ABS(LOG10(B2)-LOG10(C2)),IF(AND(B2=0,C2=0),"",ABS(LOG10(B2+1)-LOG10(C2+1)))). This formula also complies with SM 9020B in that it accounts for one result being less than the limit of quantitation by adding "1" to both results.

15.6.4 Being interconvertable with RPD, which is more standardly used by the laboratory, the log difference method is then converted to RPD using the following Excel formula: =IF(COUNT($D:$D)<15,0.2,2*ABS(1-2/(POWER(10,3.27*AVERAGE($D:$D))+1))). Note that this formula

defaults the laboratory's precision goal to 20% unless fifteen data points are provided for this calculation.

15.6.5 The formula that describes the relationship between RPD and R is as follows:

$$RPD = 2\,x\left(1 - \frac{2}{10^{3.27R} + 1}\right) x\ 100\%$$

P16. METHOD PERFORMANCE

16.1 Limit of Detection: 1.0 MPN/100 mL.

16.2 Precision: RPD limit specified by control chart calculation, described in P15.6. RPD limit ideally 20%, at high MPN values.

P17. POLLUTION PREVENTION

All wastes from these procedures shall be collected and disposed in accordance with state and federal regulations. Adjustments should be made to reduce waste.

P18. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Analysts should review all data and calculations for correctness.

18.2 P12.2: Proficiency Test

18.2.1 MPN: Proficiency test results should fall within the range established by the provider.

18.2.2 Presence/Absence: 90% of proficiency test results should match results obtained by the provider with no false negatives per analyte.

18.3 P12.3: Initial Demonstration of Capability

18.3.1 MPN: Average should fall within the acceptance range specified by the provider.

18.3.2 MPN: LCL should be greater than or equal to the lower acceptance limit specified by the provider.

18.3.3 MPN: UCL should be less than or equal to the upper acceptance limit specified by the provider.

18.3.4 Presence/Absence: 90% of results should match results obtained by the provider with no false negatives per analyte.

18.4 P12.4: On-going Demonstration of Capability

18.4.1 MPN should fall within the acceptance range specified by the provider.

18.4.2 Presence/Absence: If analyzing 4 aliquots, all results should match results obtained by the provider. If analyzing 10 aliquots, 90% of results should match results obtained by the provider with no false negatives per analyte.

18.5 P12.5: Duplicates

18.5.1 MPN: RPD should be less than the precision limit specified in the control chart (P15.6).

18.5.2 Presence/Absence: Both aliquots should have identical total coliform and E. coli qualifications.

18.6 P12.6: Sample Bottle LQC

18.6.1 Sample bottles must not auto-fluoresce.

18.6.2 Measured volume should be between 98 and 102 mL

18.6.3 Chlorine resiliency must be demonstrated at 15 mg/L for an 100 mL sample.

18.6.4 Bottles must exhibit sterility after 48 hours incubated at 35°C.

18.7 P12.7: Colilert® LQC

18.7.1 *E. coli* subspp. are the only ATCC-traceable organisms that should exhibit blue fluorescence.

18.7.2 ATCC-traceable *E. coli* and *Klebsiella* spp. should exhibit a yellow color change darker than or equal to the Colilert/Colilert-18 comparator.

18.8 P.12.10: Reagent Water Quality Control

18.8.1 Conductivity should be below 2 uS/cm.

18.8.2 Total organic carbon should be below 1 ppm.

18.8.3 Copper should be below 0.05 ppm.

18.8.4 Heterotrophic plate count should be less than 500 CFU/mL (MPN/mL).

18.8.5 Ammonia should not be detected (see reference method).

18.8.6 Total residual chlorine should be less than 0.1 mg/L.

18.9 The Quality Manager shall review all QC procedures and perform spot checks on routine analyses.

18.10 Duplicate recoveries of MPN analyses are monitored by control charting.

P19. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

19.1 The samples cannot be reanalyzed because of the 30 hour sample holding time.

P20. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 If data is unacceptable for any reason, data are taken through the corrective action process outlined by SOP-WI-30. The analyst should review their analytical technique prior to conducting this analysis again.

20.2 Analyze a quality control sample from a PT provider to ensure that any deficiencies have been resolved.

P21. WASTE MANAGEMENT

Sample bottles, empty snap packs, and pipettes are not considered infectious and may be thrown in the trash. Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal. Place all used trays in an autoclavable biohazard bag and autoclave before disposal into municipal trash.

P22. REFERENCES

- 2009 TNI V1M5
- IDEXX Colilert®
- 22 SM 9020 B
- 22 SM 9223 B

P23. TABLES, DIAGRAMS AND FLOWCHARTS

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

09-63235-01

CHANGE HISTORY LOG

Revision	Effective Date	Description of Changes
Baseline		Combined information from SOP-WI-14 and SOP-WI-15 into a single document. Incorporated 2019 VELAP audit comments about headspace and incubator thermometer placement.