QUALITY ASSURANCE MANUAL *for* INDIANA WASTEWATER LABORATORIES

2019 EDITION

INDIANA Water Environment Association

QUALITY ASSURANCE MANUAL
for
INDIANA WASTEWATER LABORATORIES

1st Edition:	**November 2002**
2nd Edition:	**July 2006**
3rd Edition:	**March 2009**
4th Edition:	**March 2019**

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ACKNOWLEDGEMENTS

It is our sincere hope that this manual will help any Indiana wastewater facility that utilizes it develop a laboratory Quality Assurance Plan that will, in turn, generate sound scientific laboratory data.

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Our mission, as a group, is to be a useful resource to all wastewater facilities throughout Indiana. We hope that this document is easy to follow and helpful.

The IWEA Lab Committee
November 2002

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INTRODUCTION

This manual is being distributed as a guidance document for laboratory personnel. The manual contains quality assurance and quality control information, detailed methods for the basic parameters that are reportable with a National Pollutant Discharge Elimination System (NPDES) permit, evaluation checklists and an appendix. This manual should be considered a tool that a wastewater laboratory can utilize to generate quality data. ***It is the responsibility of each facility to develop and follow their own unique quality manual.***

Also available on the IDEM Website are Bench Sheets examples which may be downloaded and utilized in the laboratory. Modifications have been made to some of them to reflect method revisions in this manual. ***NOTE*****: new Bench Sheets examples have been added for Hach TNTplus ammonia-N and TNTplus phosphorus-P analysis in addition to a Bench Sheet for Mixed Liquor Microbiological Examination. The example Bench Sheets are located at:** http://www.in.gov/idem/cleanwater/2443.htm

The principal parameters monitored and reported for municipal permits include Biochemical Oxygen Demand, pH and Total Suspended Solids and may also include Total Residual Chlorine, and/or Ammonia as Nitrogen, and/or Total Phosphorus. Other municipal permit parameters will include, but are not limited to, *Escherichia coli* (*E. coli*), certain metals and oil and grease.

It is an old axiom that the result of any test procedure can be no better than the sample on which is it performed. Obtaining good results will depend to a great extent upon five major activities:

1. Collecting representative samples
2. Proper sample handling and preservation
3. Adhering to adequate chain-of-custody and sample identification
4. Adequate quality assurance and quality control
5. Properly analyzing the sample

These areas are equally important for insuring the NPDES reported data is of the highest validity and quality.

Monitoring and reporting Effluent discharges under a (NPDES) permit requires specific test methods. These approved method numbers can be found in the latest edition of the CODE OF FEDERAL REGULATIONS, PROTECTION OF THE ENVIRONMENT, 40, Part 136. Only these methods are allowed for reporting purposes on the Discharge Monitoring Report (DMR) and the Monthly Report of Operations (MRO). Not every approved method is contained in this manual. The methods identified by number can be found in either: *Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020,* or *Standard Methods for the Examination of Water and Wastewater, 21st and 22nd editions.* **(*NOTE*: The 21st and 22nd editions of *Standard Methods* are now approved, and all approved methods are listed in the latest version of 40 CFR Part 136 dated 08/28/2017 with an effective date of 09/17/2017).** One or both of these references or copies of the methods should be a part of every wastewater laboratory.

Certain test methods may be specified for certain parameters in the NPDES permit. The methods specified should be capable of detecting that parameter at the limits imposed in the permit. If a method is not specified and doubt arises as to the acceptability of the method, call IDEM's Office of Water Quality, Compliance Evaluation Section.

The 4th edition of the Quality Assurance Manual for Indiana Wastewater Laboratories contains revisions to Methods in the 3rd Edition dated March 2009. In addition, the latest Definition and Procedure for the Determination of the Method Detection Limit—Revision 2 effective September 17, 2017 has been included in the Quality Assurance and Quality Control Section of the manual.

New methods added to the 4th edition are the determination of ammonia-N and Total Phosphorus referencing Hach TNTplus methodology.

An Appendix containing documents relating to Laboratory Safety, Sampling Procedures, Laboratory Grade Water, Method Detection Limit (MDL) Determination Condensed Procedure, Pipettor Calibration, Preparation of Solutions Commonly Used in the Laboratory and Temperature and Thermometer Calibration has been added to the manual.

At the time the 4th edition of the Quality Assurance Manual for Indiana Wastewater Laboratories was completed, Standard Methods 23rd edition had been released but is not yet approved in 40 CFR Part 136. Revisions made in this document are referenced to earlier editions of Standard Methods.

The information presented in this Manual has been prepared in accordance with recognized principles and is for general information only. While it is believed to be accurate, this information should not be used or relied upon for any specific application without competent professional examination and independent verification of its accuracy, suitability, and applicability.

This Manual is intended to assist wastewater facilities throughout the state of Indiana to develop a laboratory quality assurance plan for the purpose of generating sound scientific laboratory data. While it attempts to thoroughly address specific topics, it is not possible to be inclusive of all information and is therefore intended only as a guidance and/or reference document to generate quality data. Thus, the information in this Manual must be understood as a tool rather than an exhaustive statement and is not intended to create any legal obligations, which may otherwise be defined by statute, regulations, and standards. To the extent that this information references practices or procedures which are not required by a statute, regulation, or standard, the Manual cannot, and does not, create additional legal obligations.

This Manual is meant as a guideline only – the provisions contained are not a substitution for, or modification of, any specific contract or other legal requirements. Nothing in this Manual is intended to create a warranty, either express or implied, by Indiana Water Environment Association and/or any of the contributing authors. Lastly, it is not meant to replace or modify the applicable standard of care governing the performance of services related herein.

Specific brand names, products and establishments listed in this manual are given as examples only and do not represent an endorsement by the Indiana Department of Environment Management or the Indiana Water Environment Association.

QUALITY ASSURANCE and QUALITY CONTROL

I. Introduction

Environmental data produced in your laboratory is an estimate of the true values for the parameters (Biochemical Oxygen Demand, pH, Total Suspended Solids, etc.) being measured. The results you obtain are influenced by natural changes in the samples plus errors that occur during the collection and analysis of the samples. We realize, that as laboratory personnel, we have little if any control over the natural changes that occur in the samples. But we can reduce and potentially eliminate those errors that result from the human factor involved with the sample collection and analysis procedures. The focus of this section of the Quality Assurance Manual for Indiana Wastewater Laboratories is to provide you with the information necessary to help reduce those errors.

Let's begin by defining some terms relating to Quality Assurance and Quality Control:

Note: The following link has a more comprehensive Glossary of Quality Assurance Terms
https://archive.epa.gov/emap/archive-emap/web/html/qa_terms.html#ss

Accuracy - how close the measured result is to the true value for a specific test;

Bias - results that are consistently greater than or less than the true value due to systematic errors in the procedure;

Example: The barometer used to calibrate the dissolved oxygen probe reads higher than the true barometric pressure. This results in consistently high dissolved oxygen readings during the BOD_5 analysis.

Blind Standard - A blind standard is a sample obtained from an outside source whose concentration is validated and known by the supervisor but unknown to the laboratory personnel. This sample is analyzed in the laboratory and the result submitted to a supervisor. If the laboratory result is within acceptable levels, no further action is necessary. If the laboratory result is outside the acceptable levels, appropriate measures are taken to trouble shoot the problem to prevent continuing problems within the laboratory. Blind samples are analyzed every six months as part of the quality assurance/quality control program.

Calibration Check Standard - A standard prepared or purchased by the laboratory with a known/validated concentration. This type of standard is used to verify a previously established calibration curve and/or the accuracy of the analytical system. This standard should not be prepared from the same source as the calibration standards. This standard is also referred to as a "second source" standard, meaning prepared from a different lot number than the calibration standards or purchased from a different manufacturer.

Example: The laboratory analyst calibrates the ammonia apparatus using 0.1 mg/L, 1.0 mg/L and 10. mg/L standards and obtains an acceptable slope of 97%. Halfway through the analysis of 14 samples, the analyst analyzes a "second source" 3.0 mg/L Calibration Check Standard to make sure the ammonia apparatus is still in calibration. The 3.0 mg/L standard gives a result of 3.08 mg/L that is within 5% of the true value, thus the analyst continues analysis with the knowledge the ammonia apparatus is still in

calibration. NOTE: Although these may not be the exact standards used in your laboratory, any standards used should be in factors of ten and bracket the expected sample concentration(s). Refer to the determination of the individual analytical procedures for specific details.

Data Quality Objectives (DQOs) - the decision made as to how accurate analytical results must be for regulatory and/or process control purposes;

Example: A laboratory is required to analyze for Total Suspended Solids on the Influent sample as part of their NPDES discharge permit. The plant superintendent requests Volatile Suspended Solids analysis on the Influent for process control purposes. This lab might set a DQO of 10% RPD for Total Suspended Solid duplicates for reporting purposes and a DQO of 20% RPD for Volatile Suspended Solid duplicates for control purposes.

Duplicate - in this document, a duplicate is the smallest number of replicates (two) analyzed to check for precision. Another definition commonly used is: two samples collected at the same place at the same time. The analysis of this type of duplicate checks for representativeness;

Laboratory Control Sample - a measured volume of laboratory reagent water to which a known amount of the analyte being tested for is added followed by the same treatment given to the samples **(also be referred to as a Laboratory Fortified Blank)**;

Laboratory Fortified Matrix or Matrix Spike - a measured volume of sample to which a known amount of the analyte of interest is added followed by the same treatment given to all other samples in the analytical run;

Laboratory Quality Control Standard - a purchased standard certified to have a known concentration of analyte in it. Analysis of this type of standard is used to check for accuracy and bias;

Matrix - what the sample consists of (drinking water, wastewater, sludge, soil, etc.);

Method Blank - a clean sample processed simultaneously with and under the same conditions as samples containing an analyte of interest through all steps of the analytical procedure;

Precision - a measure of the agreement of results between two or more measurements of the same sample collected during the same sampling event;

Quality Assurance - the development of the procedures used during sampling and analysis needed to produce accurate and precise results, plus the review of the results to determine if the developed procedures are adequate;

Examples: Training of personnel regarding proper sampling and analysis techniques, reviewing temperature data for correct readings, writing step by step procedures for the analysis of each parameter in your NPDES discharge permit.

Quality Control - the daily functions carried out during the collection and analysis of samples to help produce accurate and precise results;

Some examples of quality control: proper cleaning of sampling equipment and bottles; proper sample collection, preservation and handling; calibration of testing instruments;

maintaining control charts and taking corrective action when needed.

Quality Control Analysis - Analysis of blanks, duplicates, standards and matrix spikes are performed on a routine basis and at the frequency listed in **Table 1**. Blanks are processed through the entire analytical procedure including the addition of any preservatives the associated samples have in them. The results from the method blanks are treated as specified in each analytical method. Results from method blanks, duplicates, standards, and matrix spike analyses are recorded on the daily log sheets and on their respective control charts.

NOTE: The Quality Control Sample Frequency shown in Table 1 may not be attainable in your laboratory. Refer to your NPDES permit and/or discuss with your permitting agency inspector the frequency required for your facility.

Table 1
Quality Control Sample Frequency

Test	Calibration	Calibration Check or other Standard	Reagent Blanks	Duplicates/ Replicates	Matrix Spikes
BOD_5	Calibrate DO meter each day samples are setup or read	3 GGA Standards with each batch of samples	*Daily	*Daily	NA
TSS	Daily - Zero and Calibrate Balance Monthly - Checked with (2) certified weights Annually - Serviced by certified technician	NA	*Daily	*Daily	NA
Ammonia-N TNTplus	NA	*Daily – see Note above	*Daily – See Note above	*Daily	*Daily – see Note above
Ammonia-N Probe	*Daily	*Daily	*Daily	*Daily	*Daily
Phosphorus-P TNTplus	NA	*Daily – see Note above	*Daily – see Note above	*Daily	*Daily – see Note above
Phosphorus-P Ascorbic Acid	Refer to the Total Phosphorus, Ascorbic Acid Method for frequency required	*Daily	*Daily	*Daily	*Daily
pH	*Daily	*Daily	NA	*Daily	NA
Residual Chlorine	Initially, and with each reagent change or if Calibration Check Standard exceeds acceptance range	*Daily – see Note above	NA	*Daily – see Note above	*Daily – see Note above
Bacteriological	NA	NA	*Daily	Weekly or 10% of samples	NA

GGA = Glucose-Glutamic Acid *Daily = Each time analysis is performed

Random Error - Error that results from inconsistent sampling and/or analytical methods;
Examples: (1) Sampler collects a sample and allows it to settle while filling out the sample collection record. The sampler then pours a portion of the sample into a composite sample bottle without mixing it, thus the sample added is not representative of the sample collected or, (2) while analyzing for Total Suspended Solids, a small twig is in the sample being filtered. By failing to remove this twig, the analyst is introducing random error into the analysis.

Reagent Blank: a sample consisting of reagent(s), without the target analyte or sample matrix, introduced into the analytical procedure at the appropriate point and carried through all subsequent steps to determine the contribution of the reagents and of the involved analytical steps to error in the observed value;

Reference Samples - samples obtained from an approved external source whose concentration is either known or unknown to the laboratory. Results from reference samples when the concentration is not known by the laboratory are submitted to the provider to determine whether the results produced by the laboratory are within the acceptable range. If the submitted results are outside the acceptable range, the laboratory must take corrective action to resolve the issue and obtain a new reference sample to verify the corrective actions were effective. Reference samples should be analyzed quarterly. A link for approved sources of reference samples may be found on the EPA website at epa.gov. (www.epa.gov/compliance/discharge-monitoring-report-quality-assurance-study-program#about).

> **A. DMR-QA Samples as Reference Samples** - Under the requirements of the NPDES program, major and selected minor permittees must participate in the DMR-QA Study. The study is conducted annually for major dischargers while minor dischargers typically participate every three years. Samples are obtained from an approved external vendor and fulfill the requirements for reference samples.

Replicate(s) - two or more analyses for the same parameter taken from a single sample;

Representativeness - measures how well the results actually reflect the sample site you are trying to monitor. Representativeness is achieved by making sure proper sampling techniques are used, using the correct analytical procedures, meeting sample holding times, and the analysis of sample duplicates/replicates;

Standard Operating Procedure (SOP) - a written document for each analytical test performed in the laboratory documenting in step-wise detail the entire procedure followed for the analysis of samples in the laboratory.

Systematic Error - Error that results from improper calibration and/or the consistent incorrect use of equipment or procedures;

> Examples: (1) In preparing calibration standards for ammonia analysis, laboratory personnel routinely use a graduated cylinder instead of a Class A volumetric flask to adjust the final volume to 100 mL. By doing this, systematic error may be introduced into the analysis or, (2) an uncertified thermometer was used in a drying oven used for Total Suspended Solids analysis and even though the thermometer read 104°C, the actual oven temperature was 112°C.

II. Quality Assurance Measures

Laboratory Cleanliness - The laboratory is to be kept clean and organized at all times. The room temperature should be kept as constant as possible. Appropriate actions are taken to maintain air quality.

Personnel Training - Training for all personnel involved with laboratory analysis must be documented showing that they are capable of meeting the Data Quality Objectives (DQOs). Initial performance can be demonstrated through the use of Quality Control Standards and internal or external split samples. Continuing training on a regular basis should be provided to help maintain competence of analytical skills.

Equipment Maintenance - Files are maintained for each piece of laboratory equipment. These files contain the operating manual, a preventative maintenance schedule, and a record of any maintenance and repairs performed. The maintenance and repair record is to contain the following information: nature of problem, date of repair, maintenance/repair performed, person performing maintenance/repair, and cost, if any. These records will help the laboratory personnel determine if results were affected by an instrument malfunction. As part of the routine equipment maintenance, analytical balances are serviced annually, and dissolved oxygen and ammonia probe membranes may need to be replaced every two to four weeks unless readings become unstable, then these membranes are replaced immediately.

Analytical Reagents - All chemicals and reagents used in the laboratory are analytical grade or better (such as ACS). Upon receipt in the laboratory, each chemical is marked in permanent ink with date received on the label and when opened for the first time. Any reagents or solutions prepared in the laboratory must have a label with the date prepared and by whom. Because labels are replaced, this information is also recorded in a permanent record as part of the three year record keeping requirement. Chemicals and reagents are stored away from direct sunlight and, if necessary, refrigerated to prevent deterioration. If refrigerated, chemicals should be brought to room temperature before aliquots are measured. Stock chemicals and reagents are transferred to a clean container prior to weighing, pipetting, etc., to prevent contamination. All solutions or reagents are discarded and ordered or prepared fresh after being opened one year unless the analytical method specifies a shorter time period for replacement.

All chemicals are stored in a safe manner and segregated by hazard type according to the Global Harmonized System, https://www.osha.gov/dsg/hazcom/global.html. The hazard types are identified with pictograms.

All flammable chemicals are to be stored in a fireproof cabinet and strong acids and bases are to be stored separately from each other.

A Safety Data Sheet (SDS) is to be on file and accessible for all purchased chemicals and reagents used by the laboratory personnel.

Laboratory Reagent Water – refer to Appendix

Labware Cleaning - After each use, glassware and plastic ware is washed with detergent, rinsed with tap water followed by a thorough rinse with laboratory reagent water. After drying, it is stored in a cabinet. There are appropriate glassware cleaning procedures for specific tests, for example, glassware used for total phosphorus analysis is washed in non-phosphate detergent, acid-washed after each use and kept separate from other laboratory glassware. (The best choice would be using a detergent that is both ammonia and phosphate free, thus eliminating having different detergents for different procedures.) Care must be taken to store BOD_5 bottles

dry to prevent the growth of bacteria or algae in the bottle. The BOD_5 siphon tubing should be cleaned monthly with dilute bleach solution (25 mL bleach/liter of laboratory reagent water) inspected daily for growth and replaced or cleaned more often if any growth is observed. All containers used to store laboratory reagent water are inspected for growth or other signs of contamination prior to withdrawing water from them. If growth or signs of contamination are observed, the laboratory reagent water is discarded, and the container cleaned with dilute hydrochloric acid followed by rinsing with tap water and a thorough rinsing with laboratory reagent water.

Instrument Calibration and Temperature Records - The pH meter, dissolved oxygen meter, and ammonia ion selective electrode are calibrated each day they are used. If they are used throughout the day, a calibration check is repeated if more than two hours has passed following the initial calibration. Temperature of incubators and refrigerators are measured on thermometers with their bulbs immersed in liquid, **see Note below**. Each day the temperature of the incubators and refrigerators are recorded on a log sheet or in a logbook. The temperatures of the drying oven(s), muffle furnace and bacteria incubation baths are recorded on the log sheet or in a logbook when these pieces of laboratory equipment are used for analysis. The temperature of the autoclave is read using a maximum reading thermometer and this reading recorded on the log sheet or in a logbook. When equipment temperatures read outside the required range, the equipment thermostats are adjusted, and this adjustment is recorded on the log sheets or in the logbook.

Thermometers used in the laboratory are calibrated annually against a thermometer traceable to an NIST (National Institute of Standards and Technology) certified thermometer. The NIST thermometer must have been certified within the past five years. Any correction factors associated with the NIST certified thermometer are recorded on the thermometer calibration log sheet and any correction factors associated with the laboratory thermometer(s) are noted on a tag attached to the thermometer. If the liquid column in the thermometer becomes separated, the thermometer in no longer accurate and must be replaced. An alternative to calibrating laboratory thermometers is to purchase factory certified thermometers traceable to NIST thermometers. These thermometers are sealed in a clear container with the bulb in a medium appropriate to the equipment temperature being measured. Each thermometer comes with a unique serial number, a certificate of NIST traceability, and the required re-certification and/or expiration date.

> **Note: Thermometers are calibrated for total immersion or partial immersion. Those calibrated for partial immersion must be immersed only to the depth of the etched circle around the stem of the thermometer just below the thermometer scale readings. Those calibrated for total immersion must be completely immersed in the matrix being measured.**

Analytical balances are zeroed daily, and the calibration checked at least monthly. One of these weights is in the milligram and one in the gram range. Documentation of the daily zeroing and monthly weight checks are recorded in a logbook. If the balance readings are not within 0.5 mg of the certified weight, a certified technician must service the balance. Balances are to be serviced at least annually.

All records relating to instrument calibrations, thermometer calibrations and temperature logs must be retained a minimum of three years.

III. Calibration

Much emphasis is placed on the analysis of known standards, reference samples, blanks, duplicates and blind standards to document accuracy, bias, and precision, but the importance of the calibration curve may be overlooked during the analytical procedure. Some instruments come with pre-programmed calibration curves, some analysts hand-draw their calibration curves or use scientific calculators to construct the curve, and the use of computer programs is increasingly being used.

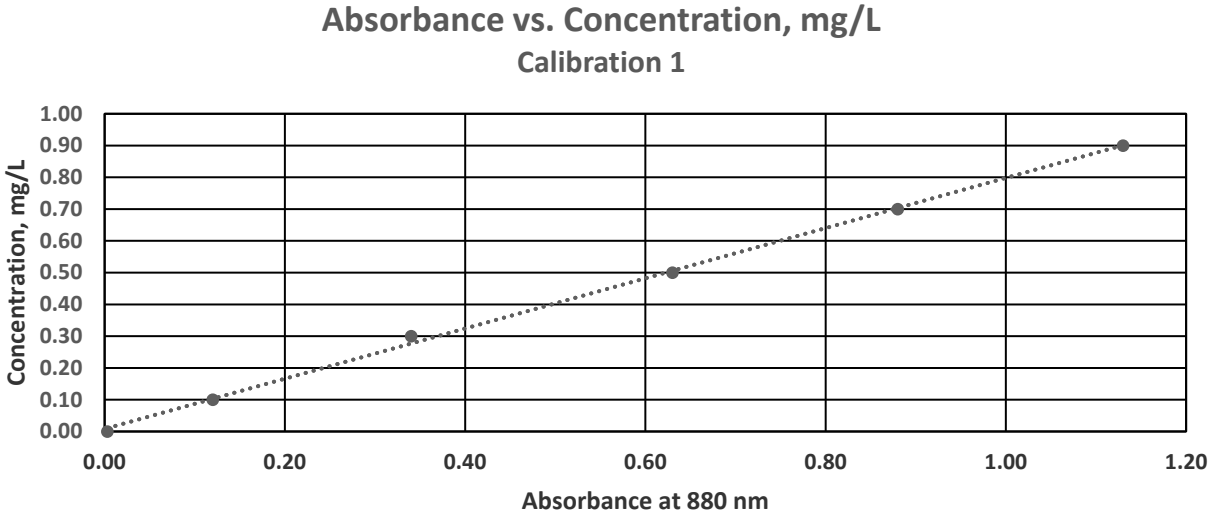
Simply put, the calibration curve shows the relationship between the instrument and/or probe response to different concentrations of analyte. The following guidelines are applied to this relationship to ensure the reporting of accurate results.

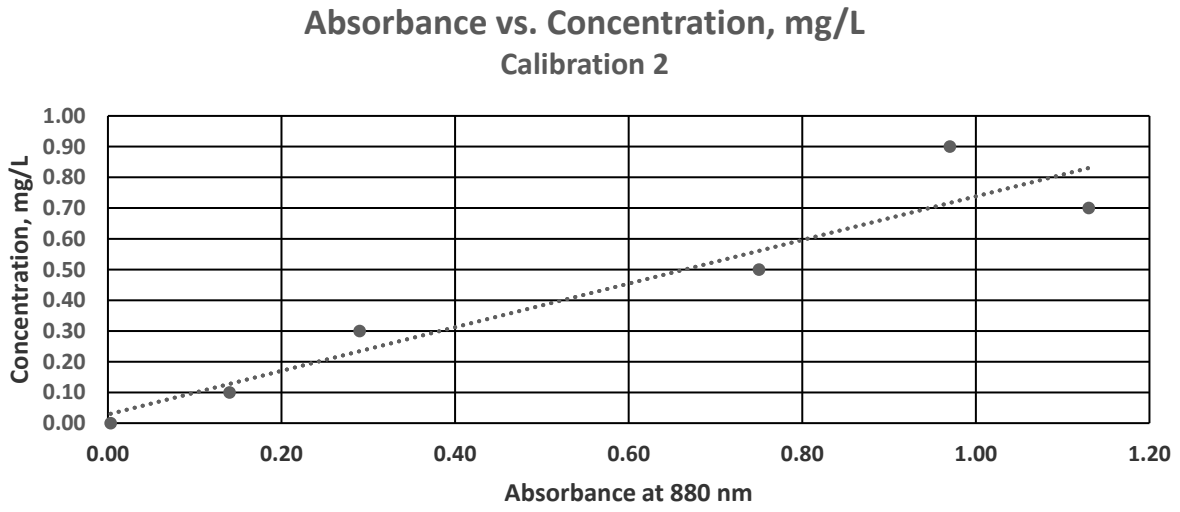
Appropriate number of standards - Calibration curves must be established using a least three standards and a blank. It is not usual practice to have a blank as part of the ammonia probe calibration. After ammonia probe calibration is established using a minimum of three standards, a blank is analyzed following the same procedure used to analyze samples. The result from the blank analysis is used to determine where the calibration curve intersects the x or y-axis in addition to checking for possible contamination problems.

Keeping it simple - linear is the key - Most of the instruments found in a wastewater laboratory respond to analyte concentrations in a linear manner. When the instrument does not follow the linear response, it is usually at the low and high extremes of the analytical curve. This usually results from overloading the instrument's detector at the high end and instrument noise and lack of sensitivity at the low end of the analytical curve. The simplest method to generate a calibration curve is using a linear regression formula and the most defensible way to do this is to plot analyte concentration on the x-axis (horizontal) and the instrument response on the y-axis (vertical).

How to handle the calibration blank - Unless the calibration algorithm (mathematical formula) you are using has a perfect fit for the calibration curve, i.e., a correlation coefficient of 1.000, you must decide what to do about the calibration blank result. A good rule to follow is: if you can adjust your instrument/probe to read zero in the presence of the blank, then the zero point should be included in the calibration curve. Including the calibration blank (zero standard) is generally acceptable for colorimetric procedures that use a spectrophotometer to measure the response to the standards and samples.

Forcing curves through zero - The practice of manipulating the data to obtain a y-intercept equal to zero usually results in biasing data that falls close to the lower end of the calibration curve. This "artificial" manipulation may result in losing important information relative to samples with low analytical signals as well as losing information relative to the limit of detection for that day's calibration. Even when the analytical method specifies using a blank as part of the calibration curve, forcing the intercept to read zero is not specified. Having said this, it is noted that the Standard Methods procedure for total phosphorus makes the following statement, "Plot absorbance vs. phosphate concentration to give a straight line passing through the origin". As a result, while this practice is discouraged in the laboratory, it is allowable to do this for total phosphorus calibrations.





Checking the calibration curve for accuracy - In the calibration graphs above, most laboratory analysts would agree that Calibration 1 is much better than Calibration 2. Methods usually read "plot concentration vs. response", so without evaluating the calibration curves, the quality of data may suffer. One means of determining the validity of a calibration is the correlation coefficient. When the correlation coefficient is at least 0.995, the calibration curve is usually considered acceptable. When the correlation coefficient is less than 0.995, the reason should be investigated, corrective action taken, and a new calibration curve established. Any scientific calculator capable of performing linear regression should display the correlation coefficient when the proper keypad entry sequence is followed. In the graphs above, Calibration 1 has an acceptable correlation coefficient of 0.9996 while Calibration 2 has an unacceptable correlation coefficient of 0.9388.

An additional means of checking the calibration curve is to use the calibration equation (i.e., slope and intercept) to convert the instrument response for the calibration standards into their respective concentrations based on the established calibration curve. There should be "reasonable" agreement between the "true" concentrations (i.e., the concentrations prepared by the analyst) and the concentrations calculated using the calibration curve. Depending on the

individual laboratory's DQOs, the calculated concentrations should be within 5 - 10% of the "true" concentrations.

Using the slope and intercept to evaluate the data used to establish Calibration 1 and 2 above, **Table 2** reflects why Calibration 1 is acceptable and Calibration 2 is unacceptable.

Table 2
Calibration 1 vs Calibration 2 Calculated Concentrations

"True Value"	0.00	0.10	0.30	0.50	0.70	0.90
Calibration 1 Calculated	0.008	0.103	0.277	0.506	0.703	0.901
Calibration 2 Calculated	0.028	0.128	0.234	0.561	0.830	0.717

Establishing the calibration range correctly - Just as we do not use a sledgehammer when installing trim around a doorway or a claw hammer to bust up large areas of concrete, we should establish a calibration curve range appropriate for the samples being analyzed. For example, when analyzing for ammonia-nitrogen, if historical data shows that sample concentrations are between 0.05 and 14.0 mg/L, we don't calibrate from 1.0 to 500. mg/L. The most accurate results are obtained when the results from the samples with unknown concentration are close to the known standards used to construct the calibration curve.

A well-constructed calibration curve should contain evenly spaced standards (at least three) with the realization that the more standards used, the better the quality of data generated. **When quantifying samples, a full calibration curve is always used**. The use of a calibration check standard or quality control standard should only be used to verify the calibration curve, but by itself, never used for quantifying samples.

Instruments with Pre-programmed Calibrations - Some of the commercially available spectrophotometers are sold with "pre-programmed" calibration curves for common wastewater tests including chlorine residual and total phosphorus. Single parameter spectrophotometers are generally referred to as colorimeters. If reference samples produce unacceptable results, recalibrating the instrument should be considered as a corrective action. Consult the manufacturer's instructions for a user-entered calibration.

Using Scientific Calculators or Computer Spreadsheets to Define the Calibration Curve - An acceptable standard practice to generate the calibration curve is to use the linear regression function of a scientific calculator or a computer spreadsheet. The use of this standardized statistical procedure will produce consistent equations for the "best-fit" line, thus eliminating the guesswork or bias with the hand-drawn line.

IV. Method Detection Limit (MDL)

One of the key questions a laboratory analyst should ask is "at what point can I distinguish a sample result from a blank result for samples with low concentrations of analyte?" This concentration has historically been designated the Method Detection Limit (MDL). When your results approach the MDL, precision is usually reduced due to instrument noise and other variables. Because of this, it is important for the analyst to understand whether there is analyte

present or not.

The determination of the MDL is required for each parameter reportable to IDEM, exceptions being MDLs for Biochemical Oxygen Demand (BOD_5), Total Suspended Solids (TSS), pH, and parameters associated with solids (sludge). Laboratories typically determine MDLs by spiking samples prepared with laboratory reagent water. However, the method of using laboratory reagent water does not take into effect how the wastewater matrix could influence the MDL results. It is recommended that each MDL for your facility be determined by adding the appropriate amount of spike to a wastewater Effluent sample. Following is the most current EPA procedure for determining MDLs.

Method Detection Limit – Introduction

The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The previously promulgated version of the MDL procedure (Revision 1.11) stated: "The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte."

The 2016 revision of the MDL procedure (Revision 2) differs from Revision 1.11 of the MDL procedure in three significant ways:

1. The MDL procedure now uses method blanks to calculate an MDL, in addition to the spiked samples that have always been used to calculate the MDL. As a result, the new definition of the MDL is: "The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results." The value calculated from the spiked samples. *Spiked samples are prepared from a clean reference matrix, such as laboratory reagent water, spiked with a known and consistent quantity of the analyte and called* MDL_s.

2. The MDL_S calculation is the same as the MDL calculation in Revision 1.11. The method blank samples are used to calculate the MDL_b, which is a very similar calculation that also calculates the 99% confidence level that the result is derived from the sample rather from contamination/noise. The MDL is the higher of the two values (either the MDL_S calculated using spiked samples or the MDL_b calculated using method blanks). EPA considers this change important because as detector sensitivity improves, the background contamination of the laboratory, consumable supplies, and equipment can be more important in determining the detection limit than the sensitivity of the instrument.

3. The MDL now requires that the samples used to calculate the MDL are representative of laboratory performance throughout the year, rather than on a single date.

A laboratory has the option to pool data from multiple instruments to calculate one MDL that represents multiple instruments.

Appendix B to 40 CFR Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 2

Definition
The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.

I. SCOPE AND APPLICATION

(1) The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The procedure requires a complete, specific, and well-defined analytical method. It is essential that all sample processing steps used by the laboratory be included in the determination of the method detection limit.

(2) The MDL procedure is *not* applicable to methods that do not produce results with a continuous distribution, such as, but not limited to, methods for Whole Effluent Toxicity, presence/absence methods, and microbiological methods that involve counting colonies. The MDL procedure also is *not* applicable to measurements such as, but not limited to, biochemical oxygen demand, color, pH, specific conductance, many titration methods, and any method where low-level spiked samples cannot be prepared. Except as described in the addendum, for the purposes of this procedure, "spiked samples" are prepared from a clean reference matrix, such as laboratory reagent water, spiked with a known and consistent quantity of the analyte. MDL determinations using spiked samples may not be appropriate for all gravimetric methods (*e.g.,* residue or Total Suspended Solids), but an MDL based on method blanks can be determined in such instances.

II. PROCEDURE

(1) **Estimate the initial MDL using one or more of the following:**

(a) The mean determined concentration plus three times the standard deviation of a set of method blanks.

(b) The concentration value that corresponds to an instrument signal-to-noise ratio in the range of 3 to 5.

(c) The concentration equivalent to three times the standard deviation of replicate instrumental measurements of spiked blanks.

(d) That region of the calibration where there is a significant change in sensitivity, *i.e.,* a break in the slope of the calibration.

(e) Instrumental limitations.

(f) Previously determined MDL.

Note: It is recognized that the experience of the analyst is important to this process. However, the analyst should include some or all of the above considerations in the initial estimate of the MDL.

(2) **Determine the initial MDL.**

Note: The Initial MDL is used when the laboratory does not have adequate data to perform the Ongoing Annual Verification specified in Section (4), typically when a new method is implemented or if a method was rarely used in the last 24 months.

(a) Select a spiking level, typically 2—10 times the estimated MDL in Section 1. Spiking levels in excess of 10 times the estimated detection limit may be required for analytes with very poor recovery (*e.g.,* for an analyte with 10% recovery, spiked at 100 micrograms/L, with mean recovery of 10 micrograms/L; the calculated MDL may be around 3 micrograms/L. Therefore, in this example, the spiking level would be 33 times the MDL, but spiking lower may result in no recovery at all).

(b) Process a minimum of seven spiked samples and seven method blank samples through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates and analyzed on three separate calendar dates. (Preparation and analysis may be on the same day.) Existing data may be used, if compliant with the requirements for at least three batches, and generated within the last twenty four months. The most recent available data for method blanks and spiked samples must be used. Statistical outlier removal procedures should not be used to remove data for the initial MDL determination, since the total number of observations is small, and the purpose of the MDL procedure is to capture routine method variability. However, documented instances of gross failures (*e.g.,* instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations, provided that at least seven spiked samples and seven method blanks are available. (The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.)

(i) If there are multiple instruments that will be assigned the same MDL, then the sample analyses must be distributed across all of the instruments.

(ii) A minimum of two spiked samples and two method blank samples prepared and analyzed on different calendar dates is required for each instrument. Each analytical batch may contain one spiked sample and one method blank sample run together. A spiked sample and a method blank sample may be analyzed in the same batch but are not required to be.

(iii) The same prepared extract may be analyzed on multiple instruments so long as the minimum requirement of seven preparations in at least three separate batches is maintained.

(c) Evaluate the spiking level: If any result for any individual analyte from the spiked samples does not meet the method qualitative identification criteria or does not provide a numerical result greater than zero, then repeat the spiked samples at a higher concentration. (Qualitative identification criteria are a set of rules or guidelines for establishing the identification or presence of an analyte using a measurement system. Qualitative identification does not ensure that quantitative results for the analyte can be obtained.)

(d) Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

(i) Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

(ii) Compute the MDL_s (the MDL based on spiked samples) as follows:
$MDL_s = t_{(n-1,\ 1-\alpha=0.99)} Ss$

Where:
MDL_s = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha=0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom. See Addendum **Table 3** 1 Single-Tailed 99th Percentile t Statistic below.

S_s = sample standard deviation of the replicate spiked sample analyses.

(iii) Compute the MDL_b (the MDL based on method blanks) as follows:

(A) If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

(B) If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where n ≥ 100, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are . . . 1.5, 1.7, 1.9, 5.0, and 10, then 164 × 0.99 = 162.36 which rounds to the 162nd method blank result. Therefore, MDL_b is 1.9 for n = 164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result).

Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

(C) If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:
$MDL_b = X + tn_{-1,1-\alpha=(0.99)}Sb$

Where: MDL_b = the MDL based on method blanks
X = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1,\ 1\alpha=0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with n−1 degrees of freedom. See Addendum **Table 3** Single-Tailed 99th Percentile t Statistic below.

S_b = sample standard deviation of the replicate method blank sample analyses.

Note: If 100 or more method blanks are available, as an option, MDL_b may be set to the concentration that is greater than or equal to the 99th percentile of the method blank results, as described in Section (2)(d)(iii)(B).

(e) Select the greater of MDL_s or MDL_b as the initial MDL.

Checking Your Method Detection Limit (MDL) Determinations ("4-Point" Check) - The MDL calculated in the laboratory can be checked using the following criteria:

1. Was the concentration of the replicate samples analyzed to determine the MDL greater than 10 times the calculated MDL? If yes, the replicate sample concentrations were too high, and a lower sample concentration needs to be prepared and the MDL determined again;

2. Is the calculated MDL higher than the concentration of the replicate samples used in the determination of the MDL? If yes, the replicate sample concentrations were too low, and a higher sample concentration needs to be prepared and the MDL determined again;

3. Is the calculated MDL greater than the discharge permit limits specified in your wastewater permit? Even the lowest discharge permit limits should be significantly greater than the MDLs calculated in the wastewater laboratory. If the MDLs calculated in your laboratory are higher than your discharge permit limits, you need to evaluate the entire analytical procedure and correct any problems causing high MDL values;

4. Are the results obtained from the analysis of the replicate samples analyzed to determine the MDL reasonable when compared to the actual concentration of the replicates analyzed? For example, if the concentration of seven replicates used to determine the MDL for ammonia-nitrogen was 0.15 mg/L and the calculated mean (average) of the seven replicates was 0.63 mg/L, the results are questionable. A good

rule of the thumb to follow is that the mean result of the replicates used for the MDL determination should be within 20 percent of the prepared/known concentration. Since the concentration of the replicates used for MDL determinations is at the lower end of the useable concentration range in the wastewater laboratory, any reference made in the Standard Operating Procedure as to the treatment of method blanks during calculations should be followed.
The following equations are useful when evaluating the calculated MDL:

Calculated MDL < **Spike Level** < 10 times the calculated MDL

Mean result (mg/L) of the 7 replicates = Prepared concentration (mg/L) ± 20%

(3) **Ongoing Data Collection.**

(a) During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked samples on each instrument, in separate batches, using the same spiking concentration used in Section 2. If any analytes are repeatedly not detected in the quarterly spiked sample analyses, or do not meet the qualitative identification criteria of the method (see section 2(c) of this procedure), then this is an indication that the spiking level is not high enough and should be adjusted upward. Note that it is not necessary to analyze additional method blanks together with the spiked samples, the method blank population should include all of the routine method blanks analyzed with each batch during the course of sample analysis.

(b) Ensure that at least seven spiked samples and seven method blanks are completed for the annual verification. If only one instrument is in use, a minimum of seven spikes are still required, but they may be drawn from the last two years of data collection.

(c) At least once per year, re-evaluate the spiking level.

(i) If more than 5% of the spiked samples do not return positive numerical results that meet all method qualitative identification criteria, then the spiking level must be increased, and the initial MDL re-determined following the procedure in section 2.

(ii) [Reserved]

(d) If the method is altered in a way that can be reasonably expected to change its sensitivity, then re-determine the initial MDL according to section 2, and the restart the ongoing data collection.

(e) If a new instrument is added to a group of instruments whose data are being pooled to create a single MDL, analyze a minimum of two spiked replicates and two method blank replicates on the new instrument. If both method blank results are below the existing MDL, then the existing MDL_b is validated. Combine the new spiked sample results to the existing spiked sample results and recalculate the MDL_s as in Section 4. If the recalculated MDL_s does not vary by more than the factor specified in section 4(f) of this procedure, then the existing MDL_s is validated. If either of these two conditions is

not met, then calculate a new MDL following the instructions in section 2.

(4) **Ongoing Annual Verification.**

(a) At least once every thirteen months, re-calculate MDL_s and MDL_b from the collected spiked samples and method blank results using the equations in section 2.

(b) Include data generated within the last twenty four months, but only data with the same spiking level. Only documented instances of gross failures (*e.g.,* instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations. (The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.) If the laboratory believes the sensitivity of the method has changed significantly, then the most recent data available may be used, maintaining compliance with the requirement for at least seven replicates in three separate batches on three separate days (see section 2b).

(c) Include the initial MDL spiked samples, if the data were generated within twenty four months.

(d) Only use data associated with acceptable calibrations and batch QC. Include all routine data, with the exception of batches that are rejected, and the associated samples reanalyzed. If the method has been altered in a way that can be reasonably expected to change its sensitivity, then use only data collected after the change.

(e) Ideally, use all method blank results from the last 24 months for the MDL_b calculation. The laboratory has the option to use only the last six months of method blank data or the fifty most recent method blanks, whichever criteria yields the greater number of method blanks.

(f) The verified MDL is the greater of the MDL_s or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

Addendum to Section II: Determination of the MDL for a Specific Matrix

The MDL may be determined in a specific sample matrix as well as in reagent water, i.e., laboratory reagent water.

(1) Analyze the sample matrix to determine the native (background) concentration of the analyte(s) of interest.

(2) If the response for the native concentration is at a signal-to-noise ratio of approximately 5-20, determine the matrix-specific MDL according to Section 2 but without spiking additional analyte.

(3) Calculate MDL_b using the method blanks, not the sample matrix.

(4) If the signal-to-noise ratio is less than 5, then the analyte(s) should be spiked into the sample matrix to obtain a concentration that will give results with a signal-to-noise ratio of approximately 10-20.

(5) If the analytes(s) of interest have signal-to-noise ratio(s) greater than approximately 20, then the resulting MDL is likely to be biased high.

Number of replicates	Degrees of freedom (n−1)	$t_{(n-1, 0.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
32	31	2.453
48	47	2.408
50	49	2.405
61	60	2.390
64	63	2.387
80	79	2.374
96	95	2.366
100	99	2.365

Table 3—Single-Tailed 99th Percentile t Statistic

III. DOCUMENTATION

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. Data and calculations used to establish the MDL must be able to be reconstructed upon request. The sample matrix used

to determine the MDL must also be identified with MDL value. Document the mean spiked and recovered analyte levels with the MDL. The rationale for removal of outlier results, if any, must be documented and maintained on file with the results of the MDL determination.

Questions and comments submitted on the 2015 proposed Methods Update Rule, and EPA's responses.

1. **Will the laboratory need to analyze significantly more samples than before to calculate the MDL?**
   No, most commercial laboratories that currently participate in analyses supporting the National Pollutant Discharge Elimination System (NPDES) calculate the MDLs for each method/instrument combination once per year.

Comparison of Number of Samples Analyzed in MDL Procedures		
Samples Required	Revision 1.11	Revision 2
Spiked samples	7/year	8/year (2/quarter)
Methods blanks (MBs)	0	0 (use routine MBs)

   Assuming the analytical instrumentation is used regularly for specific method, Revision 2 of the MDL procedure requires a 14% increase in samples analyzed for MDL determinations (1 sample per year per instrument). If some instruments are used less, then the number of spiked samples will be fewer, possibly less than performing Revision 1.11 of the MDL procedure annually.

   Additional blanks would be required for determination of the MDL only if the laboratory is implementing a new method for the first time or has just acquired a new instrument. Otherwise, no additional method blanks are required because Revision 2 of the MDL procedure uses the routine method blanks that are already being analyzed with every batch of samples.

2. **Is the laboratory required to recalculate the MDL every quarter?**
   No, the MDL is only calculated once a year. MDL spiked samples are now analyzed every quarter in which the method is used, but the calculation is only required to be performed once a year.

3. **Will laboratories have to analyze more samples for methods that are rarely used?**
   No, Revision 2 of the MDL procedure could potentially require fewer samples than Revision 1.11 of the MDL procedure for the rarely used methods. For example, if a

laboratory analyzed 7 batches of samples spread out over a 2-year period (either because they have a new instrument or because not enough analyses are conducted in a one-year period) then the laboratory would have enough sample spikes and blanks to recalculate the MDL. This would be 7 samples in 2 years, which is half of what was normally done for Revision 1.11 of the MDL procedure, if performed once a year.

4. **If the laboratory does not use a method during a quarter, will the laboratory still need to analyze low-level spiked samples?**
   No, the laboratory needs to analyze at least seven low-level spiked samples and seven method blanks for one instrument in a two-year period (spread over 3 batches), but is also supposed to analyze two spiked samples per quarter in separate batches any quarter samples are analyzed. See Sections (2)(b) and (3)(a) of the MDL procedure. A practical way for a laboratory to stay in compliance is to analyze a low-level spiked sample with the first two analytical batches every quarter. If no samples are analyzed, then there is no need to analyze spiked samples or method blanks. If one batch of samples is analyzed during a quarter, then the laboratory should include one low-level spiked sample in that batch. If two or more batches of samples are analyzed, the laboratory should include one low-level spiked sample in at least two of those batches.

5. **What happens if the laboratory has less than 7 sample spikes when calculating the MDL?**
   The minimum number of samples is 7, but more samples are used if more are available. If the analysis is performed regularly, then there will likely be 16 spiked samples per instrument (2 per quarter over 2 years) and many more blanks. If the analysis is performed very rarely, then there may be less than 7 spiked samples. In this case, the laboratory needs to perform a new initial MDL procedure, but can use the samples that are available over the last 2 years to contribute to calculating the new initial MDL. For example, if a laboratory only had 4 available spiked samples and 4 method blanks over the last 2 years, then 3 more spiked samples and 3 more method blanks would be required for determining the initial MDL. These 3 additional spiked samples and method blanks could be analyzed in one batch (assuming the existing 4 pairs of samples were in at least two batches). See Section (2)(b) for the initial MDL procedure. In this scenario, the lab would analyze 7 spiked samples in 2 years, which is less than the laboratory would have analyzed doing an MDL study every year using Revision 1.11 of the MDL procedure.

6. **Could one high blank result drastically elevate the MDL?**
   It depends. A high blank result could be eliminated in a number of ways. If there are 100 or more method blanks results, then the highest blank result is not considered (since the MDL procedure measures the 99% confidence limit). Also, a method blank result can be ignored if it is associated with a documented instance of gross failure (e.g., instrument malfunctions, mislabeled samples, cracked vials). Any data associated with rejected samples also are not used for the MDL calculation. Section (4)(d) of Revision 2 of the MDL procedure states “Include all routine data, with the exception of batches that are rejected, and the associated samples reanalyzed.”

7. **What if a laboratory buys a new instrument and wants to include it in a multi-instrument MDL?**

Provisions for a new instrument have been added to Section (3)(e) of Revision 2 of the MDL procedure. The laboratory needs to analyze a minimum of two spiked samples and two method blanks on the new instrument.

8. **If a laboratory uses a certain analysis daily, then they will have hundreds, potentially thousands, of method blanks to review for a two-year period.**
   In order to make the annual verification less labor intensive for laboratories that analyze many sample batches of certain methods, the laboratory will have the option to use only the last six months of method blank data or the fifty most recent method blanks, whichever criteria yields the greater number of method blanks.

9. **Cost of new MDL procedure.**
   The additional effort and expense is mainly in the computation, not additional sample preparation and analysis. Modern laboratories have laboratory information management systems (LIMS) that can query the data needed for the annual verification. Once the data are compiled, the MDL_S calculation is identical to the calculation in Revision 1.11. The MDL_b is a similar calculation and is simplified if blanks are almost always "non-detect." The implied requirement that the calculated MDL must be no less than one-tenth the spike value in Revision 1.11 of the MDL procedure has been eliminated in Revision 2, so laboratories will not have to redo the MDL procedure if the calculated MDL is lower than expected. This will reduce workload and is especially helpful for methods with long analyte lists. Also, the fact that the MDL samples are now analyzed throughout the year with routine samples eliminates the need to set aside a specific batch of samples and time to analyze the MDL samples.

10. **Why are acceptable calibrations and batch quality control (QC) not mentioned in the Initial MDL procedure?**
    If the laboratory is performing an initial MDL without client samples in the batch, most batch QC is not required. The spiked samples are essentially laboratory fortified blanks, and the MS/MSD samples are not required if there are no client samples in the batch. Ongoing MDL samples should be analyzed with client samples the laboratory receives, so all normal batch QC should be present. The methods already specify that calibrations must be completed before performing any analyses, so there is no need to add this requirement to the MDL procedure itself.

11. **Why is so much ongoing data collection necessary, and what additional quality is this practice of ongoing data collection providing?**
    Ongoing data collection captures instrument drift and the variation in equipment conditions throughout the year. Many laboratories currently analyze the MDL aliquots immediately after the instrument is serviced and all consumable instrument parts are new, thus yielding a best-case MDL value. Ongoing data collection leads to an MDL that represents what is actually practiced throughout the year.

12. **If the MDL and ML values change, permit limits may need to be reviewed.**
    EPA acknowledges that MDL values for some analytes may increase due to the revision of the MDL procedure. Some permits may contain limits that cannot be met by approved methods in 40 CFR Part 136. If this is the case, the permittee should use the most sensitive method allowed among the approved methods in Part 136. The NPDES

rule, "Use of Sufficiently Sensitive Test Methods for Permit Applications and Reporting" (August 19, 2014) discusses this issue in detail. (Additionally, supporting documents are available in the docket at regulations.gov, docket no. EPA-HQ-OW-2009-1019.) This situation already existed with Revision 1.11 of the MDL procedure, and permittees and permitting authorities should have addressed this issue. The new MDL procedure may cause some additional contaminants to have MLs above the permit requirements for a specific analysis. The "Sufficiently Sensitive Method" rule is very clear about what to do in this case; see 40 CFR 122.21(e)(3).

13. **How long does a laboratory have to implement the current MDL procedure after promulgation of the Methods Update Rule?**
EPA recognizes that it is not possible for any laboratory to make this change instantaneously. The laboratory should comply with the requirements of its control authority or permitting authority to implement Revision 2 of the MDL procedure.

V. Evaluating Precision

There are numerous methods by which laboratory precision can be measured. The method outlined below is suggested so that your laboratory can evaluate precision the first time duplicates are analyzed.

Duplicate analyses are performed at the frequency set forth in **Table I**. The difference between the results of duplicate analyses and the mean (average) of the duplicates is used to calculate the precision (reproducibility) of the analytical method.

Step 1 - Calculate the Range (R) for each set of duplicate samples

$$\text{Range (R)} = |\text{Sample Result} - \text{Duplicate Result}|$$

Where: $|\text{math calculation}|$ = absolute value of the "math calculation"

The absolute value simply means that negative values are expressed as positive values, thus $|14 - 18| = 4$ rather than -4. One can think of Range as the difference between two duplicate sample results always resulting in a positive value.

Step 2 - Calculate the Average, i.e., Mean for each set of duplicate samples

$$\text{Mean} = \frac{(\text{Sample Result}) + (\text{Duplicate Result})}{2}$$

Step 3 - Calculate the Relative Percent Difference (RPD)

$$\text{RPD} = \frac{\text{Range (R)} \times 100}{\text{Mean}}$$

When using Relative Percent Difference (RPD), the following Control Limits and Warning Limits are used to evaluate precision in the wastewater laboratory:

1. If the mean of the duplicates is less than or equal to five times the MDL, the MDL is the control limit and two-thirds (or 0.67 times) the MDL is the warning limit

2. If the mean of the duplicates is greater than five times and less than 20 times the MDL, the control limit is 25% RPD and the warning limit is 16.7% RPD

3. If the mean of the duplicates is greater than or equal to 20 times the MDL, the control limit is 10% RPD and the warning limit is 6.7% RPD.

For many analyses, after establishing the MDL for a given parameter, the three ranges listed above for evaluating precision in the laboratory, i.e., ≤ five times the MDL, > five to < 20 times the MDL and ≥ 20 times the MDL do not change until a new MDL is calculated. For BOD_5 and TSS analyses, that have a pre-defined MDL, the analyst needs to keep in mind the sample volumes used during analysis to accurately evaluate whether the precision between the sample and the duplicate sample are acceptable. **Table 4** illustrates how sample volume used for BOD_5 duplicate analysis affects the three ranges for calculating precision within the laboratory and **Table 5** displays the corresponding information for Total Suspended Solids (TSS) duplicate analysis. It is noted that should the mean result for the sample and sample duplicate give a result less than the MDL, calculation of precision is not necessary. The reason for this being that at very low concentrations, precision or reproducibility can be greatly influenced by instrumental background noise. Thus, any precision number calculated may reflect this instrument noise rather than the capabilities of the analyst or the analytical procedure.

For BOD_5 analysis, precision and bias is also evaluated through the analysis of the glucose-glutamic acid (GGA) check sample. Standard Methods states the recovery of this primary mixed standard should be 198 ± 30.5 mg/L. Analytical results consistently in this acceptable range demonstrate good dilution water quality, good seed effectiveness, and good technique by the analyst. Results consistently above or below the 198 mg/L value indicate bias. When this occurs, the laboratory should investigate the cause and take corrective action to eliminate the bias.

Table 4

The Effects of Sample Volume Used on Precision Ranges for BOD_5 Analysis

Greatest Sample Volume (mL) Used for Sample Duplicates	MDL mg/L	Precision Range 1 MDL to ≤ 5x MDL		Precision Range 2 > 5x MDL to < 20x MDL		Precision Range 3 ≥ 20x MDL	
		Mean of Duplicates mg/L	CL = mg/L WL = mg/L	Mean of Duplicates mg/L	CL = RPD WL = RPD	Mean of Duplicates mg/L	CL = RPD WL = RPD
300	2.0	2.0 to ≤ 10.	2.0 1.3	> 10. to < 40.	25% 16.7%	≥ 40.	10% 6.7%
200	3.0	3.0 to ≤ 15.	3.0 2.0	> 15. to < 60.	do	≥ 60.	do
150	4.0	4.0 to ≤ 20.	4.0 2.7	> 20. to < 80.	do	≥ 80.	do
100	6.0	6.0 to ≤ 30.	6.0 4.0	> 30. to < 120	do	≥ 120	do
50	12.	12. to ≤ 60.	12 8.0	> 60. to < 240	do	≥ 240	do
25	24.	24. to ≤ 120	24. 16.	> 120 to < 480	do	≥ 480	do
10	60.	60. to ≤ 300	60. 40.	> 300 to < 1200	do	≥ 1200	do

CL = Control Limit WL = Warning Limit do = same as above

Table 5

The Effects of Sample Volume Used on Precision Ranges for TSS Analysis

Greatest Sample Volume (mL) Used for Sample Duplicates	MDL mg/L	Precision Range 1 MDL to ≤ 5 x MDL		Precision Range 2 > 5x MDL to < 20 x MDL		Precision Range 3 ≥ 20 x MDL	
		Mean of Duplicates mg/L	CL = mg/L WL = mg/L	Mean of Duplicates mg/L	CL = RPD WL = RPD	Mean of Duplicates mg/L	CL = RPD WL = RPD
1000	2.5	2.5 to ≤ 12.	2.5 1.7	> 12. to < 50.	25% 16.7%	≥ 50.	10% 6.67%
500	5.0	5.0 to ≤ 25.	5.0 3.3	> 25. to < 100	do	≥ 100.	do
250	10.	10. to ≤ 50.	10. 6.7	> 50. to < 200	do	≥ 200.	do
100	25.	25. to ≤ 125	25. 17.	> 125 to < 500	do	≥ 500	do
50	50.	50. to ≤ 250	50. 33.	> 250 to < 1,000	do	≥ 1,000	do
25	100.	100 to ≤ 500	100 67.	> 500 to < 2,000	do	≥ 2,000	do
10	250	250 to ≤ 1,250	250 167	> 1,250 to < 5,000	do	≥ 5,000	do

CL = Control Limit WL = Warning Limit do = same as above

VI. Evaluating Accuracy

As defined in the Introduction of this document, accuracy measures how close the laboratory result is to the "true" value. Not only is it important for the laboratory to report accurate and legally defensible results to IDEM to help that agency control any adverse effects the treated wastewater Effluents may have on the water quality and biological community of the receiving waters, but accurate test results are also needed by the wastewater treatment plant personnel for proper process control.

Samples most commonly used in the wastewater laboratory to determine accuracy are a purchased or prepared Laboratory Check Standard, a purchased Laboratory Quality Control Standard and the Matrix Spike.

Example for a purchased Laboratory Quality Control Standard:

Standard	Units	Certified Value	Acceptance Limits
Ammonia-Nitrogen	mg/L	8.36 ± 0.13	6.59 - 10.4
Total Phosphorus	mg/L	8.60 ± 0.091	6.35 - 9.75

The laboratory result for the Ammonia-Nitrogen = 7.89 mg/L and is acceptable as it is within the Acceptance Limits of 6.59 – 10.4 mg/L.

Example for a Laboratory Check Standard:
A 5.0 mg/L Laboratory Check Standard is analyzed as part of a total phosphorus analysis using TNTplus methodology and had a result of 4.78 mg/L. The control limit is ± 10% of the actual value or 4.5 – 5.5 mg/L. The Laboratory Check Standard result is acceptable.

The Laboratory Quality Control Standard and Laboratory Check Standard are useful ways for checking the accuracy of the calibration standards used to prepare the calibration curve in addition to checking for bias (systematic error) in the analytical procedure. If the recovery of either of these type standards does not fall within the control limits, refer to Section IX, Corrective Action in the Laboratory, for guidance on appropriate corrective actions.

Matrix Spike Sample - Percent recovery data calculated from the analysis of spiked samples is used to determine the accuracy, or bias, of the analysis. Spiking a sample is the process of adding a measured amount of a known concentration of the targeted analyte to a measured amount of the sample. Samples are spiked prior to being processed through any preliminary treatment steps such as digestion or distillation. Spiked samples are analyzed at the frequency listed in **Table 1**. In this document, only ammonia-nitrogen and total phosphorus samples are discussed relative to this quality assurance and quality control procedure.

When determining the amount of spike to add to a sample, the following guidelines are useful:

1. The amount of spike should be between 1 and 5 times the known (expected) concentration of the sample being analyzed;

2. If the spiking procedure will result in an analytical result greater than the highest calibration standard, the amount of spike added should be modified; and

3. If the sample is expected to not contain the targeted analyte, the spike added should be at a level equal to the midrange of the calibration.

Table 6 can be used as a reference when determining the amount of spike to add to ammonia-nitrogen and total phosphorus samples. **Note:** If your laboratory analyzes for these parameters using the Hach TNTplus methods spiking guidelines for those parameters are found in the Methods Section of this manual for those parameters.

Table 6
Preparation of Matrix Spikes for Ammonia-Nitrogen and Total Phosphorus

Analyte	Expected Sample Concentration Range (mg/L)	Stock Standard Concentration (mg/L)	Stock Standard Volume to Add (mL)	Sample + Spike Final Volume (mL)	Final Concentration of Spike Added (mg/L)
Ammonia-Nitrogen	≤ 0.5	100	0.50	100	0.50
	> 0.5 - 1.0	100	1.0	100	1.0
	> 1.0 - 2.0	1000	0.20	100	2.0
	> 2.0 - 5.0	1000	0.50	100	5.0
	> 5.0	1000	1.0	100	10.
Total Phosphorus	≤ 0.25	50.	0.25	50	0.25
	> 0.25 - 0.50	50.	0.50	50	0.50
	> 0.50 - 2.5	100	1.0	50	2.0
	> 2.5 - 5.0	100	2.0	50	4.0
	> 5.0 - 10.	100	5.0	50	10.

Using the examples in **Table 6**, you will observe that the volume of spike added to the ammonia-nitrogen samples never exceeds 1.0 mL. When spike preparation results in a dilution of the sample by 1% or less, the dilution is considered negligible and direct subtraction of the sample concentration from the matrix spike sample concentration is considered acceptable without a correction being made for the change in volume caused by the addition of the spike. As the final volume for ammonia-nitrogen samples is 100 mL and the maximum volume of spiked added in **Table 6** is 1.0 mL, the sample dilution does not exceed the 1% level, thus the spike recovery calculation can be performed without corrections being made for the change in volume. When the amount of spike added to the sample results in a sample dilution greater than 1%, this must be taken into account during

the matrix spike percent recovery calculation - see **Equation #2** on page 33.

The addition of the added spike to a total phosphorus sample can exceed the 1.0 mL volume as total phosphorus samples are spiked prior to digestion and the final volume is adjusted to 50 mL prior to the addition of the reagents required for color development.

Note: It is extremely difficult to accurately measure spike volumes of less than 1.0 mL with a glass pipet. Measuring volumes of less than 1.0 mL can be accurately measured through the proper use of a certified Eppendorf type pipettor capable of accurately delivering volumes less than 1.0 mL.

Matrix Spike Control Limits - A control limit for matrix spike recoveries of 80% to 120% should be achievable in the wastewater laboratory. The associated warning limits would be 87% to 113%. For the purposes of this document, these limits are considered acceptable. If matrix spike recoveries do not fall within the control limits, refer to Section IX, Corrective Action in the Laboratory, for guidance on appropriate corrective actions.

Calculating Matrix Spike Recoveries

The following **Equation #1** is used to calculate the percent recovery for matrix spike samples when the spike added results in a sample dilution of ≤ 1%:

$$\% \text{ Recovery} = \frac{\text{observed - background}}{\text{spike amount}} \times 100$$

Where:

observed = the concentration measured in the **spiked** sample (mg/L)
background = the concentration measured in the **unspiked** sample (mg/L)
spike amount = the concentration of spike added to the sample (mg/L)

$$\text{spike amount} = \frac{\text{Volume of standard added (mL)} \times \text{Concentration of standard added (mg/L)}}{\text{Final volume of spiked sample (mL)}}$$

Example

An analyst transferred 100 mL of Final Effluent in a beaker labeled “background”. A separate 100 mL aliquot of the same Final Effluent sample was transferred to a separate beaker labeled “observed”. To the 100 mL Final Effluent sample in the “observed” beaker, the analyst added 1.0 mL of a 50 mg/L ammonia-nitrogen standard as a spike. Analytical results were:

“background” beaker = 0.48 mg/L
“observed” beaker = 0.93 mg/L
spike amount = 0.50 mg/L

spike amount = 1.0 mL x 50 mg/L = 0.50 mg/L
*100 mL

* - as the spike added did not dilute the sample by more than 1%, the volume of the spiked sample is not adjusted to reflect the addition of 1.0 mL of spike

Using **Equation #1** to calculate percent recovery

0.93 mg/L - 0.48 mg/L x 100 = 90% Recovery
0.50 mg/L

The following **Equation #2** is used to calculate the percent recovery for matrix spike samples when the spike added results in a sample dilution of > 1%:

$$\% \text{ Recovery} = \frac{\text{observed} - \text{background}}{\text{spike amount}} \times 100$$

Where:

observed = the concentration measured in the **spiked** sample (mg/L)
background = the concentration measured in the **unspiked** sample (mg/L)
spike amount = the concentration of spike added to the sample (mg/L)

$$\text{spike amount} = \frac{\text{Volume of standard added (mL)} \times \text{Concentration of standard added (mg/L)}}{\text{Final volume of spiked sample (mL)}}$$

Example

An analyst transferred 100 mL of Final Effluent in a beaker labeled “background”. A separate 100 mL aliquot of the same Final Effluent sample was transferred to a separate beaker labeled “observed”. To the 100 mL Final Effluent sample in the “observed” beaker, the analyst added 5.0 mL of a 10 mg/L ammonia-nitrogen standard as a spike. Analytical results were:

“background” beaker = 0.48 mg/L
“observed” beaker = 0.93 mg/L
spike amount = 0.48 mg/L

spike amount = 5.0 mL x 10. mg/L = 0.48 mg/L
*105 mL

* - as the spike added diluted the sample by more than 1%, the volume of the spiked sample is adjusted to reflect the addition of 5.0 mL of spike

Using **Equation #2** to calculate percent recovery

0.93 mg/L - 0.48 mg/L x 100 = 94% Recovery
0.48 mg/L

VII. Using Control Limits

The reasons for establishing control limits in the laboratory is to help the analyst determine if the results meet the Data Quality Objectives (DQOs) specified in the Standard Operating Procedure for each analytical method and to look for trends occurring in the laboratory which could indicate that corrective action needs to be taken prior to that specific analytical method becoming completely out-of-control, and the associated loss of defensible data. **Section VIII and Section IX,** which follow, discuss how the laboratory analyst should use the data from the quality control checks performed during analysis.

VIII. Evaluating Quality Control Charts

By charting the quality control checks associated with each analytical procedure, the analyst is able, through a scheduled review of these charts, to see the "big picture" of the analysis. For example, the analyst is keenly aware of the result for the glucose-glutamic acid check with the BOD_5 analysis on the day the sample is analyzed. By charting this quality control check, the analyst may observe that over a period of time there is a decreasing trend for this result. Further observation of this control chart shows this decreasing trend reverses itself each time the membrane is changed on the DO probe used for BOD_5 analysis. Because the analyst now can see the "big picture", a schedule can be implemented for the change of the DO probe membrane to prevent the BOD_5 analysis from becoming out-of-control. Another example might be: while looking at the "big picture" for the ammonia analysis matrix spike recovery, 3 consecutive results were greater than the established warning limit of 113% when the 10.0 mg/L standard was used as the spiking solution. A complete review of the ammonia-nitrogen records showed that the 10.0 mg/L standard had been prepared on the day prior to these occurrences happening. A fresh 10.0 mg/L standard was prepared and subsequent analysis using the fresh standard resulted in spike recoveries below the warning limit. Without the proper documentation, the analyst would not have known when the 10.0 mg/L standard was prepared. The point is that quality control is not only the process of analyzing blanks, duplicates/replicates, matrix spikes, etc., but also keeping complete records of everything that happens in the laboratory that may affect the analysis.

IX. Corrective Action in the Laboratory

When plotting the quality control results on their respective control charts, the following are examples which indicate an analysis has reached the condition of being out-of-control and corrective action is necessary:

a. The plotted point is greater than the upper control limit or less than lower control limit

> **Corrective action = repeat the analysis immediately. If the repeat analysis is within the control limit, continue analysis; if it exceeds the control limit, discontinue analysis and correct the problem;**

b. Seven consecutive plotted points are on the same side (above or below) of the mean

Corrective action = analyze another sample. If the plotted point from this sample is on the other side of the mean, continue analysis, if not, discontinue analysis and correct the problem;

c. Five or more plotted points moving in the same direction - 5 plotted points each of which is higher (or lower) than the previous point

Corrective action = Analyze another sample. If the plotted point from this sample continues the trend of the previous plotted 5 points, discontinue analysis and correct the problem, if not, continue analysis;

d. Two of three successive plotted points are greater than the upper warning limit or less than the lower warning limit

Corrective action = analyze another sample. If the plotted point from this sample result is within the warning limit, continue analysis, if this point exceeds the same warning limit, check the method for bias (systematic error) and correct the cause prior to additional analysis.

Simply stated, corrective action is required whenever any control limit is exceeded or when definite trends are observed on the control charts. The intention of corrective action is not intended to merely create more analysis in the laboratory, but rather to establish a historical record detailing the problems that occur and how these problems were corrected. The documentation of these problems and solutions is extremely important in preventing the same problems from reoccurring in the future. Without a written record to refer to, the analysts must rely on their memory of the events that occurred. Over a period of time, important information and details may be forgotten. Additionally, any new personnel in the laboratory need to have written documentation to refer to during the "trouble shooting" process involved with corrective action.

Standard Methods outlines a step-wise procedure when performing corrective action. These steps are:

1. Check data for calculation or transcription error. Correct results if error occurred;

2. Check to see if sample(s) were prepared and analyzed according to an approved method and the laboratory's Standard Operating Procedure. If not, prepare and/or analyze again;

3. Check calibration standards against an independent standard or reference material. If calibration standards fail, prepare calibration standards again and/or recalibrate instrument and reanalyze affected samples;

4. If the Laboratory Control Sample (LCS) also known as the Laboratory Fortified Blank

(LFB) fails, reanalyze another LCS;

5. If the second LCS fails, check an independent (second source) reference material. If the second source gives an acceptable result, prepare and analyze all affected sample(s) again;

6. If a Matrix Spike sample fails, check the LCS result. If the LCS is acceptable, qualify the data for the Matrix Spike sample or analyze using another method of analysis or analyze using the method of standard additions;

7. If the Matrix Spike sample and the associated LCS sample fail, prepare and analyze all affected samples again;

8. If the Method blank fails, analyze another Method blank;

9. If the second Method blank fails, prepare and analyze all affected sample(s) again.

X. Documentation and Record Keeping

All records of equipment calibration and maintenance, quality control tests, sampling events, and laboratory analysis must be retained for a minimum of three years at the wastewater treatment facility. Before reporting any data, all raw data and calculations must be reviewed for accuracy by a person other than the person who produced the data. The person reviewing the data must be experienced enough to distinguish between correct and incorrect data.

All raw data must be kept regardless of its original form. If data is transferred to a database or some other form of record, the original data records are retained also. In summary, all samples and data must be traceable back to the analyst, date collected, time collected, date analyzed, and method of analysis used. Other required information is raw data, intermediate calculations, results and the associated reports. All quality results should be traceable to all the associated sample results.

With the many tasks facing the typical wastewater treatment personnel, documentation (the paper work) is often overlooked or not performed. In order to make data defensible, documentation is extremely important. A good saying to remember is, “if you didn’t document it. . .you didn’t do it”. In other words, documentation means you can take credit for the work performed.

Records are to be kept in such a manner as to guarantee their permanence and security. All handwritten records are recorded in ink. The use of “erasable” ink or correction fluid of any type is not acceptable. When handwritten errors are made, the incorrect entry has a single line drawn through it and the correct entry is recorded. The initials of the person making the correction are written along with the date the correction was made.

METHODS

Determination of Ammonia (NH_3-N)

HACH TNTplus Nitrogen, Ammonia Salicylate Method

References: HACH Method 10205: TNTplus 830 and TNTplus 832

I. INTRODUCTION

A) GENERAL

Various compounds containing the element nitrogen are important in wastewater management programs because of the many effects that nitrogenous materials in wastewater Effluent can have on the environment. Nitrogen, in its various forms, can deplete dissolved oxygen levels in receiving water, stimulate aquatic growth, exhibit toxicity toward aquatic life, affect chlorine disinfection efficiency, present a public health hazard and affect the suitability of wastewater for reuse. Biological and chemical processes which occur in wastewater treatment plants and in the natural environment can change the chemical form in which nitrogen exists. Such changes may eliminate on effect of nitrogen while producing, or leaving unchanged, another effect. Ammonia (NH_3/NH_4) is naturally present in wastewater. It is produced largely by the deamination of organic nitrogen containing compounds and hydrolysis of urea.

B) METHOD

Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of color formed is directly proportional to the ammonia nitrogen that is in the sample. The measurement wavelength is 694 nm.

C) SCOPE & APPLICATION

This method is applicable to the measurement of 0.015 to 47. mg/L NH_3-N in potable and surface waters and domestic and industrial wastes. **Note: TNTplus 833 vials are available for a concentration range of 47 – 130 mg/L. However, measuring in this range would likely be for a side stream or biosolids and would need diluted to compensate for turbidity. After dilution, the TNTplus 832 vials could be used for measurement and the actual NH_3-N concentration be calculated based on the dilution factor.**

The implementation of the TNTplus method will only be acceptable by IDEM if the following items are on file within the laboratory:

HACH Side-By-Side Comparison of Ammonia ISE and TNTplus Reagents Study
USEPA Letter, Discussing similar findings to the HACH side-by-side study and its approval of the TNT method
*Method Detection Limit p*erformed in-lab using your personnel and equipment

II. SAMPLING, STORAGE AND PRESERVATION

Samples to be measured can be either grab or composite whichever is required in the NPDES permit.

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 hours of collection, refrigerate unacidified at ≤ 6°C. Samples to be analyzed at a later date must be preserved when collected to prevent escape of NH_3 gas. One mL of 1:1 H_2SO_4 in 500 mL of sample or concentrated HCl can be used for preservation. Samples can be checked with pH paper to ensure preservation is adequate. Preserved samples can be held for analysis up to 28 days at ≤ 6°C. Caution: although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

Acidified samples must be neutralized to pH 7 with NaOH or KOH immediately before analysis. A volume adjustment calculation is necessary when preserved samples are neutralized to account for the sample being diluted.

> Example: 50 mL of acidified sample requires 4.0 mL of 5*N* NaOH to adjust to pH 7. This means the sample being used for analysis is now 92.6% sample and 7.4% NaOH, i.e., 50 mL/54 mL = 0.926 = 92.6%. To adjust to the actual sample concentration, the following equation is used:

(Spectrophotometer result, mg/L)(100%/92.6%) = actual sample result, mg/L

Samples and standards must be brought to room temperature prior to analysis.

III. REAGENTS and EQUIPMENT

A. Reagents

1. Sulfuric Acid 1:1; add a volume of concentrated H_2S0_4 to an equal volume of laboratory reagent water. Mix well in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO PREPARE THIS SOLUTION IN THE ACID BOTTLE. Allow to cool at room temperature. This solution is used to preserve samples.
   NOTE: Always add acid to water! A highly exothermic reaction takes place when making this dilute acid. DO NOT place into cold water once the flask is hot! Prepare a cold water bath to constantly cool the mixture.
2. 5*N* sodium hydroxide NaOH (To bring pH up to 7.0 after H_2SO_4 preservation)
   5*N* NaOH = 50 g NaOH dissolved in 250 mL laboratory reagent water
3. TNTplus 830 ULR Reagent Vials – store in laboratory refrigerator
4. TNTplus 832 HR Reagent Vials – store in laboratory refrigerator
5. 100 mg/L ammonia-N Standard
6. 10.0 mg/L ammonia-N Standard – prepared from 100 mg/L Standard
7. 1.0 mg/L ammonia-N Standard – prepared from 100 mg/L Standard
8. Ammonia free, Laboratory Reagent Water

B. Equipment

1. HACH DR3900 Spectrophotometer or equivalent
2. Kimwipes

3. 100 mL Class-A Volumetric Flasks
4. 100 mL Graduated Cylinders
5. 50 mL Beakers (Acid Washed) – used to pour Stock, Check and Spiking Standards into prior to pipetting. Standards should never be pipetted directly from their container(s) to prevent the possibility of contamination.
6. 0.1 – 1.0 adjustable automatic pipettor with tips
7. 1.0 – 10. adjustable automatic pipettor with tips
8. Vial Cooling Rack

IV. PROCEDURE

1. Turn on the DR3900 and wait for instrument to perform the Self-Check.
   - This process will check the system, lamp, filter, calibration wavelength, and voltage.
   - The Main Menu will display when the Self-Check is complete, and the meter is automatically calibrated.

***NOTE 1:** When a new lot of vials is received, register that vial lot with the instrument by touching the "Target Icon" of the vial box to the "Target Icon" of the DR3900. This will allow the instrument to store that lot number and recognize the unique properties of each new lot.

Recently, Hach released Truecal Reagent Vials for TNT 830 and TNT 832 that reduce variations in results caused by variances in chemistry raw materials. A bar code on each vial contains the calibration curve data specific to each lot of chemistry, and automatically updates the calibration curve. With TNTplus products using the Truecal feature, you'll have one less thing to worry about during crucial proficiency testing or permit-limit compliance testing**.**

***NOTE 2:** Hach has the following statement in the TNTplus method: "For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with laboratory reagent water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option. Measure the reagent blank value when a new lot of reagent is used."

***Note 3:** One should use caution when subtracting a reagent blank value from sample results as the quality of laboratory reagent water used for reagent blank analysis may give "false" positive or negative values that would bias the sample results if blank correction is used.

2. Determine which vial is necessary to analyze the sample. The ULR vial will analyze concentrations between 0.15 and 2.0 mg/L. The HR vial will analyze concentrations between 2.0 and 47.0 mg/L. Remove all necessary vials from the Lab Refrigerator and bring to room temperature before analysis.

Steps 3. – 5. are only necessary when the sample(s) being analyzed have color or turbidity after being pipetted into the reagent vial and an adjustment to the sample concentration may be needed by subtracting the "Sample Blank" reading from the sample concentration.

3. Unscrew the vial cap, pipet 5.0 mL of sample when using the ULR vial or 0.2 mL when using the HR vial, replace the cap, and shake the vial 2 - 3 times. Wipe the vial with a Kimwipe and insert the vial into the cell holder.

4. Record the reading on the DR3900 as the "Sample Blank".

5. Remove the vial from the DR3900.

6. Carefully remove the foil lid from all TNTplus vials including any that have been used for "Sample Blank" determination and unscrew the DosiCap. Flip the DosiCap over so that the reagent side faces the vial. Screw the cap on tightly.

7. Shake the capped vial until the reagent dissolves. This can be verified by looking through the end of the cap and confirming that there is no white residue in the cap.

8. Wait 15 minutes.

9. Invert the sample an additional 2 - 3 times to mix.

10. Wipe the outside of the vial with a Kimwipe.

11. Insert the vial into the cell holder.

12. Once the reading is displayed and samples are being "Reagent Blank" corrected in your laboratory, readjust the result to compensate for the Reagent Blank as follows: see **Notes 2 and 3** in step 1.

a. Press "Options"
b. Press "More"
c. Press "Reagent Blank"
d. Press "On"
e. Tap on the highlighted box that displays a concentration
f. Enter the result for the corresponding Reagent Blank recorded on the bench sheet. (Enter the HR result for the HR vial results and the ULR result for the ULR vial results).
g. Press "OK"
h. Press "OK"
i. Press "Return"

13. Record this new reading as your sample result. The result is displayed in mg/L and needs no further calculation.

14. If steps 12 and 13 are not necessary and a "Sample Blank" is analyzed due to color or

turbidity, the "Sample Blank" would be subtracted from the sample result at this time to give the reportable result.

15. Analyze all necessary QA parameters at the frequency stated in the laboratory's Quality Assurance/Quality Control document. All QA parameters must be within control limits or the test is considered invalid.

V. QUALITY CONTROL – the following Quality Control samples and frequency of analysis are recommended to produce accurate data. **Refer to Note above Table I "Quality Control Sample Frequency" on page 8.**

A. Reagent Blank – See ***Note 3** in Step 1 under IV. PROCEDURE

B. Sample Blank - Samples with color or turbidity can cause high results. Samples without color or turbidity do not require sample blanks. To adjust for color or turbidity, use the steps that follow to find the sample blank.

1. Do the test procedure, but do not remove the protective foil lid from the vial
    a. Record result as Sample Blank concentration

2. Remove protective foil and do the normal test procedure.
    a. Record result

3. Subtract the Sample Blank result in 1.a. from the result in 2.a. to get the corrected sample concentration

C. Calibration Check Standards

1. 1.0 mg/L ULR Check Standard – prepare by diluting 1.0 mL of 100 mg/L ammonia-N Standard to 100 mL in a Class A Volumetric Flask with Laboratory Reagent Water

The ULR Check Standard should be analyzed at the frequency stated in the laboratory's Quality Assurance/Quality Control document. The value obtained must fall between 90% - 110% of the true value of 1.0 mg/L.

- Pipet 5.0 mL of 1.0 mg/L standard into a ULR vial and analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

- Result must be between 0.90 and 1.10 mg/L.

2. 10.0 mg/L HR Check Standard – prepare by diluting 10.0 mL of 100 mg/L ammonia-N Standard to 100 mL in a Class A Volumetric Flask with Laboratory Reagent Water

The HR Check Standard should be analyzed at the frequency stated in the laboratory's Quality Assurance/Quality Control document. The value obtained must fall between 90% - 110% of the true value of 10.0 mg/L.

- Pipet 0.2 mL of 10.0 mg/L standard into a HR vial and analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

- Result must be between 9.0 and 11.0 mg/L.

D. Duplicate

Duplicate sample(s) are analyzed at the frequency stated in the laboratory's Quality Assurance/Quality Control document. Run duplicates on Final samples only if the concentration is ≥ 0.05 mg/L ammonia-N. The duplicate results must be within the established Control Limits.

E. Spiked Samples

Spiked sample(s) are analyzed at the frequency stated in the laboratory's Quality Assurance/Quality Control document.

1. Final Effluent Spike

- Pipet 0.5 mL of the 1.0 mg/L Standard into a ULR Reagent vial.
- Pipet 4.5 mL Final Effluent into the same ULR Reagent vial

- Analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

Percent Spike Recovery (%R) Calculation

A = Final Effluent Result **with Spike** (mg/L)
B = 5.0 – Volume of Final Effluent + Spike sample (mL)
C = Final Effluent Result **without** Spike added (mg/L)
D = 4.5 – Volume of Final Effluent sample used in Spike sample (mL)
E = 1.0 Concentration of Spike added (mg/L)
F = 0.5 – Volume of Spike Added (mL)

$$\frac{[(A \times B) - (C \times D)] \times 100}{(E \times F)} = \text{Percent Recovery}$$

Recovery must be between 85% and 115%.

Example

A = Final Effluent Result **with** Spike = 0.416 mg/L
B = Volume of Final Effluent + Spike = 5.0 mL

C = Final Effluent Result **without** Spike Added = 0.354 mg/L

D = Volume of Final Effluent used in Spike sample = 4.5 mL

E = Concentration of Spike Added = 1.0 mg/L

F = Volume of Spike Added = 0.5 mL

$$\frac{[(0.416\ mg/L \times 5.0\ mL) - (0.354\ mg/L \times 4.5\ mL)] \times 100}{(1.0\ mg/L \times 0.5\ mL)} = 98.0\%\ Recovery$$

2. Raw Influent Spike

- Pipet 5.0 mL of 100 mg/L Standard into a Class A Erlenmeyer flask or graduated cylinder containing 95 mL of Raw Influent and mix thoroughly

- Pipet 0.2 mL of the above Raw Influent plus Spike solution into an HR Reagent vial

- Analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

Percent Spike Recovery (%R) Calculation

A = Raw Influent Result **with Spike** (mg/L)

B = 100 – Volume of Raw Influent + Spike sample (mL)

C = Raw Influent Result **without** Spike added (mg/L)

D = 95 - Volume of Raw Influent sample used in Spike sample (mL)

E = 100. Concentration of Spike added (mg/L)

F = 5.0 – Volume of Spike Added (mL)

$$\frac{[(A \times B) - (C \times D)] \times 100}{(E \times F)} = Percent\ Recovery$$

Recovery must be between 85% and 115%.

Example

A = Raw Influent Result **with** Spike = 16.9 mg/L

B = Volume of Raw Influent + Spike = 100 mL

C = Raw Influent Result **without** Spike Added = 12.6 mg/L

D = Volume of Raw Influent used in Spike sample = 95 mL

E = Concentration of Spike Added = 100. mg/L

F = Volume of Spike Added = 5.0 mL

$$\frac{[(16.9\ mg/L \times 100\ mL) - (12.6\ mg/L \times 95\ mL)] \times 100}{(100.\ mg/L \times 5.0\ mL)} = 98.6\%\ Recovery$$

F. Method Detection Limits

MDLs using the criteria in the latest 40 CFR Part 136 Approved Methodology must be analyzed using Ultra Low Range (ULR) TNTplus Reagent Vials at the frequency stated in 40 CFR Part 136.

G. Approved Reference Standards

Approved Reference Standards are analyzed at least quarterly and results fall within the 95% Confidence Interval and/or split samples are analyzed at least quarterly and results fall within 20% RPD.

VI. INTERFERENCES

High concentrations of dissolved ions can affect the measurement of ammonia-N using this method.

Amines are a positive interference.

Dissolved ions were individually examined to the given concentrations and do not cause interference.

No cumulative effects or influences of other ions were found.

Primary amines are found and cause high-bias results. A 10,000-fold excess of urea does not interfere.

All reducing agents interfere and cause low-bias results.

Note: *An analyte concentration that is larger than the stated range adversely influences on color formation, which results in a false reading within the method range.*

Verify measurement results with sample dilutions or standard additions.

Distillation is necessary for samples with severe interferences.

Interfering Substances & The Interference Levels:

Cl–, SO42	1000 mg/L
K+, Na+, Ca2+	500 mg/L
CO32–, NO3–, Fe3+, Cr3+, Cr6+, Zn2+, Cu2+, Co2+, Ni2+, Hg2+	50 mg/L
Fe2+	25 mg/L
Sn2+	10 mg/L
Pb2+	5 mg/L
Ag+	2 mg/L

VII. CALCULATION

NH_3-N mg/L = Direct reading of HACH DR3900 Spectrophotometer unless the sample has been preserved and neutralized. See II. SAMPLING, STORAGE AND PRESERVATION above.

VIII. WASTE MANAGEMENT

The ammonia salicylate reagent contains sodium nitroferricyanide which, when digested, is converted to total cyanide and can have an effect on total cyanide limits in the Effluent. Dispose of reacted solutions according to local, state and federal regulations.

IX. REFERENCES

HACH Method 10205. TNTplus 830 and TNTplus 832

EPA Method 351.2

Determination of Ammonia-N
Ion Selective Electrode (ISE) Method
Reference: Standard Methods 22nd ed. 4500-NH3 D.

I. INTRODUCTION

The ammonia selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride.

Dissolved ammonia (NH_3 (aq.) and NH_4) is converted by raising the pH above 11. with a strong base. NH_3 (aq.) diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

The ion selective electrode method for NH_3 is applicable to the measurement of 0.03 to 1400 mg NH_3-N/L in domestic and industrial wastes. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary in the absence of interferences. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg/L NH_3-N; hence use longer times of electrode immersion (5 to 10 min.) to obtain stable readings.

II. SAMPLING, STORAGE AND PRESERVATION

Samples to be measured can be either grab or composite whichever is required in the NPDES permit.

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 hours of collection, refrigerate unacidified at ≤ 6°C. Samples intended to be analyzed at a later date must be preserved when collected to prevent escape of NH_3 gas. Use 1 mL of 1:1 H_2SO_4 in 500 mL of sample. Samples can be checked with pH paper to ensure preservation is adequate. Preserved samples can be held for analysis up to 28 days at ≤ 6°C.

If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination. Caution: although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

Samples and standards should be at the same temperature at the time of analysis.

III. REAGENTS and EQUIPMENT

A. Reagents

1. Sulfuric Acid 1:1, slowly add a volume of concentrated H_2S0_4 to an equal volume of laboratory reagent water. Mix well in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO PREPARE THIS SOLUTION IN THE ACID BOTTLE. Allow to cool at room temperature. This solution is used to preserve samples.

   NOTE: Always add acid to water! A highly exothermic reaction takes place when making this dilute acid. DO NOT place into cold water once the flask is hot! Prepare a cold water bath to constantly cool the mixture during preparation.

2. 10*N* sodium hydroxide (NaOH), dissolve 400 g NaOH in 800 mL of laboratory reagent water, cool to room temperature and dilute to 1000 mL. This solution is used to neutralize previously preserved samples or can also be used to raise the pH to > 11 during analysis in place of the pH adjusting ISA.

3. A pH adjusting ISA (5M NaOH, 10% methanol, color indicator, 0.05 M disodium EDTA) such as Orion Cat. No. 951211.

4. Electrode internal filling solution such as Orion Cat. No. 951202.

5. Electrode storage solution. Use the 1000 mg/L ammonia-N standard or manufacturer's recommended storage solution.

6. 1000 mg/L ammonia-N Standard such as Orion No. 951007.
   Additional standards may be prepared from this standard (see procedure) or purchased separately in the needed concentrations.

   6.a. The 1000 mg/L ammonia-N standard may be prepared in lab by drying anhydrous ammonium chloride (NH_4Cl) at 100^0C, then weighing 3.819 g and diluting to 1 liter with ammonia-free water.

7. Ammonia-free laboratory reagent water for making all dilutions and blanks.

B. Equipment

1. 150 mL glass beakers
2. 100 mL and 1 liter volumetric flasks: Class A
3. 1 mL, 3 mL, 5 mL, 10 mL volumetric pipettes: Class A
4. 5 mL, 10 mL, 25 mL wide bore graduated pipettes
5. Thermally insulated magnetic stirrer and Teflon coated stirring bars; or Orion Stirrer Probe, Orion Cat. No. 096019.
6. Ammonia ion selective electrode, such as Orion model 9512BNWP, Orion Cat. No. 951201.
7. pH/ISE meter, such as Orion model 940A or Thermo Scientific 4 Star pH/ISE meter

IV. PROCEDURE

A. Preparation of Standards

1. 1000 mg/L ammonia-N (NH_3-N) stock standard solution:
   purchase commercially, Orion 951007 or equivalent (check expiration date) OR prepared in laboratory as follows: dry anhydrous ammonium chloride (NH_4Cl) at 100°C then cool to room temperature in a desiccator before weighing 3.819 g and diluting to 1 liter with ammonia-free laboratory reagent water.
   May be stored up to 6 months

2. 100 mg/L ammonia-N (NH_3-N) standard solution:
   purchase commercially (check expiration date) OR prepare by diluting 100. mL of 1000 mg/L ammonia-N (NH_3-N) stock standard solution to 1 liter with ammonia-free water.
   May be stored up to 6 months.

3. 10 mg/L ammonia-N (NH_3-N) standard solution:
   purchase commercially (check expiration date) OR prepare by diluting 10. mL of 1000 mg/L ammonia-N (NH_3-N) stock standard solution to 1 liter with ammonia-free water.
   May be stored up to 2 weeks.

4. 1.0 mg/L ammonia-N (NH_3-N) standard solution:
   purchase commercially (check expiration date); OR prepare by diluting 10. mL of 100 mg/L standard solution to 1 liter with ammonia-free water.
   May be stored up to 2 weeks.

5. 0.1 mg/L ammonia-N (NH_3-N) standard solution:
   purchase commercially (check expiration date); OR prepare by diluting 10. mL of 1.0 mg/L standard solution to 100 mL with ammonia-free water.
   Make fresh each time meter is calibrated.

6. 3.0 mg/L ammonia-N (NH_3-N) standard solution (used to check calibration curve):
   purchase commercially (check expiration date); OR prepare by diluting 3.0 mL of *1000 mg/L NH_3-N to 1 liter with ammonia-free water.
   May be stored up to 2 weeks.
   *The 3.0 mg/L calibration check standard should trace back to a different lot number than the lot number of the purchased standards or those standards prepared in the laboratory used to establish the calibration curve - referred to as a Second Source Standard.

B. Meter Calibration and Analysis (example given is for an Orion 940A – follow the manufacturer's instructions for your specific meter)

1. Pressing any key when it is in stand-by mode turns on the meter. Immerse the probe in ammonia-free water for 1 to 2 minutes while stirring. Do not stir so rapidly that a vortex is created. Creating a vortex may introduce air bubbles into the solution. If they become trapped on the electrode membrane they will cause interference. Stir just fast enough to mix the solution well. Maintain the same stirring rate throughout the calibration and testing procedures.

2. To calibrate the meter, start by pressing the "Calibrate key". The meter will display the date and time of last calibration.

3. The display asks for the number of standards to be used in the calibration. Press the number "3" key and then press the "yes" button.

4. Now proceed with the introduction of the standards. Calibrate starting with 100 mL of the lowest standard (0.1 mg/L).

5. Add sufficient volume of the ISA (usually 2 mL is sufficient) to raise the pH above 11. The sample will turn blue (if using a color indicating ISA) when the pH is 11 or greater. **Do not add** the ISA before immersing electrode as ammonia-N may be lost from the basic solution. If the electrode is not immersed it cannot detect the ammonia-N that is released during this time.

6. Keep the electrode in solution until a stable reading is obtained (typically 2 to 3 minutes but longer times may be required for standards and samples of low concentrations).

7. After the meter stabilizes and a ready message comes on the screen, key in the standard's concentration value and then press the "yes" key so the meter will accept the value.

8. Check and record the millivolt reading by pressing the "2^{nd}" button and then the "mV" key**.** Return to the concentration mode after recording mV by pressing the measure key.

9. Repeat the steps from above for the remaining standards, proceeding from lowest to highest in concentration. Note: If the electrode is functioning properly a tenfold change of NH_3-N concentration results in an mV potential change of -57 ± 3 mV at 20°C.

10. After the calibration is complete, check the curve with a 3.0 mg/L Second Source ammonia-N standard. This standard, for QC purposes, should fall between 95% to 105% recovery (2.85 mg/L —3.15 mg/L).

11. A method blank sample should also be analyzed. It is important to show that your ammonia-free laboratory reagent water measures < the established Method Detection Limit.

12. If all the above meet criteria, proceed with analysis of samples.

13. All sample volumes are based on a 100 mL final volume. Record sample concentration values from a stable meter display. Dilute samples if necessary to bring the ammonia-N concentration to within the calibration curve range (0.1 mg/L to 10. mg/L if the calibration curve was established using 0.1, 1.0 and 10. mg/L standards). If an aliquot of sample needs to be diluted to 100 mL, the dilution factor, i.e., (100 mL/sample mL used in the dilution) will need to be used to calculate the original sample concentration by multiplying the analytical result times the dilution factor.

14. Always make sure you rinse the probe between samples to prevent carryover, but do not rub the electrode membrane.

15. Check the slope of the calibration curve over time by analyzing the 3.0 mg/L standard periodically throughout and at the end of the analysis - acceptable result is 2.85 – 3.15 mg/L.

16. When sample analysis is complete, place the meter in Standby and rinse the probe with ammonia-free water then immerse it in the manufacturer's recommended storage solution for short term storage.

V. QUALITY CONTROL

A. A 3.0 mg/L standard of a different lot number or source than the calibration standards should be analyzed each time the meter is calibrated. The value obtained must fall between 95% - 105% of the true value of 3.0 mg/L. This standard should also be analyzed periodically throughout and at the end of the analysis.

B. A duplicate sample is analyzed with each analytical run on the Raw and the Final. Analyze duplicates on Final samples only if its concentration falls above the Method Detection Limit. All measured duplicates should fall within the current control limits.

C. Spiked samples should be analyzed on both Raw and Final samples. Once again, only analyze a Final spike if the concentration of the Final sample is above the Method Detection Limit. A general rule of thumb is the spiked sample should have about twice the concentration of the original sample. All measured spikes should fall within the current control limits.

D. Approved Reference Standards should be analyzed at least quarterly and results fall within the 95% Confidence Interval and/or split samples are analyzed at least quarterly and results fall within 20% RPD.

VI. SPIKE CALCULATIONS

$$\% \text{ Spike Recovery} = \frac{(B - A) \times 100}{C}$$

Where:

A = sample concentration, mg/L x total volume of sample, mL

B = sample + spike concentration, mg/L x total volume of sample + spike analyzed, mL

C = concentration of spike added, mg/L x volume of spike added, mL

Note: concentration, mg/L x total volume, mL = total µg of analyte of interest

Example 1 for sample that is not diluted prior to analysis:

100 mL of Final Effluent is analyzed and the result is 0.27 mg/L NH_3-N

100 mL of the same Final Effluent is spiked with 1.0 mL of 10 mg/L NH_3-N standard and the result is 0.36 mg/L. As the spike added is not > 1.0 mL, a sample volume adjustment does not need to be made.

A = 0.27 mg/L x 100 mL = 27. µg

B = 0.36 mg/L x 100 mL = 36. µg

C = 10 mg/L x 1.0 mL = 10. µg

$$\% \text{ Spike Recovery} = \frac{(B - A) \times 100}{C}$$

$$\% \text{ Spike Recovery} = \frac{(36.\ \mu g - 27.\ \mu g) \times 100}{10.\ \mu g} = 90\%$$

Example 2 for diluted samples when the spike is added before diluting to 100 mL:

25 mL of Raw Influent is diluted to 100 mL with ammonia-free water and analysis gives a result of 3.68 mg/L. **Note: final volume = 100 mL**

25 mL of the same Raw Influent is spiked with 10 mL of 10 mg/L NH_3-N standard and then brought up to 100 mL with ammonia-free water and analysis gives a result of 4.76 mg/L. **Note: final volume = 100 mL**

A = 3.68 mg/L x 100 mL = 368 µg

B = 4.76 mg/L x 100 mL = 476 µg

C = 10 mg/L x 10 mL = 100 µg

$$\% \text{ Spike Recovery} = \frac{(B - A) \times 100}{C}$$

$$\% \text{ Spike Recovery} = \frac{(476\ \mu g - 368\ \mu g) \times 100}{100\ \mu g} = 108\%$$

Example 3 for diluted samples when the spike is added after the sample is diluted to 100 mL and the volume of spike added is > 1.0 mL:

25 mL of Raw Influent is diluted to 100 mL with ammonia-free water and analysis gives a result of 3.68 mg/L. **Note: final volume = 100 mL**

25 mL of the same Raw Influent is brought up to 100 mL with ammonia-free water and then spiked with 10 mL of 10 mg/L standard. Analysis gives a result of 4.27 mg/L. **Note: final volume of the spiked sample = 110 mL**

A = 3.68 mg/L x 100 mL = 368 µg

B = 4.27 mg/L x 110 mL = 470 µg

C = 10 mg/L x 10 mL = 100 µg

$$\% \text{ Spike Recovery} = \frac{(B - A) \times 100}{C}$$

$$\% \text{ Spike Recovery} = \frac{(470\ \mu g - 368\ \mu g) \times 100}{100\ \mu g} = 102\%$$

Note: the dilution factor must be taken into account for Examples 2 and 3 when reporting the Raw Influent concentration:

Dilution factor = final volume of diluted sample ÷ sample volume used
= 100 mL ÷ 25 mL = 4

Concentration to report: 3.68 mg/L x 4 = 14.7 mg/L

VII. METHOD DETECTION LIMIT (MDL)

A Method Detection Limit using the criteria in the latest 40 CFR Part 136 Approved Methodology must be analyzed at the frequency stated.

VIII. TROUBLE SHOOTING

A. Standards and samples should be at room temperature before analysis. A 1°C difference in temperature will result in about a 2% measurement error.

B. The probe's membrane should be free of air bubbles while immersed in samples. Immersing the probe at a 45 degree angle should help prevent bubbles.

C. Unusually long periods of time for meter reading stabilization and membrane holes or discoloration may indicate the internal filling solution and membrane needs to be changed.

D. Ammonia-N concentrations < 1.0 mg/L need longer stabilization times, from 5 to 10 minutes, to obtain a more accurate reading.

E. If a power outage occurs while the meter is in standby or in operation, the memory may be lost. The meter will need to be re-programmed and recalibrated before the analysis can be continued. If this happens, unplug the meter to reset the electronics before proceeding with programming.

F. Never touch the membrane with the hands. Oils from the hand can coat the membrane and interfere with normal operation.

G. Place a piece of insulating material, such as Styrofoam or cardboard, between the magnetic stir plate and the beaker to prevent measurement errors from the transfer of heat to the sample.

H. Final Effluent sample should be checked for chlorine and dechlorinated if necessary.

I. For problems, other than the ones mentioned, refer to the electrode manual (Model 95-12 Ammonia Electrode Instruction Manual) or instrument instruction booklet (Orion Bench Top pH/ISE Meter Instruction Manual Model 920A).

IX. INTERFERENCES

High concentrations of dissolved ions can affect the measurement of ammonia using this method. Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution is used.

X. PRECISION AND BIAS

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH_3-N/L, standard deviations were ±0.037, ±0.017, ±0.007, and ±0.003, respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 mg NH_3-N/L, recoveries were 96% and 91%, respectively.

XI. REFERENCES

A. Standard Methods for the Examination of Water and Wastewater, 22nd Edition.

B. Orion Laboratory Products Group Bench Top pH/ISE Meter Instruction Manual, Model 940A, (1991).

C. Ammonia Ion Selective Electrode, Thermo Fisher Scientific (2002).

Ammonia Distillation Process
and
Instructions for Performing the Comparability Study
for Distilled and Undistilled Samples

DO YOU KNOW WHERE YOUR AMMONIA COMPARABILITY DATA IS?

Guidelines establishing test procedures for the analysis of pollutants found at 40 CFR Part 136 contain a footnote (6) regarding the manual distillation procedure for ammonia-N (*Methods for Chemical Analysis of Water and Wastes* 350.2 or *Standard Methods for the Examination of Water & Wastewater* 21st ed. 4500-NH_3 B.). The footnote reads as follows:

> "Manual distillation is not required if comparability data on representative Final Effluent samples are on company file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies".

Although the footnote contains no reference as to when this comparability data should be updated, IDEM recommends facilities over 4.0 MGD to redo the comparability data biennially or, as warranted, if a major change in the waste streams occurs. (If a specific Quality Assurance Program at a facility sets this update at a more frequent interval that program would override this recommendation.)

Any questions regarding this policy should be directed to Becky Ruark at 317-691-1909.

The following sections contain instructions for conducting the comparability study.

There are two set-up procedures for conducting the Ammonia Comparability Study:

Set-up Procedure 1

1. Collect at least three Final Effluent samples (one gallon each) on different days of the week and preserve with sulfuric acid (8.0 mL of 1:1 H_2SO_4 per gallon) and put in cold storage (≤ 6° C). No sample preservation is necessary if the test is conducted immediately.

2. Divide each sample into four aliquots.

3. Test each aliquot for NH_3-N with and without prior distillation using the laboratory's ammonia-N method. Testing should be conducted within seven days of sample collection but testing within 24 hours is preferred to avoid possible effect of storage on the nitrogenous matter in sewage.

4. Compare the average for NH_3-N values of each sample (four NH_3-N values without distillation and four for NH_3-N values with distillation). There will be a total of 24 NH_3-N values (three samples x four aliquots/sample without distillation and three samples x four aliquots/sample with distillation).

5. If the difference of NH_3-N values is < 5% of the average NH_3-N values on the average of three samples, one can consider the comparative data acceptable.

6. Compile the comparative data generated by the foregoing steps in a tabular form and retain in your files for a minimum of three years.

Set-up Procedure 2

1. Each sample is divided into three portions

    a. Portion A to be analyzed after distillation

    b. Portion B to be analyzed after distillation

    c. Portion C to be analyzed without distillation

2. Determine the standard deviation (SD) of the differences of Portion A and Portion B on twenty different samples

3. Determine the average value of Portion A and Portion B for each sample

4. Compare the average value of Portion A and B to the value for Portion C. If the value for Portion C is consistently within three (3) standard deviations of the average of Portion A and B, then distillation would not be required.

AMMONIA NITROGEN DISTILLATION METHOD

DISCUSSION: The distillation of samples prior to analysis for ammonia nitrogen removes the ammonia from components of the sample which would present interferences. A borate buffer solution is added to the sample before distillation which buffers at a pH of 9.5. This minimizes the hydrolysis of cyanates and organic nitrogen compounds which would increase the ammonia concentration in the sample. Ammonia distilled out of the sample is absorbed into either boric acid or sulfuric acid. Boric acid must be the absorbing solution if either the titration or nesslerization method will be used to determine ammonia nitrogen concentration; sulfuric acid must be the absorbing solution if the ion selective electrode (ISE) will be used.

While prior distillation is a requirement for the titrimetric procedure, it may be omitted under certain conditions for the nesslerization and ISE methods. For purposes of NPDES reporting, the EPA requires distillation of all samples unless data on representative Final Effluent samples are on file that show that comparable results are obtained without distillation.

1. APPARATUS

    a. Distillation apparatus – a Pyrex flask of 800 – 2000 mL capacity attached to a vertical condenser

    b. pH meter

2. REAGENTS

    a. Ammonia-free laboratory reagent water for dilution of samples and preparation of reagents and standards

    b. Borate buffer solution, pH 9.5 – Dissolve 9.5 grams sodium tetraborate decahydrate, $Na_2B_4O_7 \cdot 10\ H_2O$ in laboratory reagent water and dilute to 1 liter. To 500 mL of this solution, add 88 mL of 0.1*N* sodium hydroxide, NaOH and dilute to 1 liter with laboratory reagent water

    c. Absorbing Solution – for the ISE method use 0.04*N* sulfuric acid; for the titrimetric and nesslerization methods use boric acid
        i. Sulfuric acid, 0.04*N* – dilute 1.0 mL concentrated sulfuric acid, H_2SO_4 to 1 liter
        ii. Boric acid – Dissolve 20 grams H_3BO_3 in laboratory reagent water and dilute to 1 liter with laboratory reagent water

    d. Sulfuric acid, 1*N* – Add 28 mL of concentrated sulfuric acid, H_2SO_4 to 500 mL laboratory reagent water and dilute to 1 liter with laboratory reagent water

    e. Sodium hydroxide, 1*N* – Dissolve 40 grams of sodium hydroxide, NaOH in laboratory reagent water and dilute to 1 liter with laboratory reagent water

f. Sodium hydroxide, 0.1*N* – Dissolve 4.0 grams of sodium hydroxide, NaOH in laboratory reagent water and dilute to 1 liter with laboratory reagent water

g. Dechlorinating agent, either of the following may be used:
    i. Sodium thiosulfate, 0.014*N* – Dissolve 3.5 grams sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5\ H_2O$ in laboratory reagent water and dilute to 1 liter with laboratory reagent water. Prepare fresh weekly.
    ii. Sodium sulfite, 0.014*N* – Dissolve 0.9 grams sodium sulfite, Na_2SO_3 in laboratory reagent water and dilute to 1 liter with laboratory reagent water. Prepare fresh daily.

3. PROCEDURE

a. If more than 4 hours have elapsed since the last use of the distillation apparatus, add 500 mL of laboratory reagent water and 25 mL of borate buffer solution and a few boiling chips to the distillation flask. Steam out the distillation apparatus until at least 300 mL of distillate has been collected.

b. Measure out 500 mL of sample or, if necessary, an aliquot of sample diluted to 500 mL. If the ammonia nitrogen concentration of the sample it expected to be < 0.1 mg/L, use a sample volume of 1000 mL. If sample has a chlorine residual, dechlorinate using the appropriate amount of 0.014*N* sodium sulfite or sodium thiosulfate (1 mL of 0.014*N* solution removes 1 mg/L residual chlorine in 500 mL sample).

c. Raise or lower the pH of the sample to 9.5 with 1*N* sodium hydroxide, NaOH or 1*N* sulfuric acid, H_2SO_4.

d. Remove the water from the steamed-out flask and pour in treated sample.

e. Add 25 mL borate buffer.

f. Distill at a rate of 6 – 10 mL/minute with the tip of the delivery tube submerged in 50 mL of the absorbing solution in a 500 mL Erlenmeyer receiving flask.

g. Collect at least 300 mL of distillate.

h. Lower the receiving flask so that the end of the delivery tube no longer contacts the liquid in the flask and continue distilling for a couple of minutes to clean out the apparatus.

i. Dilute the distillate to 500 mL with ammonia-free laboratory reagent water and mix well. (NOTE: If the titrimetric method is to be used, it is not necessary to dilute the distillate to 500 mL; the volume of distillate collected may be titrated directly.)

DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND (BOD_5)

Reference: Standard Methods 22nd ed. 5210 B.

This procedure is seperated into several sections. This has been done to aid in simplyfying what can seem like a complicated procedure. The sections are seperated as follows:

SECTION #	DESCRIPTION	PAGE #
1	Dissolved Oxygen Determination - SM4500-O G. Membrane-Electrode Method - SM4500-O H. Optical-Probe Method	59
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3	Pretreatment of Samples	61
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1. Dissolved Oxygen Determination:

Dissolved Oxygen (DO) and BOD_5 Determinations:
Whichever method is used Membrane-Electrode Method or Optical-Probe Method follow the manufacturer's procedures for complete operation and calibration of the DO meter and probe. Steps 1 – 8 gives a general description of performing a DO measurement when performing the BOD_5 analysis.

1. Membrane-Electrode Method - With each use of the DO probe, check the following:

✓ Wrinkles
✓ Discolorations
✓ Bubbles
✓ Suspended matter in filling solution
✓ Surface slime

If any of the above are observed, replace the membrane per specific manufacturer's instructions.

2. Optical-Probe Method - With each use of the DO probe, check the following:

✓ Surface slime

Typically, the caps on these probes are replaced less frequently then membrane caps. In the event, the cap needs replaced follow the manufacturer's instructions.

3. Read and record the barometric pressure each day of analysis. This can be from a barometer in the laboratory. Barometric pressure readings should <u>not</u> be corrected to sea level.

4. Calibrate the instrument used for BOD_5 analysis each day of use and document. Note: The BOD_5 bench sheet located on IDEM's Website: http://www.in.gov/idem/cleanwater/2443.htm indicates the information that needs to be recorded when calibrating the instrument.

5. Determine the Initial DO of all bottles in the analytical run and record on bench sheet.

6. Fill water seals with dilution water and snap on plastic caps to reduce evaporation from seals. Place all bottles in the analytical run in a 20 ± 1°C incubator for 5 days ± 6 hours.

7. After removing from the incubator and before removing the stoppers, pour off excess water.

8. After 5 days ± 6 hours determine the DO of all samples in the analytical run and record the result as Final DO.

2. Dilution Water Preparation:

Apparatus:

1. 300 mL BOD bottles
2. 2 - 5 liter glass bottle with siphon
3. 20 ± 1°C incubator
4. DO meter and probe
5. Buret
6. Class A pipets

Source Water and Reagents:

1. **Source water for preparing BOD dilution water:** Obtain water from a suitable source such as deionized, distilled, tap, or natural water for making sample dilutions. If the dilution water blanks show a DO depletion greater than 0.20 mg/L, obtain satisfactory water by improving purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light in attempts to bring the dilution blank into range.

2. **Phosphate buffer:** Dissolve 8.5 g monopotassium phosphate (KH_2PO_4), 21.75 g dipotassium phosphate (K_2HPO_4), 33.4 g disodium phosphate ($Na_2HPO_4 \bullet 7H_2O$), and 1.7 g ammonium chloride (NH_4Cl) in about 500 mL laboratory reagent water and dilute to 1 L. with laboratory reagent water. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g monopotassium phosphate (KH_2PO_4) and 1.7 g ammonium chloride (NH_4Cl) in about 700 mL laboratory reagent water. Adjust pH to 7.2 with 30% sodium hydroxide (NaOH) and dilute to 1 L with laboratory reagent water.

3. **Magnesium sulfate solution:** Dissolve 22.5 g ($MgSO_4 \bullet 7H_2O$) in laboratory reagent water. Dilute to 1 L with laboratory reagent water.

4. **Calcium chloride solution:** Dissolve 27.5 g ($CaCl_2$) in laboratory reagent water. Dilute to 1 L with laboratory reagent water.

5. **Ferric chloride solution:** Dissolve 0.25 g ($FeCl_3 \bullet 6H_2O$) in laboratory reagent water. Dilute to 1 L with laboratory reagent water.

Note: Prepared reagents or BOD Nutrient Buffer Pillows containing the nutrients for specific volumes of BOD dilution water, i.e., 300 mL, 3 L, 4 L, 6 L or 19 L can be purchased as an alternative.

Preparation of Dilution Water:

1. Dilution water is prepared by adding 1.0 mL of each nutrient solution or the appropriate Nutrient Buffer Pillow/L of source water. Dilution water should be allowed to equilibrate in the incubator or in the lab at room temperature (20-22°C or 68-72°F).

2. It is important that dilution water is saturated with oxygen. Saturation can be accomplished by bubbling filtered, compressed air into the dilution water.

3. Completely fill one or more bottles (two or more is recommended) with dilution water to be incubated as blanks. More details to this step are given in Section 8 BOD_5 Setup.
   $CBOD_5$ – DO NOT add nitrification inhibitor to the blanks.

4. The BOD_5 bench sheet located on IDEM's Website: http://www.in.gov/idem/cleanwater/2443.htm indicates the information that needs to be recorded when analyzing BOD_5.

3. Pretreatment of Samples:

Reagents:

1. **1*N* Sodium hydroxide (NaOH**): Dissolved 4.0 grams NaOH in 60 mL laboratory reagent water and after cooling to room temperature dilute to 100 mL with laboratory reagent water.

2. **1*N* Sulfuric acid (H_2SO_4):** Dilute 2.8 mL concentrated H_2SO_4 in 60 mL laboratory reagent water and after cooling to room temperature dilute to 100 mL with laboratory reagent water.

Sample Adjustments:

1. Sample temperature: Bring sample temperature to 20 ± 3°C.

2. The diluted sample used to determine BOD_5 must have a pH between 6.0 and 8.0. If the pH is not within the 6.0 to 8.0 range then adjust the pH to 7.0 to 7.2. This can be accomplished by neutralizing samples with 1*N* sulfuric acid or 1*N* sodium hydroxide (base). Do not dilute the sample with the acid or base by more than 0.5% (1.5 mL in a 300 mL BOD bottle).

3. If any type of chlorination process is employed during treatment, Final Effluent samples are initially tested for the presence of residual chorine. Dechlorination is required if a chlorine residual is present. Residual chlorine can kill the microorganisms that are critical to BOD_5 analysis.

4. Samples supersaturated with dissolved oxygen, over 9.0 mg/L at 20°C, may be encountered during winter months in localities where algae is actively growing (lagoons) or when samples have been preserved by cooling to ≤ 6.0°C . To prevent excess loss of oxygen due to supersaturation during incubation of these samples, the DO should be reduced by warming them to room temperature than shaking the samples or aerating them with filtered compressed air. *These types of samples often have a high concentration of nitrifying organisms which can lead to bias in BOD_5 results.*

5. Samples of industrial wastes, **disinfected wastes**, high temperature wastes, or wastes with extreme pH values may not contain enough microorganisms to oxidize the biodegradable matter in the samples. Such samples must be seeded. See BOD_5 Seeding Procedure in Section 7.

6. If carbonaceous BOD_5 is required in your NPDES permit, it is necessary to add nitrification inhibitor to <u>all</u> samples. DO NOT add nitrification inhibitor to the blanks.

    a. 1) Nitrification inhibition using 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Add 10 mg TCMP/L to diluted sample or 3 mg TCMP to each 300-mL bottle or sample dilution vessel, or proportional amounts to other sized bottles, after initial sample dilution but before final filling of the bottles with dilution water.

    b. Nitrification inhibition using allylthiourea (ATU)—Add 1 mL ATU solution/L diluted sample or 0.3 mL/300mL test bottle or sample dilution vessel. Do not add ATU to BOD bottles until they are at least two-thirds filled with diluted sample.

4. Pretreatment of Chlorinated BOD Samples:

Reagents:

1. **Acetic acid solution, 1+1**: Carefully add 500 mL acetic acid ($C_2H_3O_2$), concentrated (glacial) to 500 mL of laboratory reagent water.

2. **Potassium Iodide Solution**: Dissolve 10 grams potassium Iodide (KI), in a 100 mL volumetric flask. Bring to volume with laboratory reagent water.

3. **Sodium Sulfite Solution, 0.0250N**: Dissolve 1.575 grams anhydrous sodium sulfite, (Na_2SO_3), in a 1,000 mL volumetric flask. Dilute to volume with laboratory reagent water.

   Note: This solution is not stable and must be prepared daily.

4. **Starch Indicator Solution (For Analysis with Iodine):** Prepare an emulsion of 5 g soluble starch in a mortar of beaker with a small amount of laboratory reagent water. Pour this emulsion into 1 L of boiling laboratory reagent water, stir, and let settle overnight. Use the clear supernatant. This solution may be preserved by the addition of 1.25 g salicylic acid/L and stored at 4°C.

Procedure:

1. Conduct a chlorine residual analysis on a portion of the sample collected. *Potassium iodide/starch paper can be used as a quick qualitative test for residual chlorine*. If no residual is found, proceed with the BOD_5 analysis utilizing seeded dilution water. If a chlorine residual is observed, proceed with the following steps before initiating the BOD_5 test.

2. Determination of volume of sodium sulfite needed to neutralize the chlorine residual in the sample prior to BOD_5 analysis.

   Determine required volume of (Na_2SO_3) solution on a 100 to 1000 mL portion of neutralized sample by adding 10 mL 1+1 acetic acid or 1+50 (H_2SO_4), 10 mL potassium iodide (KI) solution per 1000 mL sample and titrating with (Na_2SO_3) solution to the starch-iodine end point for residual. Add to neutralized sample the proportional volume of (Na_2SO_3) solution determined by the above test, mix, and after 10 to 20 minutes check sample for residual chlorine.
   NOTE: Excess (Na_2SO_3) exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.

Procedure for Dechlorinating Final Effluent BOD_5 Samples *(quick method)*

Residual chlorine may dissipate in chlorinated samples if the sample is allowed to stand for one to two hours prior to BOD_5 analysis. For those samples where this does not occur, chlorine residual is destroyed by the addition of a 10% sodium thiosulfate ($Na_2S_2O_3 \bullet 5H_2O$) solution.

Reagents and Equipment:

1. DPD Powder Pop Dispenser or DPD Powder pillows for chlorine for use with 10 mL of sample

2. Sodium thiosulfate ($Na_2S_2O_3 \bullet 5H_2O$) 10% solution: Prepare by dissolving 10 g sodium thiosulfate pentahydrate in 50 mL laboratory reagent water. Dilute to volume in a 100 mL Class A volumetric flask. Store in a refrigerator. Prepare fresh weekly.

Procedure:

1. Transfer 10 mL chlorinated sample to a clean test tube/vial. Add the contents of one DPD Powder pillow or one shot of DPD reagent from the DPD Powder Pop Dispenser. If a pink color develops, chlorine residual is present in the sample.

2. Add one drop of 10% sodium thiosulfate to the sample designated for BOD_5 analysis and mix thoroughly.

3. Check the sample in #2 using the procedure in #1.

4. If no pink color develops, the sample is ready for BOD_5 analysis if all other sample pretreatment procedures have been performed.

5. If a pink color develops, repeat steps #2 and #3 until no pink color is observed.

As sodium thiosulfate is very effective in neutralizing the chlorine, usually one drop is sufficient to remove chlorine from chlorinated samples.

<u>Use the sodium thiosulfate with caution as it has an oxygen demand and excess amounts will result in elevated BOD_5 results</u>.

After the Final Effluent sample is dechlorinated, it must be seeded during the BOD_5 sample setup procedure. Use the BOD seeding procedure found in this document.

5. Dilution Technique (prepare a least three dilutions of each sample):

1. Estimate the BOD_5 of the sample and select suitable dilutions from the following table:

Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)
< 5.0	200, 250*, 300*
< 10.	100, 150, 200
10-30	25, 50, 100
60 - 90	10, 15, 25

Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)
90 - 150	5, 10, 15
150 - 300	1, 3, 5**
300 - 1500	0.5, 1, 3**
1500 - 3000	0.25, 0.5, 1**

[*] When a bottle contains more than 67%, i.e., > 201 mL of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle) or use a commercially prepared Nutrient Buffer Pillow designed to dose the appropriate bottle size.

[**]When < 3.0 mL of sample is going to be used for analysis due to the strength of the sample, a preliminary dilution must be made prior to pipetting the sample into the BOD bottle (See 2. below) and the dilution factor taken into account when calculating the mg/L BOD.[**]

Example: a sample is expected to have a BOD of approximately 500 mg/L. The analyst decides to set up dilutions of 0.5 mL, 1.0 mL and 3.0 mL in an attempt to have a DO depletion ≥ 2.0 mg/L and also have ≥ 1.0 mg/L DO remaining after incubation.

1. For the 3.0 mL dilution, the analyst pipets 3.0 mL of well mixed sample directly into a BOD bottle

2. For the 1.0 mL and 0.5 mL dilutions, the analyst prepares a 1:10 (10% dilution) preliminary dilution by pipetting 10 mL of well mixed sample using a wide-tipped pipet into a Class A 100 mL graduated cylinder and diluting to 100 mL laboratory reagent water.

a. 10 mL of well mixed diluted sample prepared in 2. is pipetted into a BOD bottle for the 1.0 mL dilution (10 mL of a 10% dilution = 1.0 mL of original sample).

b. 5.0 mL of well mixed diluted sample prepared in 2. is pipetted into a BOD bottle for the 0.5 mL dilution (5.0 mL of a 10% dilution = 0.5 mL of original sample).

When preparing duplicate samples for quality control purposes, prepare the duplicates at the same dilutions as the original sample if there is sufficient sample volume to do this.

2. Using a **wide-tipped** pipet for samples < 50 mL or a graduated cylinder for larger sample volumes, measure the proper amount of well-mixed sample into thoroughly cleaned and rinsed 300 mL bottles.

3. Each BOD bottle is filled slowly, preventing the introduction of air bubbles and adding dilution water so that the stopper can be inserted without leaving an air bubble. The siphon hose must be made of surgical gum (latex rubber), polypropylene or polyethylene. (NO OTHER MATERIALS MAY BE USED)

4. $CBOD_5$ – nitrification inhibitor must be added to the BOD bottles where $CBOD_5$ is being determined.

5. The BOD_5 bench sheet located on IDEM's Website: http://www.in.gov/idem/cleanwater/2443/htm indicates the information that needs to be recorded when analyzing BOD_5.

Note: Make a series of dilutions of prepared sample estimated to produce a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after the 5 day incubation.

6. Preparation of Glucose-Glutamic Acid Standard (GGA)

Reagents:

Note: The glucose-glutamic acid solution can be purchased commercially. If purchasing this standard, check to make sure that each liter of standard contains 150 mg of glucose and 150 mg of glutamic acid.

1. Reagent grade glucose, ($C_6H_{12}O_6$)

2. Glutamic acid, ($HOCOCH_2CH_2CH(NH_2)COOH$)

Procedure:

1. Dry reagent grade glucose and glutamic acid at 103° C for one hour. Cool for one hour in the desiccator.

2. Dissolve 150 mg (0.150 g) of glucose and 150 mg (0.150 g) glutamic acid in laboratory reagent water and dilute to 1000 mL with laboratory reagent water in a Class A volumetric flask.

   Note: This solution will become contaminated quickly and must be used immediately unless the following is done:

   Place into each of several milk dilution bottles or test tubes/vials the quantity of GGA standard that will be used each time this standard is analyzed.

   Seal by placing caps on the bottles or test tubes/vials loosely (about a quarter turn) so they can't readily fall off but can still vent steam and sterilize for thirty minutes. Use caution, if the caps are too tight the vessels WILL explode. These sterilized portions can then be cooled to room temperature, then TIGHTEN the caps and store at ≤ 6°C. These sterilized standards can be used for up six months if not opened.

   When analyzing this standard, pipet six mL of GGA standard into all three BOD bottles being used for analysis of the GGA standard. This is critical! The BOD_5 concentration of 198 ± 30.5 mg/L for this standard is based on a 2% dilution of GGA (6.0 mL/300 mL BOD bottle). It is important NOT to use dilutions other than the 2% dilution.

   Prepare the GGA samples in the following order.

   1. Fill the BOD bottles 2/3 full with dilution water.
   2. Add the 6 mL of GGA to the BOD bottle.
   3. Add the appropriate seed volume to the BOD bottle.

   Note: The seed volume added to GGA samples should be the same as the seed volume added to seeded samples.

4. If $CBOD_5$ is to be determined on the GGA samples – add the appropriate amount of nitrification inhibitor to the BOD bottle.
5. Top off the BOD bottle with dilution water.
6. Measure the initial DO and record your results.
7. Place a glass stopper on the BOD bottle.
Note: Make sure there is a water seal around the stopper.
8. Place a cap on the BOD bottle.
9. These bottles are incubated and BOD_5 is determined in the same manner as other BOD_5 or $CBOD_5$ samples.

The acceptable BOD_5 value of the standard is 198 ± 30.5 mg/L. The acceptable $CBOD_5$ value may differ from this. Consult the Certificate of Analysis (COA) for the acceptable $CBOD_5$ value. If the calculated result falls outside this range, the cause of the problem must be identified. Consistently high values can indicate the use of too much seed suspension, contaminated dilution water, or the occurrence of nitrification.
Consistently low values can indicate poor seed quality, the quantity of seed used or the presence of a toxic material. If low values persist, prepare a new solution of glucose-glutamic acid to check the source of dilution water and source of seed. Treatment plant sample results obtained using the same seed and dilution water as were used in analyzing the GGA standard may need to be qualified. Once the problem has been identified and corrected through additional analysis of the GGA standard, you should document what caused the problem for future reference.

7. BOD_5 Seeding Procedure

Before planning to seed samples, conduct a study to determine the amount of seed to add to seed controls and samples. Instructions for the study:

Preparation of Seed:

Collect a Raw Influent grab sample the day before performing the test. If the Raw Influent contains significant industrial loading, settled mixed liquor may provide a better seed than Raw Influent. If used for seed, settled mixed liquor does not need to be incubated at 20°C overnight. Seed can also be commercially obtained. There are at least two products widely used: BioSeed, and PolySeed. Follow the manufacturer's preparation guidelines if you are using commercially obtained seed.

NOTE: Raw Influent grab sample should be taken at the same time of day each time seeding material is needed. This will help ensure that samples are somewhat uniform.

1. Place Raw Influent grab sample in the 20°C incubator overnight.

Preparation of seed controls – Initial Study (Example given is when Raw Influent is used for seeding. The same procedure would be used for commercially obtained seed)

1. Take the incubated Raw Influent sample out of the incubator -- DO NOT MIX.

2. Pipet 3, 6, 9, 12, 15, and 18 mL of the clear supernatant into six BOD bottles respectively.

3. Fill these six bottles with BOD dilution water.

4. Determine the initial dissolved oxygen ($DO_{initial}$) on each of the six bottles.

Calculation of Seed Correction-Initial Study

1. After the 5 day incubation, determine the final dissolved oxygen (DO_{final}) on each of the six seed controls set up in the section above.

2. Ideally, one of the six seed controls will have close to 50% dissolved oxygen depletion. If this 50% dissolved oxygen depletion is not obtained, repeat the **Initial Study** using larger volumes of the clear supernatant until the 50% dissolved oxygen is obtained. NOTE: each study will require that you start with a new Raw Influent grab sample that has been incubated and allowed to settle overnight.

3. For each seed control dilution analyzed, calculate the DO lost per mL of seed used as follows:

$$\frac{DO_{initial} - DO_{final}}{\text{mL Raw Influent supernatant used}}$$

Example: 9 mL of incubated Raw Influent supernatant was added to a 300 mL BOD bottle and the bottle was then filled with BOD dilution water. The $DO_{initial}$ = 8.8 mg/L. After the 5 day incubation period, the DO_{final} = 4.3 mg/L. Using the formula above:

$$\frac{8.8 \text{ mg/L} - 4.3 \text{ mg/L}}{9 \text{ mL seed added}} = \frac{4.5 \text{ mg/L}}{9 \text{ mL}} = 0.5 \text{ mg/L DO lost per mL of seed added}$$

4. Use the same rule for DO depletion criteria as in all other BODs (at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO after 5 days).

5. If more than one of the seed controls meets the DO depletion criteria, referred to in #4, calculate the average DO lost per mL of seed (**Table 1** follows).

Table 1

Initial Study to Determine how many milliliters of Incubated Raw Influent Supernatant (seed) to Use in Seed Controls

Bottle #	Seed Added (mL)	$DO_{initial}$ (mg/L)	DO_{final} (mg/L)	DO Lost (mg/L)	DO Lost per mL of Seed
A	3	8.9	7.4	1.5	***
B	6	8.8	5.9	2.9	0.48
C	9	8.8	4.2	4.6	0.51
D	12	8.7	2.8	5.9	0.49
E	15	8.8	1.4	7.4	0.49
F	18	8.8	0.2	8.6	***

*** = Did not meet the criteria of 2.0 mg/L DO loss or 1.0 mg/L DO residual

In Table 1 above, it is observed that the sample with 9 mL of Seed Added lost approximately 50% of the $DO_{initial}$, thus by setting up seed controls with 6, 9, and 12 mL respectively, you can be fairly confident that at least one of the seed controls will give you a DO depletion that meets the criteria referenced in #4.

Using Table 1, the <u>average</u> DO lost per mL of seed Added =

$$\frac{0.48 + 0.51 + 0.49 + 0.49}{\text{Number of valid results } 4} = \frac{1.97}{4} = \text{average of } 0.49 \text{ DO lost/mL of seed added}$$

<u>This 0.49 DO lost/mL of seed added, or rounded off, 0.5 can now be used to determine how many mL of seed to add to the samples to obtain a Seed Correction in the range of 0.6 – 1.0 mg/L</u>

Calculating Amount of seed to add to the Final Effluent Sample, GGA and other samples requiring seeding – Initial Study

If the average seed loss/mL of seed added in the seed controls falls in the range of 0.6 to 1.0 mg/L DO lost, it should be sufficient to add 1.0 mL of seed to each of your BOD bottles when you are conducting your usual tests to obtain a Seed Correction in the acceptable range.

If the average seed loss/mL of seed added in the seed controls falls below 0.6 mg/L DO lost and the seed controls met the DO depletion criteria, the amount of seed added to each of your BOD bottles will need to be such that the number of mL added multiplied by the seed loss/mL DO lost falls within the range of 0.6 to 1.0 DO mg/L loss.

For example: using the seed loss/mL of seed added calculated in **Table 1** above of 0.5 mg/L, if 2.0 mL is added to BOD_5 samples then

0.5 mg/L DO lost x 2.0 mL = 1.0 Seed Correction

Another example: if the average seed loss/mL in the seed controls is 0.3 mg/L, if 2.0 mL is added to BOD_5 samples then

0.3 mg/L DO lost x 2.0 mL = 0.6 Seed Correction

And another example: if the average seed loss/mL in the seed controls is 0.7 mg/L, then 1.0 mL is sufficient to add to BOD_5 samples

Now you should have a reasonable idea of what volumes of seed will be needed to add to your Final Effluent BOD_5 samples to meet the depletion criteria and how to figure the Seed Correction number that will be subtracted from the BOD_5 calculation.

At this point you should have the following steps completed:

1. DO Meter is calibrated and ready for use. **(Section 1)**
2. Dilution water prepared **(Section 2)**
3. Samples prepared with a plan of sample dilutions **(Sections 3 – 5)**
4. GGA Standard prepared, or purchased **(Section 6)**
5. Predetermined seed volumes for the Seed Correction, samples, and GGA **(Section 7)**

8. BOD_5 SETUP

Note: Write down the volume(s) of the seed, GGA, and sample(s) after placing the required volume in the bottle. This can aid in tracking what has been done and what remains.

Preparation of Blanks

1. Fill one or more (two or more is recommended) 300 mL BOD bottles with dilution water to be incubated as Blank(s).

2. Follow steps 5 – 8 in **Section 1**.

Seeding Procedure for your Seed Controls, GGA, Final Effluent and other samples requiring seeding

Preparation of Seed (Seed Controls)

1. Follow steps 1 and 2 in Initial Study under Preparation of seed.

2. Fill a minimum of 3 bottles 2/3 with dilution water.

3. Using calculations determined in your Initial Study that include the volumes that gave approximately 50% depletion, follow steps 1 through 4 in the Initial Study instructions under Preparation of seed controls. NOTE: ideally you will only be using 3 dilutions, not 6 as in the Initial Study.

4. Pipet the appropriate seed volumes in to each bottle.

5. $CBOD_5$ – Add nitrification inhibitor to each bottle.

6. Top off the bottle with dilution water.

7. Follow steps 5 – 8 in **Section 1**.

Preparation of GGA

1. Fill 3 bottles approximately 2/3 with dilution water.

2. Add 6.0 mL of GGA to each of the bottles.

3. Pipet amount of seed (supernatant) that you have already determined will give the needed seed depletion of 0.6 to 1.0 mg/L into each of your GGA sample bottles.

4. $CBOD_5$ – Add nitrification inhibitor to each bottle if $CBOD_5$ is the desired result.

   Note: the $CBOD_5$ acceptable value and range may be different than 198 ± 30.5 mg/L

5. Complete the filling of the BOD bottles with dilution water.

6. Follow steps 5 – 8 in **Section 1**.

Preparation of Raw Influent and Final Effluent BOD_5 Samples (other samples may be required by your NPDES Permit)

1. Place dilution water in each bottle following dilution techniques in Section 5.
   * *When a bottle contains more than 67%, i.e., > 201 mL of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle) or use commercially prepared solutions designed to dose the appropriate bottle size.*

2. Add the appropriate amount of sample (Raw Influent and Final Effluent) to each of the bottles.

3. Pipet the mL of seed supernatant that you have determined will give the recommended depletion of 0.6 to 1.0 mg/L into each of your Final Effluent BOD sample bottles. Do not seed the Raw Influent samples.

4. If $CBOD_5$ is the desired result – add nitrification inhibitor to each bottle.

5. Complete the filling of the BOD bottles with dilution water.

6. Follow steps 5 – 8 in **Section 1**.

9. Calculations

For unseeded samples, use the following formula:

BOD_5 mg/L = (Initial DO, mg/L - Final DO, mg/L) × Dilution Factor

$$\textbf{Dilution Factor} = \frac{\textbf{Bottle Volume (300 mL)}}{\textbf{Sample Volume, mL}}$$

For seeded samples, use the following formula:

BOD_5 mg/L = [(Initial DO, mg/L – Final DO, mg/L) - Seed Correction)] x Dilution Factor

$$\textbf{Dilution factor} = \frac{\textbf{Bottle Volume (300 mL)}}{\textbf{Sample Volume, mL}}$$

Note: In the event all the dilutions analyzed for BOD_5 have too much oxygen depletion, i.e., there is < 1.0 mg/L oxygen remaining after the five day incubation period, the analyst is to use the lowest sample volume used to calculate the result and report the result as > the calculated value.

Also, if the DO depletion is < 2.0 mg/L and sample concentration is 100% (no dilution except for seed and nutrient, mineral and buffer solutions), actual Seed Corrected DO depletion may be reported as the BOD_5 even if it is < 2.0 mg/L.

BOD_5 Lower Detection Limits (LDLs)

1. Unseeded Samples
    a. The lower detection limit for unseeded samples that require dilution is 2.0 mg/L multiplied by the dilution factor as established by the requirement for a minimum DO depletion of 2.0 mg/L

$$LDL = \frac{2.0 \text{ mg/L} \times 300 \text{ mL}}{\text{Sample Volume, mL}}$$

****Example for an unseeded 150 mL sample**

$$\textbf{LDL} = \frac{\textbf{2.0 mg/L x 300 mL}}{\textbf{Sample Volume, 150 mL}} = \textbf{4.0 mg/L}$$

b. The lower detection limit for unseeded samples that require no dilution is equal to the detection limit of the DO measurement method (~0.1 mg/L)

$$\text{LDL} = \frac{\sim 0.1 \text{ mg/L} \times 300 \text{ mL}}{\text{Sample Volume, 300 mL}}$$

****Example for an unseeded 300 mL sample**

$\text{LDL} = \frac{\sim 0.1 \text{ mg/L} \times 300 \text{ mL}}{\text{Sample Volume, 300 mL}} = \sim 0.1 \text{ mg/L}$

2. Seeded Samples
   a. The lower detection limit for seeded samples that require dilution is ~1.0 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum Seed Correction which should be ≤ 1.0 mg/L

$$\text{LDL} = \frac{(2.0 \text{ mg/L} - \text{Seed Correction, mg/L}) \times 300 \text{ mL}}{\text{Sample Volume, mL}}$$

****Example for a seeded 250 mL sample where the Seed Correction = 0.8 mg/L**

$\text{LDL} = \frac{(2.0 \text{ mg/L} - 0.8 \text{ mg/L}) \times 300 \text{ mL}}{\text{Sample Volume, 250 mL}} = 1.4 \text{ mg/L}$

   b. The lower detection limit for seeded samples that require no dilution can calculate to as low as 0.0 mg/L as established by the difference between the sample DO depletion and the Seed Correction

$$\text{LDL} = \frac{[(\text{Initial DO, mg/L} - \text{Final DO, mg/L}) - \text{Seed Correction}] \times 300 \text{ mL}}{\text{Sample Volume, 300 mL}}$$

**** Example for a seeded 300 mL sample**

Initial DO = 8.4 mg/L

Final DO = 6.8 mg/L

Seed Correction = 0.7 mg/L

$\text{LDL} = \frac{[(8.4 \text{ mg/L} - 6.8 \text{ mg/L}) - 0.7 \text{ mg/L}] \times 300 \text{ mL}}{\text{Sample Volume, 300 mL}} = 0.9 \text{ mg/L}$

NOTE: EXCEL SPREADSHEETS SEE A < SIGN AS TEXT AND NOT A NUMBER AND WILL NOT USE THE RESULT WHEN CALCULATING THE AVERAGE.

10. QA/QC Requirements

1. **Section 1**: DO Meter

   The DO meter is calibrated daily or with each use. Calibration is documented and if the meter fails calibration corrective action is taken.

2. **Section 2**: Dilution Water

   The source water used to prepare dilution water should be allowed to equilibrate, preferably in a glass container, for 24 hours at 20 ± 3° C and have a DO of at least 7.5 mg/L before adding the phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ reagents, 1 mL of each/L of source water. Alternatively, there are prepackaged BOD Nutrient Pillows for the volume of dilution water being prepared available from Hach Company. Dilution water is prepared immediately before use UNLESS documentation shows that acceptable BOD blank depletions of ≤ 0.20 mg/L after 5 day incubation after longer holding times can be achieved. Initial DO of blanks should be 7.5 - 9.0 mg/L and pH between 6.0 – 8.0. Temperature should be 20 ± 3°C and DO depletion of blanks is ≤ 0.20 mg/L. The DO depletion is the average of all blank samples. A control chart is used for tracking BOD dilution water blank depletions and corrective action is documented when results fall outside of control limit of 0.20 mg/L. The steps taken in the preparation of dilution water are documented.

3. **Sections 3 – 5**: Sample Preparation

   Sample pH has been adjusted to 6.0 – 8.0. Chlorine has been removed if present. Initial DO is between 7.00 and 9.00 mg/L. The volume of seed added to samples is the same as is used in the bottles used for GGA analysis. Duplicates have been setup and control limits have been established using historical data.

4. **Section 6**: 6.0 mL GGA Standard

   Initial DO is between 7.00 – 9.00 mg/L. The same seed volume is used in the sample(s) and GGA bottles. A minimum of three (6.0 mL GGA) samples have been setup. The average result of the three samples is within the established control limit of 198 ± 30.5. A percent recovery control chart can be utilized to identify trends and identify results falling outside of the established control limits. Corrective action steps are taken and documented when results fall outside of control limits.

5. **Section 7**: Seeding

   Three seed bottles have been setup and meet the minimum oxygen depletion and minimum oxygen residual DO requirements. The Seed Correction is between 0.60 – 1.00 mg/L. Corrective action steps are taken and documented when results fall outside these control limits.

6. **Section 8**: BOD_5 Setup

   Initial DO of all bottles is between 7.00 – 9.00 mg/L. Proper setup steps have been taken. Required documentation has been recorded on the BOD_5 bench sheet.

   $CBOD_5$ – Nitrification Inhibitor has been added to all sample bottles except for dilution water blank samples. The use of Nitrification Inhibitor is documented.

Seed is added to all samples that have Nitrification Inhibitor in them except for Raw Influent samples.

Notes:

1. Standard Methods recommends that the dilution water blank depletion not exceed 0.20 mg/L, if the DO depletion in the dilution water blanks exceeds 0.20 mg/L the results of the test are questionable. Do not subtract blank values from sample results.

2. Only dilutions with DO depletions of at least 2.0 mg/L (except as stated above for 100% dilutions), and a final DO of at least 1.0 mg/L may be used to calculate BOD_5.

3. If there is no evidence of toxic effects, average the results from all dilutions that meet the minimum oxygen depletion and minimum oxygen residual DO requirements.

4. A toxic effect is indicated when BOD_5 increases significantly as the sample dilution increases (sample volume decreases) or sample replicates show more than a 30% difference between high and low values. This is often referred to as “sliding BODs.

Determination of Chlorine (Residual) DPD Colorimetric Method

Reference: Standard Methods 21st ed. 4500-Cl G.

Apparatus:

1. Photometric equipment - one of the following is required:
   a. Spectrophotometer, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.
   b. Filter photometer equipped with a filter having a maximum transmission in the wavelength range of 490 to 530 nm and providing a light path of 1 cm or longer.
   c. Colorimeter designed for chlorine analysis
2. Sample cells/cuvettes

Reagents:

1. DPD Total Chlorine Powder Pillow

Sample Collection:

1. Collect sample and analyze **IMMEDIATELY but no longer than 15 minutes after sample collection!**
2. Sample must be a grab.
3. Sample should not be mixed or unnecessarily agitated after collection.
4. Sample should be shielded from sunlight to decrease the reduction of chlorine.

Procedure:

1. Press the Power key to turn on the meter (meter operational modes differ, follow the manufacturer's procedure for your meter).

   NOTE: If the meter reads in different concentration ranges, set the meter to read in the concentration range for the expected sample concentration.

2. Rinse a 10 mL sample cell three times with sample.

3. Fill the 10 mL sample cell to the 10 mL line with sample and cap.

4. Wipe the liquid off the sample cell and place in the cell holder with the diamond mark facing you. Tightly cover the cell with the instrument cap.

5. Press **Zero**. The display will show; 0.00 mg/L Cl_2.

   NOTE: Zeroing on samples before the DPD is added reduces the chance for false positive readings due to from turbidity.

6. Remove the sample cell from the cell holder and add the contents of one DPD Total Chlorine Powder Pillow.

7. Swirl the sample cell for 20 seconds to mix.

8. Wipe the liquid off the sample cell and place in the cell holder with the diamond mark facing you. Tightly cover the cell with the instrument cap.

9. Wait two minutes.

10. Press **Read**. Meter will read mg/L Cl_2 Record the concentration.

11. Repeat Steps 2 - 10 for any additional samples or quality control samples.

    NOTE: If the sample temporarily turns yellow after DPD addition or shows over range, make a dilution on a fresh sample with laboratory reagent water and repeat the test. Some loss of chlorine may occur. Multiply the result by the dilution factor.

Calculations:

Cl_2 (mg/L) = Reading displayed on meter

Cl_2 (mg/L) for diluted sample = Reading displayed on meter x DF

Dilution Factor (DF) = $\frac{\text{Final volume of diluted sample, mL}}{\text{Sample volume used in diluted sample, mL}}$

Quality Assurance/Quality Control:

1. A sample duplicate is analyzed at the frequency required by the IDEM Inspector

2. A quality control sample or calibration check sample is analyzed at the frequency required by the IDEM Inspector

3. If more than one sample cell/cuvette is used during analysis, use only those that are from a matched set

DETERMINATION OF *E. coli*

Memorandum – Policy for Reporting TNTC Data

To: **All NPDES Permittees Who Must Perform *E. coli* Testing**

From: **Bruno Pigott, Assistant Commissioner**
Office of Water Quality

Subject: Policy for Reporting "Too Numerous to Count" (TNTC) Data for *E. coli* Testing

Date: **July 1, 2005**

Utilization of this TNTC policy should not become necessary in any but the rarest of situations. Any questions regarding this policy should be directed to Becky Ruark at 317-691-1909 or bruark@idem.in.gov

For Testing Methods Utilizing a Membrane Filter:

NPDES permits require that the monthly average of *E. coli* be less than 125 colonies per 100 milliliters (mL) of filtered sample. *Standard Methods for the Examination of Water and Wastewater* 20th Edition – pages 9-59, indicate that the allowable maximum number of colonies per plate (filter) is 200. The optimum count is in the range of 20 to 80 colonies, with an ideal sample yielding about 50 colonies. (If no filter has a count falling in the optimum range, meaning in the range of 20 to 80 colonies, total the colonies on all filters and report as number per 100 mL) (See 20th Edition – pages 9-61 for detailed examples.)

Even though filtration of 100 milliliters (or lesser volumes) normally produces an acceptable colony count for disinfected Final Effluent, occasionally the count for these normally acceptable dilutions may exceed 200 colonies per plate.

To prevent such an occurrence, we are strongly recommending that laboratory personnel *routinely* run a 1.0 milliliter dilution *along with* the normally acceptable dilutions for each test.

If all dilutions for that test, including the 1.0 mL dilution, result in plates (filters) that are deemed TNTC, the number reported for the 1.0 mL test should be 63,200. This number should be reported on the Monthly Report of Operations (MRO) as the *E. coli* result for that day and should be included in the monthly average calculation.

Justification: If the 1.0 mL plate is deemed TNTC, then the actual count is likely to be somewhere between 20,000 (maximum count for a 1.0 mL dilution) and 200,000 (maximum count for a 0.1 mL dilution). **The TNTC number of 63,200 is the geometric mean of those two numbers.**

For the Colilert® Method Using Quanti-tray Procedure:

Even though filtration of 100 milliliters (or a lesser volume that you have determined) normally produces an acceptable Quanti-tray, very occasionally these normally acceptable dilutions may result in all wells on the Quanti-tray fluorescing.

To prevent such an occurrence, we are strongly recommending that laboratory personnel *routinely* run a 10.0 milliliter dilution *along with* the normally acceptable dilution for each test.

If all dilutions for that test, including the 10.0 mL dilution, result in trays that are fully fluoresced, the number reported for the 10.0 mL test should be 76,000. This number should be reported on the MRO as the *E. coli* result for that day and included in the monthly average calculation. (However, if your facility is using an Excel spreadsheet MRO supplied by IDEM, you do not have to alter the default value assigned to TNTC results by the spreadsheet.)

Justification: If the 10.0 mL tray is fully fluoresced, then the actual count is likely to be somewhere between 24,192 (maximum count for a 10.0 mL dilution) and 241,920 (maximum count for a 1.0 mL dilution). **The reportable number of 76,000 is the approximate geometric mean of those two numbers.**

THE COLISCAN® MEMBRANE FILTER METHOD
of
Micrology Laboratories LLC
for
***ESCHERICHIA COLI* and TOTAL COLIFORMS**

1.0 Scope and Application

1.1 The Coliscan® Membrane Filter method consists of a medium which detects the presence or absence of *E. coli* and total coliforms simultaneously and also allows the enumeration of each.

1.2 The method allows the detection and enumeration of *E. coli* and other coliforms in 24 hours or less and does not require a confirmation step.

1.3 The detection limit is one target CFU/sample.

2.0 Summary of the Method

2.1 The Coliscan® MF medium determines the presence or absence (and enumeration) of *E. coli* and other coliforms in any size water sample (100 mL is required for drinking water). The sample (diluted or not) is passed through a 0.45 µm pore size, 47 mm diameter membrane filter using standard equipment and methodology. The filter is then placed into a 50 mm plate containing a pad saturated with the medium (if in broth form) or a layer of the medium which has been solidified with an added agar gelling agent. Incubate for 24 hours at 35° ± 0.5°C. *E. coli* CFUs will appear as blue/purple and other coliform CFUs will appear pink/magenta.

2.2 Coliscan® MF medium (broth or agar) contains nutrients to assure the growth of the target organisms, buffers to maintain appropriate pH, and inhibitors to reduce the growth of nontarget organisms. It is similar to the modification of m-TEC described by Duncanson and Cabelli (1986 paper presented at the National Meeting of AWWA). *E. coli* colonies growing on the medium appear blue to purple due to the combination of the enzymes glucuronidase and galactosidase affecting their respective substrates, 5-Bromo-4-Chloro-3-Indolyl-B-D-glucuronide (X-gluc) and 6-Chloro-3-Indolyl-B-D-galactoside (Salmon-gal). The teal green product of X-gluc hydrolysis combines with the pink/magenta product of the Salmon-gal hydrolysis to produce the blue to purple appearance of *E. coli* colonies. Coliform colonies (other than *E. coli*) are colored pink/magenta as a result of producing only galactosidase which acts on the Salmon-gal only.

3.0 Definitions

3.1 *Escherichia coli* - Those bacteria which grow as blue/purple colonies on the Coliscan® MF medium as a result of the production of both glucuronidase and galactosidase enzymes. These bacteria are of fecal origin.

3.2 Total Coliforms - Those bacteria which make up the sum of the *E. coli* (blue/purple colonies) and other coliforms. The other coliforms will appear as pink/magenta colonies on the Coliscan® MF medium because they produce galactosidase, but not glucuronidase and so cleave only the Salsmon-gal substrate. Species of the genera *Citrobacter, Enterobacter, and Klebsiella* are main groups (other than *Escherichia*) of coliform bacteria.

3.3 Non-Coliforms **-** Bacteria that form colonies which are not blue/purple or pink/magenta on

Coliscan® MF medium.

4.0 Interferences

4.1 No known chemical substances normally encountered in drinking water or source waters have been observed to affect the color of *E. coli* or other coliform colonies on the Coliscan® MF medium. If particulate or colloidal materials are suspended in water samples, they may interfere with filtering efficiency by clogging filter pores and they may cause some spreading of bacterial colonies as they grow on the filter surface during incubation. However, these materials would very rarely prevent the accurate determination of the bacterial population.

4.2 Colonies exhibiting the color(s) of the target organisms should not be included in the *E. coli* or other coliform counts if they are less than 0.5 mm diameter (except when the entire colony population is very large due to excessive crowding on the plate. In such a case the sample should be rerun at a higher dilution.). Small colored colonies of this nature should not be counted unless they are isolated into pure culture and then verified by approved procedures.

4.3 Colonies exhibiting a teal green color which is indicative of the production of glucuronidase without the production of galactosidase should not be counted as *E. coli* without isolation into pure culture and verification by approved procedures.

4.4 It cannot be safely assumed that colonies can be picked directly from the surface of the filter and used to inoculate confirmatory media directly, particularly if the colonies are blue/purple or teal green, as they may be contaminated by cells from adjoining colonies that have traveled on the filter surface. Therefore, questionable colonies should be picked and streaked onto the surface of a differential medium such as Coliscan® Easygel® to ensure their purity before further testing.

4.5 Unlike media which utilize a fluorogen (such as MUG or MUGal) and a chromogen, where the fluorogen tends to diffuse rapidly into the surrounding medium, thus making the timing of reading the test results critical (before excessive diffusion occurs which may make neighboring colonies appear as false positives), the chromophores of the chromogens used in Coliscan® MF are quite insoluble and little or no diffusion away from the target colonies occurs.

5.0 Safety

5.1 Standard safety practices should be observed by persons using these materials in the laboratory.

5.2 Any materials containing living or viable microbes should be disinfected or sterilized by accepted standard methods before being discarded.

5.3. Refer to the SDS for specific product information.

6.0 Equipment and supplies

6.1 Incubator set at 35°± 0.5°C with provision for maintaining materials at above 80% humidity

6.2 Filter funnel apparatus for 47 mm membrane filters, with a vacuum source

6.3 Dissecting microscope (10-15X) with built-in light sources

6.4 Sterile disposable or properly cleaned (by well-known standard methods) glass or plasticware including 1 and 10 mL pipettes, sample collection containers, flasks, graduated cylinders, and diluent containers

6.5. Sterile forceps

6.6 Sterile 50 mm diameter petri dishes with absorbent pads

6.7 Sterile 0.45 µm pore size, 47 mm diameter micropore filters for sample filtering

6.8 Sterile Dilution and Rinse water prepared in accordance with Standard Methods.

6.9 Biohazard bag

6.10 Water bath set at 44.5 ± 0.2°C (Optional) – **see bold information in Section 11.1**

6.11 Whirl pacs (Optional) – **see bold information in Section 11.1**

7.0 Reagents and Standards

7.1 Coliscan® MF medium of Micrology Laboratories LLC is provided in a broth for using 1.8 - 2 mL/dish. The medium should be kept frozen (2 - 6°C) and has an expiration time of 6 months.

7.2 Preparation of the Medium for use:
The broth medium should be thawed before use. The thawed medium can be kept refrigerated for up to 2 weeks.

7.3 Make a 10% solution of sodium thiosulfate using laboratory reagent water.

8.0 Sample collection, De-chlorination, Preservation, Shipment and Storage

8.1 Collect samples in a sterile, clean wide mouth glass or heat resistant plastic bottle with a leakproof closure, all of which is non-toxic in use. A pre-sterilized, sealable, non-toxic plastic bag may also be used for sample collection.

8.2 For potable water, open the tap and allow the water to run for 2 - 3 minutes and then collect the sample using aseptic technique to avoid contamination. For other sample types, aseptically collect water that is representative of the source.

8.3 Samples with residual chlorine should be neutralized at the time of collection by adding 1 mL of a 10% solution of sodium thiosulfate (or the equivalent) per liter of sample.

8.4 Samples should be tested as soon as possible after collection. If processing is not done within 1 hour, the sample should be held on ice or refrigerated at 2 - 10°C. Potable water samples should be tested or processed within a maximum holding time of 30 hours of collection and non-potable samples should be tested or processed within a maximum holding time of 8 hours of collection.

9.0 Quality Control

9.1 Coliscan® MF is tested for quality control at the time of manufacture and is certified to meet specifications. Each lot should be tested by the using laboratory by preparing three plates of the

medium, one to serve as a positive control, one to serve as a negative control, and one to serve as an uninoculated control.

Prepare 24 hour tryptone broth cultures of typical *E. coli, Enterobacter aerogenes or Klebsiellsa pneumonia, and Pseudomonas aeruginosa or Salmonella typhimurium.* Prepare serial dilutions of *E. coli and Enterobacter or Klebsiella combined* so that the combined inoculum will result in 20 - 80 CFU/100 mL and filter. Place the filter on the surface of one of the plates of Coliscan® MF medium. Prepare serial dilutions of the *Pseudomonas or Salmonella* so that the inoculum will result in 20 - 80 CFU/100 mL and filter. Place the filter on the surface of the second plate of Coliscan® MF medium. Filter 100 mL of sterile diluent and place the filter on the surface of the third plate of Coliscan® MF medium. Use sterile filter apparatus for each prep.

Incubate the plates 24 hr. at 35° ± 0.5°C. The *E. coli/Enterobacter or Klebsiella* control should have both blue/purple (*E. coli*) and pink/magenta (*Enterobacter or Klebsiella*) colonies, the *Pseudomonas or Salmonella* control should have colorless colonies, and the diluent blank control should have no colonies.

Colonies from the controls may be picked and tested further with various diagnostic media if desired.

10.0 Calibration and Standardization

10.1 Coliscan® MF calibration or standardization is not required.

10.2 Incubators should be tested daily to assure maintenance of proper temperature. Thermometers used should be tested at least annually against an NIST certified thermometer.

11.0 Procedure

11.1 Test Procedure

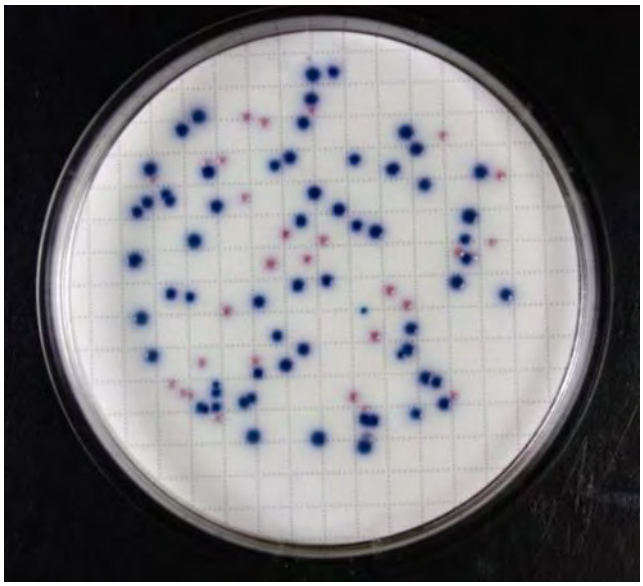
11.1.1 Using proper technique, filter the sample through a 47 mm, 0.45 µm pore size membrane filter. Rinse the filter funnel twice with at least 20 mL of sterile diluent/rinse to complete the filtration. Transfer the filter to a petri dish containing a pad saturated with 1.8 - 2 mL of the Coliscan® MF broth, invert the dish and incubate at 35° ± 0.5°C for 24 hours. (The pad should only be saturated and should not have pooled broth when tilted. Depending on the brand of dishes/pads, the proper amount of broth may vary. Too much broth results in possible spreaders on the membrane surface.) **If *E. coli* is the only target organism desired and the other coliforms and non-coliforms are undesired and may interfere with the growth of the *E. coli*, it is suggested that an initial "resuscitation period" by incubation for 2 hours at 35° ± 0.5° C., followed by transferring the dishes to a Whirl pac(s) making sure there is a water tight seal and submerging inverted for the remaining incubation in a water bath at 44.5 ± 0.2°C. will lessen the non-E.coli colony growth without negatively affecting the *E. coli* growth as *E. coli* is noted to grow at 44.5°C. Other coliforms and many other microbes will not grow well at 44.5°C.**

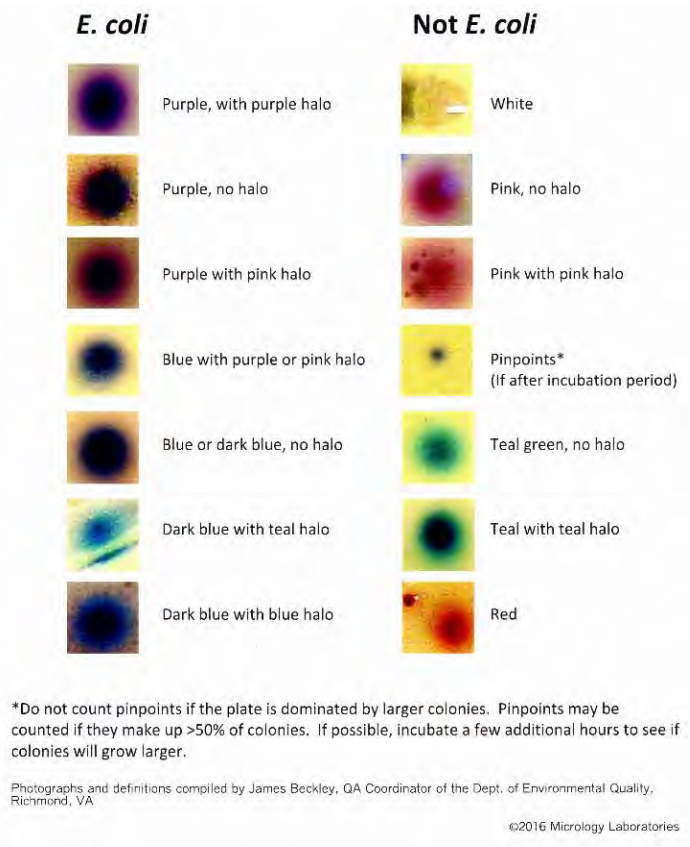
11.2 Interpretation

11.2.1 Check the filter surface for colony forming units. Generally, colonies are obvious and can be observed with the unaided eye in normal room or daylight. However, the use of a 10 - 15X magnifying device is recommended for critical analysis.

11.2.2 Here is a Coliscan® MF dish inoculated with E. coli and Enterobacter aerogenes (a common coliform). The blue are E. coli and the pink are Enterobacter. Non-coliform gram negatives such as Salmonella and Proterus will grow as colorless CFUs. This photo is typical of appearance at 24 hour incubation at 35-37 Celsius.

Submitted by Dr. Jonathan Roth – Micrology Labs





12.0 Data Analysis, Calculation, Interpretation and Reporting Results

12.1 Presence/Absence Test

12.1.1 The presence of at least one blue/purple or pink/magenta colony at least 0.5 mm in diameter indicates the sample is total coliform positive. The presence of at least one blue/purple colony indicates the sample is positive for *E. coli.* The presence of at least one pink/magenta colony indicates the sample is positive for general coliforms.

12.2 General Coliform (excludes *E. coli*) - Quantitative Test

12.2.1 Count the number of pink/magenta CFUs present on the membrane filter and record as the number/amount of sample used for that test. For example, if the amount of sample was 10 mL and there were 20 pink CFUs, record as 20 per 10 mL. Then translate to the number of CFUs/100 mL. In this case, the 10 mL sample is 10% of 100, so the 20 CFUs should be multiplied X 10, giving 200 CFU/100 mL sample. All pink/magenta CFUs should be counted as general coliforms. (Colonies should be at least 0.5 mm diameter to be counted.)

12.3 *E. coli* (Fecal) - Quantitative Test

12.3.1 Count the number of blue/purple CFUs present on the membrane filter and record as the number of *E. coli*/amount of sample used for that test. Then translate to the number of *E. coli* CFUs/100 mL of sample (see 12.1.1). All blue/purple CFUs should be counted as *E. coli.* (Colonies should be at least 0.5 mm diameter to be counted.)

12.4 Total Coliforms - Quantitative Test

12.4.1 The sum of the number of general coliform CFUs and the number of *E. coli* CFUs from one sample equals the number of total coliforms in that sample. That is, the total number of pink/magenta CFUs and blue/purple CFUs = the total coliforms for that sample.

13.0 Method Performance Characteristics

13.1 Specificity - In a study done to compare Coliscan® MF to the m-TEC Method for the detection and enumeration of *E. coli* from disinfected Final Effluent wastewater, the false positive error was 3.8% and the false negative error was less than 1.0%. That is, of 105 CFUs judged to be *E. coli* (blue/purple) which were picked and subjected to Enterotube® analysis, only 4 were identified as other than *E. coli*. And of 131 CFUs judged to be coliforms other than *E. coli* (pink/magenta) which were picked and subjected to Enterotube analysis, only 1 was identified as other than a true coliform.

13.2 Comparability - The Pearson Coefficient for the parallel analyses on the Coliscan® MF and the m-TEC methods within the same laboratory was 0.928. T- test analyses indicated no significant differences between the methods at the 95% confidence level.

14.0 Pollution Prevention

14.1 Laboratory personnel should use pollution control techniques to minimize waste generation wherever possible. Where this is not possible at the source, recycling should be practiced.

15.0 Waste Management

15.1 Each laboratory is responsible to comply with all federal, state and local regulations governing waste management. Special emphasis should be placed on hazardous waste identification rules and land disposal restrictions and to protecting the air, water, and land by minimizing and controlling all release from fume hoods and bench operations. Compliance is also required with any sewerage discharge permits and regulations. Federal, state or local authorities should be contacted for further specific information.

16.0 Troubleshooting

Count only the blue and purple colonies. Some operators may initially be confused in differentiating between purple and pink colonies as purple/blue colonies are a combination of the product of the color from both enzyme substrates (green-teal and pink-magenta). Normally, the purple/blue colonies appear first on the membrane because they contain color from both substrates, and the pink are very much lighter colored. However, for operators who are unsure of the colors, Micrology Labs has available test media to differentiate whether the color is from only a single substrate (not *E. coli*) or a combination of both substrates (*E. coli*). This can be ordered as a separate item from Micrology Laboratories (Cat. #25101 Confirmation Media).

17.0 References

16.1 APHA (1995) Standard Methods for the Examination of Water and Wastewater. Edition 19.

16.2 Roth, J.N., W.J. Ferguson. (1993) Method Test Media and Chromogenic Compounds for Identifying and Differentiating General Coliforms and *Escherichia coli* Bacteria. United States Patent #5,210,022.

16.3 Umble, A.K., et al. (1999) Elkhart, Ind., Tests an Improved, Simplified Membrane Filtration Method for *Escherichia coli* Detection and Enumeration. Water Environment Tech. Vol.11, No.4, 57-59.

MICROLOGY LABORATORIES LLC 1-888-327-9435 www.micrologylabs.com

Determination and Enumeration of *E. coli* in Wastewater utilizing Colilert® and Quanti-Tray®/2000

SM 9223 B. Enzyme Substrate Test

BACKGROUND INFORMATION:

Colilert® was originally developed for use in clinical laboratories to enumerate and identify bacteria in urine. It was then adapted for the simultaneous detection and confirmation of total coliforms and *E. coli* in the analysis of drinking water.

PRINCIPAL:

As total coliforms and *E. coli* bacteria metabolize the nutrient indicators in Colilert that produces two distinct reactions. The two nutrient indicators are: (1) o-nitrophenyl- ∃-D-galacto-pyranoside that changes from clear to yellow for total coliform and (2) 4-methylum-belliferyl-∃-D-glucuronide that will emit a definite blue fluorescence when using a long-wavelength 366 *nm* ultraviolet light for *E. coli*.

APPARATUS:

A. Reagents:

WHEN PERFORMING ALL TESTS USE APPROPRIATE TECHNIQUES AND TAKE ALL SAFETY PRECAUTIONS NECESSARY. REFER TO THE SDS ON FILE FOR ADDITIONAL SAFETY INFORMATION.

1. Colilert© Reagent-Snap Pack [IDEXX Laboratories]- for 100 mL water samples. Shelf life: up to 12 months. Store at 2-30°C, away from light.

2. Color Comparator [IDEXX Laboratories] is a liquid color reference reagent used to distinguish a minimum positive from negative test results at 24-28 hours (for Quanti-Tray 2000).

B. Equipment*:*

- Quanti-Tray Sealer [IDEXX Laboratories]
- Quanti- Tray/2000 Comparator
- Quanti- Tray/2000 97-well rubber insert
- Quanti-Tray/2000, (97-wells) [IDEXX Laboratories]
- 100 mL disposable vessel with Sodium Thiosulfate
- 10 mL sterile pipet
- Pipet bulb
- Incubator (35 ± 0.5°C)
- Autoclave
- Autoclavable Bio-Hazard bags
- 6-watt long wave (366-nm) UV Lamp
- UV Absorbing safety glasses

PROCEDURE:

When performing all tests, use aseptic techniques. Take all safety precautions necessary.

Procedural Notes:

- Avoid touching the top of the media pack after it has been opened or the inside of the sample bottle or cap.
- Avoid prolonged exposure of the inoculated Colilert to direct sunlight. The indicator compounds may be hydrolyzed, creating a false positive total coliform result.
- Upon adding Colilert to the sample, a transient blue color may appear in samples containing an excessive amount of free chlorine.
- IDEM recommends analyzing a sample of 10 mL of disinfected Final Effluent and 90 mL of sterilized dilution water to ensure that a result of TNTC is not obtained. (Please consult IDEM Memorandum from July 1, 2005, for specific information on reporting TNTC).

A. SAMPLE ANALYSES: MULTI-WELL PROCEDURE

1. Collect at least 110 mL sample in sterilized 100 mL disposable vessel containing sodium thiosulfate for dechlorination. Sodium thiosulfate is not required if chlorine is not used for disinfection.
2. Turn the sealer power switch on (the amber colored light should turn on). This must warm up for up to ten minutes before it is ready. The green Ready light will turn on when the sealer is ready.
3. Set up a 100 mL for each sample collected. IDEM recommends also setting up a 10 mL sample dilution, i.e., 10 mL sample + 90 mL laboratory reagent water – if this is done, make sure that sterilized laboratory reagent water and a sterile pipet is used to set up the 10 mL dilution). Use the 100 mL indicator on the vessel for meniscus measurement. No glassware is permitted due to contamination risk.
4. Carefully separate one Colilert reagent snap pack and tap to make sure all the Colilert powder is in the bottom part of the pack. Open one pack by snapping back the top at the score line.
5. Add the reagent to the vessel containing the sample.
6. Aseptically cap and shake the vessel until all powder is dissolved.
7. Label the Quanti-Tray/2000 with the sample name, dilution, set up date and time, and initials of the technician preparing the tray.
8. Pour the sample reagent mixture directly into the tray avoiding contact with the foil tab.
9. Place the sample-filled Quanti-Tray onto the rubber insert of the sealer with the well side of the Quanti-Tray facing down. Make sure the tray is properly seated in the rubber insert, and with each well of the Tray in its corresponding rubber insert hole.
10. Make sure the green light on the sealer is illuminated, indicating it is ready.
11. Slide the rubber insert into the sealer until the motor grabs the rubber insert and begins to draw it into the sealer. In approximately 15 seconds, the tray will be sealed and partially ejected from the rear of the sealer.
12. Remove the rubber insert and Tray from the rear of the sealer.

13. Turn off the sealer and place the Quanti-Tray in an incubator at 35° ± 0.5° C. Incubate for 24 – 28 hours.
14. Remove trays from incubator and place side by side to the comparator under the UV lamp so that the lamp is within 5 inches from the tray.
15. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of *E. coli* is confirmed. Count the number of positive large and positive small wells.
16. Use the Quanti-Tray/2000 MPN table to determine the most probable number of colonies and record the results in the Log Book.
17. Place the Quanti-Tray in a Bio-Hazard bag and autoclave. The tray is now ready for disposal into trash receptacle

B. QC VALIDATION OF MATERIALS & DILUTION WATER

Performing Quality Control on each lot of media, vessels, trays, and batch of dilution water will eliminate QC on each sample run. When ordering for the year, ask for all of one lot number so that numerous QC analyses will not have to be done.

1. Warm up the sealer per manufacturer's instructions.
2. To one of two sterile vessels:
   - Add 100 mL of sterile water

   To the other sterile vessels:
   - Add 100 mL of wastewater collected prior to disinfection.
3. Add one pack of reagent to each of the samples. "Flip" the pack to loosen the media, then snap open the top and pour aseptically into the sample bottle.
4. Cap and shake the sample bottles. Wait a few minutes for the reagent to dissolve.
5. Label each Quanti-Tray/2000 with the date, time and sample ID.
6. Re-shake the sample, remove the cap, and take the corresponding Quanti-Tray/2000 (carefully squeezing on the outer edges of the tray to open it) and pour the sample into the Quanti-Tray.
7. Run the sample through the tray sealer, the sealer will dispense the sample into the wells and seal the package.
8. Incubate for 24 – 28 hours at (35 ± 0.5°C].
9. Read the Quanti-Tray for color and fluorescence as described in A. Sample Analysis above.

NOTE: Positive checks should result in multiple fluorescent wells per tray while negative checks (Sterility Checks) should result in zero yellow wells and fluoresced wells per tray. If a tray does not match the expected results, check for errors in technique. Such as: contamination of the bottle or Quanti-Tray during the procedure or transferring the culture to an incorrectly labeled bottle. Repeat procedure. If it fails again call the manufacturer to see if there have been problems with those specific lots. Record all lot numbers and results.

C. QUALITY CONTROL

1. Dilution Water Sterility (Blank Analysis)

Sterility of dilution water should be checked monthly. To verify sterility, analyze a sterile water control (100 mL of dilution water) in addition to the routine analysis. If the control exhibits faint fluorescence, discard the batch and use a new substrate of dilution water.

- IDEM recommends analyzing a sample of 10 mL of disinfected Final Effluent and 90 mL of sterilized dilution water to ensure that a result of TNTC is not obtained. (Please consult IDEM Memorandum from July 1, 2005, for specific information on reporting TNTC).

2. Precision (Duplicate Analysis)

A duplicate analysis should be analyzed on 10% of samples and on at least one sample per test run. If the lab conducts less than 10 tests per week, analyze a duplicate on at least one sample each week.

D. SAMPLE COLLECTION

Collect the *E. coli* sample at the same time and place for the disinfected Final Effluent sample**.** Collect a 100 mL sample in a sterile vessel containing sodium thiosulfate. Take the sample directly to the lab and analyze immediately or refrigerate sample prior to analysis. Holding time prior to analysis cannot exceed 8 hours.

E. INCUBATION

Place in a 35± 0.5°C incubator for 24 - 28 hours Record daily in a logbook or bench sheet.

F. ENUMERATION AND IDENTIFICATION

After 24 hours of incubation, remove the trays from the incubator. Use the color comparator to compare the yellow and fluorescent signal. Sample wells that are clear (no color change) are negative for total coliform and *E. coli*. All yellow wells, greater than or equal to the comparators are counted as positive for total coliform. Next examine all total coliform-positive wells for fluorescence using the 366 nm UV lamp. Yellow and blue-fluorescent wells, greater than or equal to the comparator, are counted as positive for *E. coli*. Record the number of positive large and small wells for Quanti-Tray/2000.

G. CALCULATIONS

1. Use the MPN (Most Possible Number) table provided by IDEXX and determine the MPN/100 mL for *E. coli.*

2. For Quanti-Tray/2000, find the number of positive small wells across the top of the Table. Then go down the left side of the chart and find the number of positive large wells. The MPN/100 mL value is where the value for the positive large and positive small wells intercepts. MPN is a number, based on certain probability formulas and is an estimate of the mean density of coliforms in the sample. If any dilution of the sample is made, multiply

the MPN/100 mL value by the dilution factor to obtain the final MPN/100 mL value.
3. Record results on the appropriate bench sheet. Initial, date and record time for all results.

REFERENCES

Standard Methods 21st ed. 9223B.
IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, ME 04092

DETERMINATION OF pH

Electrometric Method

Reference: Standard Methods 21st ed. 4500-H^+ B.

Apparatus:

1. pH Meter
2. Combination electrode
3. Automatic Temperature Compensator (ATC)
4. Stirring Apparatus (optional) – see *Note 3* below
5. Teflon coated stirring bars (optional) – see *Note 3* below
6. Beakers
7. Insulating material (optional) – see *Note 3* below

Reagents:

1. pH buffer 4.0 (red); purchased commercially
2. pH buffer 7.0 (yellow); purchased commercially
3. pH buffer 10.0 (blue); purchased commercially
4. Electrode storage solution

Sample Collection:

1. Samples are collected as a grab sample in a clean plastic or glass bottle and brought immediately to the lab for pH measurements – samples must be analyzed within 15 minutes of collection time.

Calibration:

NOTE 1: These calibration instructions apply only to a sample pH meter. You should consult the manufacturer's instructions for directions on how to calibrate your specific pH meter. In all cases, the meter should be calibrated with three buffers or a minimum of two buffers that bracket the samples measured pH within 1 to 2 pH units, and then verified with a third second source buffer that falls between the two buffers used for calibration. ***Note:*** *a second source buffer is one that has a different lot number than the buffers used for calibration.*

NOTE 2: If your pH meter does not allow calibration with pH buffers that are more than 3 pH units apart, you can calibrate with the 4.0 and 7.0 pH buffer and then check the calibration using either a second source 4.0, 7.0, or another pH buffer that is in the range between the two. Alternatively, for samples with pH values between 7.0 and 10.0, you can calibrate with the 7.0 and 10.0 and then check the calibration using a second source 7.0, 10.0, or another pH buffer that is in the range between the two.

NOTE 3: Not all manufacturers suggest stirring samples, but it can improve electrode response time and sample homogeneity. If samples are stirred, pH buffers/standards should also be stirred in the same fashion. The stirring action should be gentle to minimize carbon dioxide entrapment. Carbon dioxide entrapment can affect pH readings in samples that lack adequate

buffering capacity (i.e., low alkalinities). Some magnetic stirrers generate enough heat to increase the temperature of the sample. To avoid this, place piece of insulating material between the stirrer and the beaker.

NOTE 4: If only occasional measurements are made, calibrate before each measurement.

1. Turn pH meter on.
2. Remove electrode from storage solution, rinse, and blot dry with a soft lint-free tissue.
3. Select the "calibration" function on the initial startup screen of the pH meter.
4. If prompted, enter "3" for the number of buffers being used during calibration.
5. Place the 3 pH buffers (4.0, 7.0, and 10.0) in clean glass beakers. Use a sufficient volume (> 50 mL) to cover the sensing elements of the electrode(s). The volume should allow for the sensing elements of the electrode(s) to be submerged, but still about one-half inch above the bottom.
6. Immerse the electrode in the first buffer solution (pH 4.0) and wait until electrode has stabilized. Read buffer and calibrate as pH 4.0 or at the pH value on the bottle corresponding to the temperature of the buffer.
7. Immerse the electrode in the first buffer solution (pH 7.0) and wait until electrode has stabilized. Read buffer and calibrate as pH 7.0 or at the pH value on the bottle corresponding to the temperature of the buffer.
8. Immerse the electrode in the first buffer solution (pH 10.0) and wait until electrode has stabilized. Read buffer and calibrate as pH 10.0 or at the pH value on the bottle corresponding to the temperature of the buffer.
9. Push done to view calibration summary and record % slope. Acceptable is usually 92 – 102%.
10. Store the calibration to accept the calibration data and return to the measurement mode.
11. After calibration, immerse the electrode again into a second source pH 7.0 or other second source buffer that has a pH value within the calibration range to verify the accuracy of the calibration.
12. Read and record the pH value to the nearest 0.1 unit.
13. If the measured pH of the verification buffer falls within ± 0.1 pH units of the true value then the calibration is acceptable, and you can begin sample analysis.

Note: Buffers and samples should be analyzed in the same type beaker, i.e., glass or plastic

NOTE: The buffer and samples readings must all be compensated for their respective temperatures. The best way to do this is to use an Automatic Temperature Compensator (ATC) probe during calibration and analysis.

NOTE: Always rinse the electrode off with laboratory reagent water between operations to achieve maximum precision.

NOTE: The % slope is a way of expressing how far away the calibration is from the theoretically perfect mV value of 59.2 mV. Consult the manufacturer's specifications for the specific slope

tolerances of your electrode. In most instances, an acceptable slope is 92 – 102%.

NOTE: If the slope is not in the acceptable range, pour fresh buffers (check expiration dates) and repeat the calibration. If the slope is still not acceptable, this usually means the electrode needs cleaned or replaced.

Procedure:

1. Calibrate the electrode as described above.
2. Place the sample in a clean glass beaker. Use a sufficient volume (> 50 mL) to cover the sensing elements of the electrode(s). The sample volume should allow for the sensing elements of the electrode to be submerged, but still about one-half inch above the bottom.
3. Immerse the electrode(s) in the sample. Wait for the reading to stabilize and record the pH value to the nearest 0.1 unit. Always report temperature at which pH is measured.
   NOTE: DMRQA samples require reporting to the nearest 0.01 unit.
4. Perform a duplicate pH analysis to ensure precision and accuracy. Wait for the reading to stabilize and record the pH value to the nearest 0.1 unit.
5. Rinse the electrode with laboratory reagent water. Store in 4.0 M potassium chlorine (KCl) or storage solution recommended by the manufacturer.

NOTE: The electrode storing solution varies with the type of electrode used. Generally, a storage solution will have a conductivity greater than 4000 µmhos/cm but to prevent faster deterioration of the electrode (and more costs to the lab), store the electrode in the storing solution recommended by the manufacturer. Electrodes should be keep wet by returning them to the storage solution whenever the pH meter is not in use.

Determination of Total Phosphorus (PO_4^{3-}-P)

HACH TNTplus Spectrophotometric Measurement Method

References: HACH Method 10210: TNTplus 843, TNTplus 844 and TNTplus 845

I. INTRODUCTION

A. GENERAL

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. Phosphates arise from a variety of sources. Small amounts are added to some water supplies during treatment while larger quantities may be added during laundering or other cleaning processes. Even more, phosphates are used extensively in the treatment of boiler waters. Orthophosphates as fertilizers are carried into surface waters with storm runoff and melting snow. Organic phosphates are formed primarily by biological processes such as body waste and food residue or formed from orthophosphates during biological treatment processes or receiving-water biota. Phosphorus is essential to the growth of organisms and can be, conversely, the nutrient that limits the primary productivity of a receiving body of water. In the instance of growth limitation, the discharge of Raw or treated wastewater may stimulate the growth of photosynthetic aquatic organisms in nuisance quantities.

B. METHOD

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration and is measured at 650 or 880 ± 5 nm. Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion.

C. SCOPE

Method is applicable for the range of 0.05 to 20 mg/L, depending on the range of TNTplus vials used.

II. SAMPLING, PRESERVATION, & PREPARATION

A. SAMPLE COLLECTION

For grab sampling, use only glass containers that have been cleansed with 1:1 HCl and rinsed with laboratory reagent water. If glassware is used solely for PO_4^{3-}-P analysis, acid cleaning may be required occasionally while intermediary cleansing may be done with phosphate-free glassware-washer detergent.

For 24-hour composite sampling, all samples are collected in plastic containers that are conducive to the automatic samplers. All Influent and Effluent results that are reported on the MRO and DMR shall be produced using 24-hour, flow-proportioned, composite samples.

B. SAMPLE PRESERVATION

All samples should be analyzed the day they are collected. It is not typical to need sample preservation.

• If preservation is necessary, lower pH to ≤ 2 with concentrated H_2SO_4 and refrigerate at ≤ 6°C for ≤ 28 days.

C. SAMPLE PREPARATION

Adjust pH to between 2 and 10 with 5*N* sodium hydroxide (NaOH). Warm samples and standards to room temperature before analysis.

III. <u>REAGENTS, STANDARDS and EQUIPMENT</u>

A. REAGENTS

1) Sulfuric Acid (Preservation Only)
2) 5*N* Sodium hydroxide (To bring sample pH between 2 - 10 after H_2SO_4 preservation)
   • 50 g NaOH dissolved in 250 mL laboratory reagent water
3) Laboratory Reagent Water
4) 50 mg/L Phosphate Standard (LabChem #LC186007)
5) TNTplus (843) Low Range (LR) Vials – store at room temperature
6) TNTplus (844) High Range (HR) Vials – store at room temperature
7) TNTplus (845) Ultra High Range (UHR) Vials – store at room temperature
8) TNTplus Reagent B (Ammonium Molybdate & Sulfuric Acid) – store at room temperature
9) TNTplus DosiCap C (Ascorbic Acid) – store at room temperature

B. STANDARDS

1) Stock Standard
   - 50 mg/L Phosphate Standard (LabChem #LC186007) or equivalent
2) LR and HR QA Check Standard
   - 1.0 mg/L Phosphate Standard
     Pipet 2.0 mL 50 mg/L Stock Standard and dilute to 100 mL in a Class A Volumetric Flask with laboratory reagent water
3) UHR Check Standard
   - 5.0 mg/L Phosphate Standard
     Pipet 10 mL of 50 mg/L Stock Standard and dilute to 100 mL in a Class A Volumetric Flask with laboratory reagent water

B. EQUIPMENT

1. HACH DR3900 Spectrophotometer
2. HACH DRB200 Reactor
3. Vial Cooling Rack
4. 0.1 to 1.0 mL Adjustable Pipettor with appropriate tips
5. 1.0 to 10. mL Adjustable Pipettor with appropriate tips
6. 100 mL Class A Volumetric Flasks (Acid Washed)
7. 100 mL Class A Graduated Cylinders

8. 50 mL Beakers (Acid Washed) – used to pour Stock, Check and Spiking Standards into prior to pipetting as standards should never be pipetted directly from their container(s) to prevent the possibility of contaminating them.

Note: All glassware used should be acid washed with hot 1:1 HCl and rinsed with laboratory reagent water. Preferably, this glassware should be used only for the determination of phosphorus and after use should be rinsed with laboratory reagent water and kept covered until needed again. If this is done, the treatment with 1:1 HCl is only occasionally required. Only phosphate free commercial detergents should be used when cleaning the glassware.

IV. PROCEDURE

1) Prepare the workspace to ascertain vial/sample identification and location.
   The TNTplus vials may NOT be written on and it is difficult to write on the cooling rack. Therefore, you must take precaution to keep track of and not misplace any vial during the entire procedure. This may be done by labeling each sample/vial, on your workspace, directly in front of each vial as it sits in the cooling rack and placing them in the same order when in the DRB Reactor.

2) Turn on the DR3900 and wait for instrument to perform the Self-Check.
   - This process will check the system, lamp, filter, calibration wavelength, and voltage.
   - The Main Menu will display when the Self-Check is complete, and the meter is automatically calibrated.

***NOTE 1:** When a new lot of vials is received, register that vial lot with the instrument by touching the "Target Icon" of the vial box to the "Target Icon" of the DR3900. This will allow the instrument to store that lot number and recognize the unique properties of each new lot.

Recently, Hach released Truecal Reagent Vials for TNT 843 and TNT 845 that reduce variations in results caused by variances in chemistry raw materials. A bar code on each vial contains the calibration curve data specific to each lot of chemistry, and automatically updates the calibration curve. With TNTplus products using the Truecal feature, you'll have one less thing to worry about during crucial proficiency testing or permit-limit compliance testing.

***NOTE 2:** Hach has the following statement in the TNTplus method: "For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with laboratory reagent water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option. Measure the reagent blank value when a new lot of reagent is used."

***NOTE 3:** One should use caution when subtracting a reagent blank value from sample results as the quality of laboratory reagent water used for reagent blank analysis may give

"false" positive or negative values that would bias the sample results if blank correction is used.

3) Sample Blank: Refer to Section V. QUALITY ASSURANCE/QUALITY CONTROL B. SAMPLE BLANK on page 100

4) Turn on the DRB200 Reactor via the switch on the back.

Heating the Reactor to 100°C:

a. 100° should be your default setting. Select the ▼.

b. Select 100°

c. Select 60'

d. Select OK - 100° and Start should be displayed on the screen

e. Select Start - The Reactor will automatically heat to 100°. When it reaches that temp, it will beep twice.

5) Determine which vials are necessary to analyze the samples. The LR vial will analyze concentrations between 0.05 and 1.5 mg/L. The HR vial will analyze concentrations between 0.5 and 5.0 mg/L. The UHR vial will analyze concentrations between 2.0 and 20.0 mg/L.

6) Remove the vial cap, pipet 2.0 mL of sample into the **LR vials,** pipet 0.5 mL of sample into the **HR vials** and pipet 0.4 mL of sample into the **UHR vials** and replace the vial caps on their respective vials**.**

7) Carefully remove the foil lid from the vials and unscrew the DosiCap. Flip the DosiCap over so that the reagent side faces the vial. Screw the cap on tightly.

8) Shake the capped vial until the reagent dissolves. This can be verified by looking through the end of the cap and confirming that there is no white residue in the cap.

9) Insert vials into the reactor and close the lid.

NOTE: You must take note of your vial placement! There is no means to label the vials in or on the Reactor. Notation of vial placement may be done by drawing a diagram of the Reactor on your workspace where each of the Reactor circles are uniquely labeled with their respective vial identities.

10) Operating the Reactor (Digestion at 100°C for 60 minutes):

1. Make sure the Reactor has been set to digest the vials for 60 minutes.
2. Once the Reactor has heated to 100°, it will beep twice.
3. Select "Start"

a. It will automatically time the 60 minute digestion time. A clock and 60' will be displayed on the screen with the 60 counting down to 0 over the succeeding hour.

b. The Reactor will beep again when the 60 minute digestion has completed.

11) Remove the vials from the Reactor and place them in the cooling rack, again taking note of vial ID and placement.

12) Allow vials to cool to room temperature (approximately 30 minutes).

13) Using previously calibrated automatic pipettors and appropriate tips, pipet Reagent B into each vial using the reagent bottle assigned to each vial type/lot:

- LR Vials: 0.2 mL of Reagent B
- HR Vials: 0.2 mL of Reagent B
- UHR Vials: 0.5 mL of Reagent B

NOTE: Immediately close Reagent B containers when done pipetting!

14) Retrieve the appropriate number/ratio of grey DosiCaps from their respective Reagent C containers.

15) Screw the DosiCaps onto each vial and invert 2-3 times to dissolve the reagent in each cap.

16) Using a lab timer, allow the reagent to react for 10 minutes.

17) Invert the sample an additional 2 - 3 times to mix.

18) Wipe the outside of the vial with a Kimwipe.

19) Insert the vial into the cell holder of the DR3900.

20) When the reading is displayed, **make sure the spec is measuring TOTAL Phosphorus (PO_4^{3-}-P).** If it is not, proceed as follows:

Note: This must be done for each type of TNTplus vials used, i.e., TNTplus 843, TNTplus 844 and TNTplus 845

a. Press “Options”
b. Press “More”
c. Press “Chemical Form”
d. Press “PO_4^{3-}-P”
e. Press “OK”
f. Press “Return”

21) If the decision is made to use Reagent Blank correction for the samples **refer to NOTES 2 and 3 under <u>IV. PROCEDURE - 2)</u> above**, readjust the results to compensate for the Reagent Blank as follows:

a. Press “Options”
b. Press “More”
c. Press “Reagent Blank”
d. Press “On”
e. Tap on the highlighted box that displays a concentration

f. Enter the result for the corresponding Reagent Blank recorded on the bench sheet**. (Enter the LR Reagent Blank result for the LR vials, HR Reagent Blank result for the HR vials and the UHR Reagent Blank result for the UHR vials).**
g. Press "OK"
h. Press "OK"
i. Press "Return"

22) If Reagent Blank correction is being used, record this new reading as your sample result. If not, record the result displayed in step 20). Results are displayed in mg/L and needs no further calculation.

23) Analyze all necessary QA parameters as indicated by the bench sheet. All QA parameters must be within established control limits or the test is considered invalid.

V. QUALITY ASSURANCE/QUALITY CONTROL – the following Quality Assurance/Quality Control samples and frequency of analysis are recommended to produce accurate data. **Refer to NOTE above Table I "Quality Control Sample Frequency" on page 8.**

- All standard solutions must be analyzed at the same temperature as the samples.

A. REAGENT BLANK

Refer to NOTE 3: under PROCEDURE - 2) above

To analyze a reagent blank, use laboratory reagent water in place of sample and analyze according to the procedure listed above.

B. SAMPLE BLANK

A sample blank is not typically necessary due to the small volume of sample used in each TNTplus reagent vial and sample turbidity and color is normally removed during the digestion process . If a sample requiring a LR TNTplus reagent vial is particularly turbid, however, a sample blank may be necessary. This analysis will compensate for color and turbidity of the sample. If the sample blank result is a positive number, the sample blank will be subtracted from the sample result in lieu of the reagent blank if blank correction is being used; and if the sample blank is a negative number, the sample blank will be added to the sample result in lieu of the reagent blank if blank correction is being used.

To analyze a sample blank, add the correct volume of sample to the vial but do NOT remove the foil from the DosiCap or flip it over to release the reagent. Simply replace the cap. Process the sample blank reagent vial(s) in the same procedure used for the samples and record the result(s).

C. LR and HR QA CHECK STANDARD (1.0 mg/L) – refer to Section III B. 2)

This standard must be analyzed with each sample batch that is not accompanied by a Certified Reference Standard.

- Pipet **2.0 mL** of the 1.0 mg/L standard into a **LR vial** or **0.5 mL** of the 1.0 mg/L standard into a **HR vial** and analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

- Result must be between 0.90 and 1.10 mg/L.

D. UHR QA CHECK STANDARD (5.0 mg/L) – refer to Section III B. 3)

This standard must be analyzed with each sample batch that is not accompanied by a Certified Reference Standard.

- Pipet 0.4 mL of the 5.0 mg/L standard into an UHR vial and analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

- Result must be between 4.5 and 5.5 mg/L.

E. DUPLICATES

A duplicate must be analyzed for each TNTplus vial range used in an analysis or at the frequency stated in the laboratory's Quality Assurance/Quality Control document. The results must be within the established control limits.

F. FINAL EFFLUENT SPIKE

This parameter is analyzed at the frequency stated in the laboratory's Quality Assurance/Quality Control document.

- Pipet 1.0 mL of 50 mg/L phosphate Stock Standard and dilute 100 mL with Final Effluent in a Class A Volumetric Flask or Graduated Cylinder – mix thoroughly.
- Pipet 0.5 mL of the Final Effluent + Spike solution into a HR vial.
- Analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

Percent Spike Recovery (%R) Calculation

A = Final Effluent Result **with Spike** (mg/L)
B = 100 – Volume of Final Effluent + Spike sample (mL)
C = Final Effluent Result **without** Spike added (mg/L)
D = 99 – Volume of Final Effluent sample used in Spike sample (mL)
E = 50. Concentration of Spike added (mg/L)
F = 1.0 – Volume of Spike Added

$$\frac{[(A \times B) - (C \times D)] \times 100}{(E \times F)} = \text{Percent Recovery}$$

Recovery must be between 85% and 115%

Example

A = Final Effluent Result **with** Spike = 1.38 mg/L
B = Volume of Final Effluent + Spike = 100 mL
C = Final Effluent Result **without** Spike Added = 0.931 mg/L
D = Volume of Final Effluent used in Spike sample = 99 mL
E = Concentration of Spike Added = 50. mg/L
F = Volume of Spike Added = 1.0 mL

$$\frac{[(1.38\ mg/L \times 100\ mL) - (0.931\ mg/L \times 99\ mL)] \times 100}{(50.\ mg/L \times 1.0\ mL)} = 91.6\%\ Recovery$$

G. RAW INFLUENT SPIKE

This parameter should be analyzed with the Raw Influent at the frequency stated in the laboratory's Quality Assurance/Quality Control document.

- Pipet 4.0 mL of 50 mg/L phosphate Stock Standard and dilute to 100 mL with Raw Influent in a Class A Volumetric Flask or Graduated Cylinder.
- Pipet 0.4 mL of this solution into a UHR vial.
- Analyze as usual (Including the correction for the reagent blank if the reagent blank adjust option is being used).

Percent Spike Recovery (%R) Calculation

A = Raw Influent Result **with Spike** (mg/L)
B = 100 – Volume of Raw Influent + Spike sample (mL)
C = Raw Influent Result **without** Spike added (mg/L)
D = 96 – Volume of Raw Influent sample used in Spike sample (mL)
E = 50. Concentration of Spike added (mg/L)
F = 4.0 – Volume of Spike Added (mL)

$$\frac{[(A \times B) - (C \times D)] \times 100}{(E \times F)} = Percent\ Recovery$$

Recovery must be between 85% and 115%

Example

A = Raw Influent Result **with** Spike = 4.27 mg/L
B = Volume of Raw Influent + Spike = 100 mL
C = Raw Influent Result **without** Spike Added = 2.23 mg/L
D = Volume of Raw Influent used in Spike sample = 96 mL
E = Concentration of Spike Added = 50. mg/L
F = Volume of Spike Added = 4.0 mL

$$\frac{(4.27\ mg/L \times 100\ mL) - (2.23\ mg/L \times 96\ mL) \times 100}{(50.\ mg/L \times 4.0\ mL)} = 106\%\ Recovery$$

Recovery must be between 85% and 115%.

H. METHOD DETECTION LIMIT (MDL)

An MDL using the criteria in the latest 40 CFR Part 136 Approved Methodology must be analyzed using Low Range (LR) TNTplus Reagent Vials at the frequency stated in 40 CFR Part 136.

I. APPROVED REFERENCE STANDARDS

Approved Reference Standards are analyzed at least quarterly and results fall within the

95% Confidence Interval and/or split samples are analyzed at least quarterly and results fall within 20% Relative Percent Difference (RPD).

VI. INTERFERENCES

There are no interferences caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in seawater. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in seawater, it does not interfere.

VII. CALCULATION

Total Phosphorus, mg/L = Direct reading of HACH DR3900 Spectrophotometer

VIII. WASTE MANAGEMENT

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

IX. REFERENCES

1. Standard Methods 21st ed. Method 4500-P Pages 4-146 to 4-149
2. Standard Methods 21st ed. Method 4500-P B. Pages 4-149 to 4-151
3. HACH Method 10210: (TNTplus 843), (TNTplus 844) and (TNTplus 845)
4. EPA Method 365.3

Determination of Total Phosphorus, Ascorbic Acid Method

References: Standard Methods 21st ed. 4500-P A., B.(5) & E.

INTRODUCTION

Excerpt from SM 21st ed.

Phosphorus occurs in natural waters and in wastewater almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates, and organically bound phosphates. They occur in solutions,, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatments. Larger quantities of the same compounds may be added during laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface water with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving-water biota. Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of Raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic form and incorporated into organic compounds. (End of excerpt)

To analyze the phosphorus level in a sample, one must convert the phosphorus to dissolved orthophosphate and then carry out a colorimetric determination of this orthophosphate.

Phosphorus may occur in combination with organic matter, therefore it is essential to use a digestion method that will effectively oxidize organic matter to release the orthophosphate. The ascorbic acid method of colorimetric determination uses ammonium molybdate and antimony potassium tartrate. They react with orthophosphate to form a heteropoly acid, phosphomolybdic acid, that is reduced to intensely colored molybdenum blue by ascorbic acid. The concentration of molybdenum blue is measured spectrophotometrically, and its color intensity is directly proportional to the concentration of orthophosphate in the sample.

Phosphates in unpreserved samples may be absorbed into the wall of plastic storage bottles, therefore, it is necessary to be sure that each sample has been preserved with 1:1 H_2SO_4 and cooled to ≤ 6°C. The holding time for phosphorus samples is 28 days.

Reagents/Chemicals and Equipment

A. Reagents

1. Ammonium persulfate, $(NH_4)_2S_2O_8$, solid

2. Sulfuric acid solutions - always slowly add acid to water!
   (a) 10.8*N* for Persulfate Digestion Method – slowly dilute 300 mL concentrated H_2SO_4 to 600 ml laboratory reagent water while stirring. Cool to room temperature than dilute to 1 L with laboratory reagent water
   (b) 1+1 for preserving phosphorus samples – slowly add an equal volume of concentrated H_2SO_4 to the same volume of laboratory reagent water

3. Phenolphthalein indicator aqueous solution
   Dissolve 5.0 g phenolphthalein in 1 L laboratory reagent water.

4. Sodium hydroxide Solutions
   (a) 10*N* - Dissolve 400 g NaOH in 600 mL laboratory reagent water. After cooling to room temperature, dilute to 1 L with laboratory reagent water
   (b) 5*N* – Dissolve 200 g NaOH in laboratory reagent water and dilute to 1 L
   (c) 1*N* – Dissolve 40. g NaOH in laboratory reagent water and dilute to 1 L

5. Solutions needed to prepare Combined Reagent:
   (a) Sulfuric acid, 5*N* solution – dilute 140 mL conc. H_2SO_4 to 1 L with laboratory reagent water
   (b) Antimony potassium tartrate solution – dissolve 1.3715 g $K(SbO)C_4H_4O_6 \cdot ½\ H_2O$ in laboratory reagent water and dilute to 500 mL in a Class A volumetric flask. Store in a glass stoppered container.
   (c) Ammonium molybdate solution – dissolve 40. g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1 L laboratory reagent water. Store in a glass stoppered container.
   (d) Ascorbic acid, 0.1 M solution – dissolve 1.76 g ascorbic acid solid in 100 mL laboratory reagent water. This solution is stable for about one week if stored at 4°C.

6. Stock Phosphate Solution – 50.0 mg/L PO_4^{3-}-P
   Dissolve 219.5 mg (0.2195 g) anhydrous KH_2PO_4 (that has been dried at 105°C) in laboratory reagent water and dilute to 1 L in a Class A volumetric flask. 1.00 mL = 50.0 µg PO_4^{3-}-P. You will need two Stock Phosphate Solutions prepared from different lot numbers. The second one to be labeled Second Source Stock Phosphate Solution. These standards can be purchased.

7. Standard Phosphate Solution – 1.0 mg/L PO_4^{3-}-P
   Using a Class A pipet, pipet 10. mL of the Stock Phosphate Solution and dilute to 500 mL in a Class A volumetric flask with laboratory reagent water. 1.00 mL = 1.0 µg PO_4^{3-}-P. You will need two of these solutions with the appropriate labels, one from each lot number used in #6.

8. 1+1 Hydrochloric acid – for acid washing phosphorus glassware
   Mix equal volumes of HCl and laboratory reagent water together

B. Equipment

1. 125 mL Erlenmeyer Flasks

2. 5.0, 10. and 25. mL Class A Volumetric Pipets or a 1.0 to 10. mL adjustable pipettor with tips

3. 500 mL and 1000 mL Class A Volumetric Flasks

4. 50 mL and 100 mL Graduated Cylinders

5. 10 mL wide bore pipet for pipetting Raw samples

6. 1.0, 8.0 and 20. mL Bottle Top Dispensers with Bottles

7. Glass dropper bottles with glass droppers

8. Glass Scoop – (0.2 g ammonium persulfate capacity)

9. 50 mL Nessler tubes and stoppers

10. Hot Plate

11. Spectrophotometer, with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer or Filter photometer, equipped with a red color filter and a light path of 0.5 cm or longer

12. Appropriately sized matched set of sample cells/cuvettes/vials

Calibration curve

A new standard curve is prepared each time the following occur:

1. A new stock standard is purchased or prepared
2. Solutions used to prepare Combined Reagent have been prepared
3. The spectrophotometer or cuvettes/tubes/vials have been changed
4. The analysis of the second source calibration standard exceeds the control limit or exceeds the warning limit on two out of three analyses

To establish the calibration curve, the following suggested standards prepared from the Standard Phosphate Solution are analyzed:

1. 0.0 mg/L using 50 mL laboratory reagent water – calibration blank
2. 0.1 mg/L - pipet 5.0 mL of Standard Phosphate Solution and dilute to 50 ml with laboratory reagent water
3. 0.3 mg/L - pipet 15. mL of Standard Phosphate Solution and dilute to 50 ml with laboratory reagent water
4. 0.5 mg/L - pipet 25. mL of Standard Phosphate Solution and dilute to 50 mL with laboratory reagent water
5. 0.7 mg/L - pipet 35. mL of Standard Phosphate Solution and dilute to 50 mL with laboratory reagent water
6. 0.9 mg/L - pipet 45. mL of Standard Phosphate Solution and dilute to 50 mL with laboratory reagent water

Note: the following only applies to a HACH DR3900 spectrophotometer. If your lab has a different type spectrophotometer or your spectrophotometer is not capable of storing a calibration curve, you will need to calculate the Slope, Intercept and Correlation Coefficient (must be > 0.995) based on the Absorbance or Transmittance readings obtained when establishing the calibration curve.

To create a new curve in the HACH DR3900 spectrophotometer follow these steps:

a) After highlighting phosphorus from the user programs, touch PROGRAM OPTIONS instead of START.
b) Touch EDIT, highlight Calibration, and touch EDIT again. Choose Read Standards and begin by zeroing with the blank.
c) After reading each of the standards, touch NEXT and then touch STORE to keep the new calibration information in the program.
d) Touch START when you are ready to read your blank and remaining samples.

Persulfate Digestion Method

1. Turn on the hot plate.

2. Set up 125 mL Erlenmeyer flasks for each sample to be tested and be sure they are labeled with the appropriate ID.

3. Use the Class A volumetric pipets or adjustable pipettors to transfer standards into their respective flasks:
   a. Control Standard, 0.40 mg/L = 20. mL of the Standard Phosphate Solution
   b. Laboratory Fortified Blank (LFB), 0.20 mg/L = 10. mL of the Second Source Standard Phosphate Solution
   c. When spiking the Final sample, pipet 10. mL of the Standard Phosphate Solution into the Final spike flask for a 0.2 mg/L spike
   d. When spiking the Raw sample, pipet 10 mL of the Standard Phosphate Solution into the Raw spike flask for a 0.2 mg/L spike

4. Choose sample dilutions that will yield results that fall within the range of your calibration curve (it would be 0.1 to 0.9 mg/L if using the standards listed above in CALIBRATION CURVE). Final volume is 50 mL prior to colorization, therefore, a dilution factor of 1 is 50 mL of sample, a dilution factor of 10 is 5.0 mL of sample diluted to 50 mL with laboratory reagent water and so on. Generally, use 50 mL of Final for a dilution of 1 and use 10. mL of Raw for a dilution factor of 5. Based on the suggested calibration curve above, if the Raw has an expected concentration > 4.5 mg/L after the dilution factor is applied, use 5.0 mL of Raw for a dilution factor of 10.

5. Run one Method Detection Limit (MDL) sample every week, making sure the sample has an appropriate concentration. Refer to #2 under EVALUATION/QUALITY CONTROL

6. Fill an Erlenmeyer flask labeled “B” for Blank with approximately 50 mL of laboratory reagent water. Dilute all the other flasks that contain < 50 mL of sample up to about 50 mL with the laboratory reagent water. The Erlenmeyer flasks are not volumetric, and the markings are only approximate. Fill the flasks to a point between the 50 mL and 75 mL markings.

7. Add one drop of the phenolphthalein solution to each flask. If the sample turns pink, add 10.8*N* sulfuric acid dropwise until the color is discharged. (This should not occur if the sample was properly preserved to pH < 2.0)

8. Using the scoop, add 2 level scoops (or 0.4 g) of ammonium persulfate to each flask.

9. Add 1 mL 10.8*N* sulfuric acid to each flask, using a bottle top dispenser pipettor. Give the dispenser one or more pumps in the sink before starting to add the solution to the flasks to make sure the dispensing tube is full. Alternatively, you can use an adjustable pipettor to add the 1 mL.

10. Mix the samples well and place them on the hot plate for digestion. Be sure that the hood is on and that the sash is in the proper position to avoid fumes in the lab.

11. Digest samples until about 10 mL remains in the flask. Remove each flask as it reaches this volume and allow to cool under the hood.

Colorization and Analysis Procedure

1. Place cooled flasks on the counter you will use for the colorization process. It is easiest if you place the flasks in the order that they will be read on the spectrophotometer.

2. Add 20 mL of laboratory reagent water to each flask, using the bottle top dispenser.

3. Add one drop of phenolphthalein to each flask.

4. Add one dropper full of 10N NaOH to each flask and swirl to mix

5. Add one to two droppers full of 5N NaOH to each flask and swirl. Then add the 5N NaOH dropwise until a faint pink color persists after swirling.

6. Pour each sample into a corresponding 50 mL Nessler tube. Rinse each flask with laboratory reagent water and pour the rinse into the tube. Then fill the tube to the 50 mL line with laboratory reagent water.

7. Turn on the HACH DR3900 spectrophotometer. **Note**: steps 7. and 8. are only relevant if your laboratory uses a HACH DR3900 spectrophotometer.

8. Touch USER PROGRAM on the touch screen display. Touch to highlight phosphorus from the list and then touch START. Slide open the cell compartment cover and remove the light shield. The spectrophotometer is now ready to use. If the phosphorus program isn't available, or you need to create a new one for any reason, follow these steps:
    a) On the Main Menu screen touch USER PROGRAMS, then touch PROGRAM OPTIONS.
    b) Touch the NEW key, then you will need to input the Program Number (the next sequence number available), the Program Name, the Program Type (Single or Multi-

Wavelength), the Units, the Wavelength (nm), Concentration Resolution, and Chemical Form which is optional.
c) The screen then prompts you to calibrate. Detailed calibration instructions are shown under CALIBRATION CURVE.

9. Prepare the Combined Reagent as follow (volumes shown are to prepare 100 mL – you can adjust the volumes accordingly if more is needed):
   a. Sulfuric acid, 5N solution: 50 mL
   b. Antimony potassium tartrate solution: 5 mL
   c. Ammonium molybdate solution: 15 mL
   d. Ascorbic acid, 0.1M solution: 30 ml

   Prepare in the designated reagent bottle and swirl to mix. You will need 8 mL of this for each sample. Prepare enough for a few more samples than you are analyzing to insure the dispenser is full for each pump if using a bottle top dispenser.

10. Using the bottle top dispenser, squirt one or more pumps of Combined Reagent into the sink to make sure the delivery tubing is full then begin adding 8 mL to each Nessler tube. Alternatively, the 8 mL can be added using an adjustable pipettor. Stopper each Nessler tube and mix thoroughly by inverting several times. It is a good idea to do only those Nessler tubes that can be read on the spectrophotometer within the 20 minute window. Addition of the Combined Reagent can be staggered so that all samples are read in their 20 minute window.

11. After at least 10 but no more than 30 minutes, take readings for each sample on the spectrophotometer. Invert each Nessler tube, remove the stopper, rinse the sample cell/cuvette/tube/vial with the sample and discard the rinse. Refill the sample cell/cuvette/tube/vial, wipe dry with a Kim Wipe and place it in the spectrophotometer. Slide the cell compartment cover over or close the sample compartment cover and take the reading. If your spectrophotometer has the capability, you will need to zero it with the digested blank by touching the ZERO display key before reading the samples. All following samples can be read in the same manner by touching the READ display key after insertion.

12. Enter all data onto the appropriate bench sheet.

13. Rinse all glassware with water and clean with warm 1+1 HCl then rinse at least four times with tap water followed by rinsing with laboratory reagent water. It is best that glassware used for phosphorus be reserved for this analysis.

Helpful Hints

1. Get your combined reagent solutions out of the refrigerator when you begin the analysis so they can come to room temperature before using them. If they are still cold when you are ready to use them, warm to room temperature in the sink. Using them cold can lead to inaccurate colorization.

2. If running phosphorus daily it is easier to make a larger amount of Ascorbic acid, 3.52 g to 200 mL for example, and use it for the whole week.

3. Sometimes a precipitate may form after digestion. Do not filter, it should re-dissolve under the acid conditions of the combined reagent.

4. The following calculations are used for phosphorus:

$$\text{Dilution factor} = \frac{\text{Final Volume, 50 mL}}{\text{Sample Volume Used, mL}}$$

$$\text{Total Phosphorus, mg/L} = \text{Phosphorus, mg/L} \times \text{Dilution Factor}$$

$$\text{Percent Removal for Daily Sample} = \frac{(\text{Concentration P in Raw} - \text{Concentration P in Final})(100)}{\text{Concentration P in Raw}}$$

Evaluation/Quality Control

9 Quality Control Elements

1. Demonstration of Capability (DOC)
    - Analysts run a proficiency test sample from an outside source quarterly.
    - A Laboratory Fortified Blank (0.2 mg/L) from the Second Source Standard Phosphate Solution is analyzed with each run.
    - A Control Standard (0.4 mg/L) from the Standard Phosphate Solution is analyzed with each run.
    - If either the 0.2 mg/L LFB or the 0.4 mg/L Control Standard is out of control the analysis is still valid, if both are out of control then the analysis is invalid.

2. Method Detection Limit (MDL)
    - MDLs are analyzed at the frequency and using the criteria in the latest 40 CFR Part 136 Approved Methodology.

3. Laboratory Reagent Blank (LRB) aka Method Blank (MB)
    - A method blank is run each day the test is performed, consisting of 50 mL laboratory reagent water and carried through the entire analysis like any other sample. The digested blank is used to zero the spectrophotometer if the spectrophotometer has the capability.

4. Laboratory Fortified Blank (LFB)
    - Analyze a laboratory fortified blank with every run.
    - The LFB is 10. mL of the second source standard diluted to 50 mL and taken through the entire digestion and analysis procedure.

5. Duplicates and Matrix Spikes
    - For each phosphorus run, analyze duplicates and spikes for Raw and Final samples. When running phosphorus daily alternate which samples are spiked and duplicated. One day analyze Final duplicates and a Final spike, the next day analyze Raw duplicates and a Raw spike.

6. Continuing Calibration Verification
    - The Laboratory Fortified Blank (0.20 mg/L) and the Control Standard (0.40 mg/L) are used as continuing verification of the calibration curve for each run.

7. Control Charts and Trend Analysis
    - Maintained daily for all QC parameters analyzed

8. QC Acceptance Criteria
    - If a duplicate or spike sample is out of control then that sample must be analyzed again. All other data in the run is acceptable.
    - If multiple QC points are out of control then the whole run must be analyzed again.

9. Definition of Preparation/Analytical Batches that Drive QC Frequencies
    - The analytical batch usually consists of Final and Raw samples. Each day of analysis there is a set of duplicates and a spike analyzed on either the Raw or Final sample. These are in addition to the LRB and LFB samples and the MDL sample analyzed at the frequency stated in 40 CFR Part 136.

Preventative Maintenance

1. Keep the area for the sample cells/cuvettes/tubes/vials free of dust and debris.

References

Standard Methods for the Examination of Water and Wastewater, Method 4500-P A., B. and E.

Determination of Total Suspended Solids (TSS)
Dried at 103-105°C
Reference: Standard Methods 21st ed. 2540 D.

1. SCOPE AND APPLICATION

1.1. Total Suspended Solids (TSS) also referred to as Non-Filterable Residue is defined as the amount of solids in a sample that are larger than 2 microns. This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of determination is 4 mg/L to 20,000 mg/L. This method is based on Standard Methods, 21st edition, Procedure 2540 D. Referencing Standard Methods 19th, 20th ed. is also acceptable.

2. SUMMARY OF METHOD

2.1. A well-mixed sample is filtered through a pre-weighed glass fiber filter. The filter and any residue are then dried to a constant weight at 103 – 105°C. The filter is cooled in a desiccator, weighed and the results used to compute the TSS of the sample.

Note: If the filtrate of this analysis is collected in a clean filtering flask, it may be used for a Total Dissolved Solids (TDS) determination if the sample requires both tests.

3. APPARATUS

3.1. Glass fiber filters (without organic binder), 934 AH, or equivalent i.e., Whatman GF/C
3.2. Filter Holder or Gooch crucibles or Buchner funnel
3.3. Aluminum weigh dishes (not needed if using Gooch crucibles or Buchner funnel)
3.4. Membrane Filter Funnel (not needed if using Gooch crucibles or Buchner funnel)
3.5. Filtering Flask
3.6. Filter Support: Filtering apparatus with reservoir and a coarse fritted disc as a filter support (not needed if using Gooch crucibles or Buchner funnels)
3.7. Drying Oven with an adjustable temperature control knob capable of heating up to 105°C
3.8. Desiccator
3.9. Analytical Balance capable of weighing to 0.0001 g (0.1 mg)
3.10. Forceps and/or tongs
3.11. Aluminum foil
3.12. Graduated Cylinders (assorted sizes)
3.13. Wide Bore Pipets (Blowout type)
3.14 Vacuum Pump
3.15 Vacuum Tubing

4. REAGENTS AND CHEMICALS

4.1. Laboratory Reagent Water

4.2. Desiccant – granules containing a color indicator of moisture concentration – usually blue in color when dry or a commercially available self-contained desiccant package

4.2.1. The desiccant is placed in the bottom of the desiccator used to store filters, crucibles, and aluminum weigh dishes

4.2.2. When the desiccant turns from blue to pink (indicating water absorption), heat the desiccant in an 180°C oven for a minimum of one hour. After cooling the desiccant, it is ready to be reused. Reuse the desiccant three times. After three times, discard the old desiccant and replace with new desiccant. The old desiccant may be disposed with the normal laboratory waste. If using a commercially available self-contained desiccant package, consult the manufacturer's instructions for the correct heating temperature. Commercially packaged desiccants usually involve the use of plastics that may require a lower heating temperature and a long drying time.

5. SAMPLE HANDLING AND PRESERVATION

5.1. When analysis cannot be performed within two hours of sample collection, preservation by refrigeration or icing to ≤ 6°C is required to minimize the microbiological decomposition of solids. Analysis should begin as soon as possible after collection.

5.2. Non-representative solids such as grit, seeds, leaves, sticks, and lumps of fecal matter should be removed from the sample prior to analysis if the goal of the analysis is not to include non-representative solids in the final result. Floating oil and grease, if present, should be included in the sample and dispersed by vigorous mixing before measuring the sample aliquot to be analyzed.

5.3. Samples stored at ≤ 6°C should be brought to room temperature before processing.

5.4. Sample must be analyzed within seven days of collection.

6. SAMPLE PREPARATION AND HANDLING PROCEDURES

Note: Be sure to record all the necessary information neatly and accurately on the TSS bench sheet.

6.1. Preparation of the glass fiber filters

6.1.1. Place each glass fiber filter on the membrane filter apparatus rough side up (if using Gooch crucibles or Buchner funnels, place glass fiber filter rough side up in a clean Gooch crucible or Buchner funnel). While applying a vacuum, wash each filter with three successive 20 mL volumes of laboratory reagent water allowing the water to be completely pulled through the filter between washings.

6.1.2. Using filter forceps (use tongs when handling Gooch crucibles), remove the filter from the filtering apparatus, place on clean aluminum foil and dry in the oven at 103 – 105°C for a minimum of one hour (Gooch crucibles do not need placed on clean aluminum foil). The thermometer used to check the temperature of the oven must have a Certificate of Traceability to a NIST certified thermometer or the thermometer must be compared to a NIST traceable thermometer on an annual schedule with any correction factor recorded and included with the thermometer. After heating at 103 – 105°C for a minimum of one hour, cool the filters and/or

Gooch crucibles containing filters in a desiccator for at least 30 minutes prior to weighing.

6.1.3. After verifying the calibration of the analytical balance, weigh the filters and/or Gooch crucibles and record the weight. Store in the desiccator until ready to use.

6.2. When ready to filter a sample, assemble the filtering apparatus: Connect the vacuum hose to the filter flask. Place the filter support (or Gooch crucible holder or Buchner funnel) on the filter flask. Put the filter, rough side up (or Gooch crucible or Buchner funnel) on the filtering apparatus. If using a filter, place the membrane filter funnel on top of the filter. Begin vacuum suction.

6.3. While the filtering apparatus is operating, shake the sample vigorously and transfer an aliquot of the well-mixed sample to the filtering apparatus using a clean graduated cylinder or wide bore pipet. The volume of sample filtered should be such that it gives a solids weight of at least 0.0025 g (2.5 mg) but not more than 0.2 g (200 mg). If the volume filtered fails to meet the minimum yield, increase the sample volume up to a maximum of 1 L. If complete filtration takes more than 10 minutes, increase the filter diameter or decrease the sample volume. Record volume of sample used on the TSS bench sheet. For sample volumes < 25 mL, a wide bore pipet is recommended for measuring the volume of the sample to be filtered. **Note:** If TDS is also going to be determined, be sure to remove the filtrate from the filtering flask prior to rinsing the graduated cylinder or pipet, filter, and sides of the filtering device. This will ensure the filtrate used for TDS is not diluted. After all the liquid has pass through the filter, wash the graduated cylinder or pipet, filter, and sides of the filtering device with three 10 mL portions of laboratory reagent water. Continue to apply the vacuum for three minutes after the sample has completely passed through and the filter has been rinsed, to assure that all traces of water have been removed.

6.4. Carefully remove the filter with forceps (remove Gooch crucibles with tongs) and place filter into aluminum weigh dish labeled with the sample location. Place aluminum weigh dish and/or Gooch crucible in an oven set at 103–105^{o}C. Wash the graduated cylinder or pipet, the filter holder, and membrane filter funnel then rinse with laboratory reagent water before filtering the next sample to avoid sample carryover. **Note:** If also analyzing for TDS, the filter flask must also be washed and rinsed with laboratory reagent water. Dry the sample filters and/or Gooch crucibles for a minimum of one hour (overnight drying ensures thorough drying).

6.5. Remove the dried aluminum weigh dishes containing filters and/or Gooch crucibles and place in desiccator to cool for at least 30 minutes. Check the calibration of the balance before proceeding. Weigh the filters and/or Gooch crucibles and record weight on TSS bench sheet. Do not leave the desiccator open too long. If the filters or crucibles are exposed to the laboratory air for a long period of time, the sample results may be affected.

6.6. On a routine basis, repeat the drying cycle in the 103 – 105^{o}C oven for one more hour,

then cool in the desiccator and weigh again using the same procedures described in step 6.5. The weight obtained in step 6.6. should agree within 4% or 0.0005 g (0.5 mg) of the weight recorded in step 6.5. If it does not, longer drying times may be required in step 6.4. to ensure complete drying of the samples.

6.7. Determination of Total Suspended Solids

Total Suspended Solids, mg/L is calculated using the one of the following equations:

$$\text{If weights are in grams (g) - TSS, mg/L} = \frac{\text{Residue Weight (g)} \times 1{,}000{,}000}{\text{Sample Volume (mL)}}$$

$$\text{If weights are in milligrams (mg) TSS, mg/L} = \frac{\text{Residue Weight (mg)} \times 1000}{\text{Sample Volume (mL)}}$$

7. METHOD DETECTION LIMIT (MDL)

7.1 Standard Methods states the following: "Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume to 1 L." Based on that, the lower reporting limit could be presumed to be 2.5 mg/L for TSS.

7.2 40 CFR Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 2 that became effective 9/17/2017 has the following statement in it: "MDL determinations using spiked samples may not be appropriate for all gravimetric methods (*i.e.,* residue or Total Suspended Solids), but a MDL_b based on method blanks can be determined in such instances." Following the procedure for MDLs using method blanks required in Appendix B to Part 136 (found under Method Detection Limit in the Quality Assurance and Quality Section of this manual), the laboratory may be able to establish a TSS Lower Reporting Limit of < 2.5 mg/L when a 1000 mL sample volume is filtered.

7.3 In the case the laboratory chooses to use the minimum yield of 2.5 mg dried residue stated in Standard Methods, the following equation would be used for the TSS Lower Reporting Limit, mg/L when sample volumes filtered were other than 1000 mL.

$$\textbf{TSS Lower Reporting Limit, mg/L} = \frac{\textbf{2.5 mg} \times \textbf{1000 mL}}{\textbf{Sample Volume Filtered, mL}}$$

7.4 In the case the laboratory chooses to establish a MDL_b specific for their facility, the following equation would be used for the TSS Lower Reporting Limit, mg/L when sample volumes filtered were other than 1000 mL.

$$\textbf{TSS Lower Reporting Limit, mg/L} = \frac{\textbf{MDL}_b \times \textbf{1000 mL}}{\textbf{Sample Volume Filtered, mL}}$$

8. QUALITY CONTROL

8.1 One laboratory reagent water blank per batch of 20 samples or less. The result for the blank must be below the current Method Detection Limit (MDL) to report the results for the batch of samples without qualification. If the result for the blank is > than the MDL, the blank and all associated samples should be rerun until the blank is ≤ MDL.

8.2 One set of sample duplicates per batch or per 5% of samples analyzed, whichever is greater. The calculated precision value (RPD) must meet the control limit established in the laboratory. If the duplicates exceed the acceptable control limit, they must be rerun until an acceptable RPD result is achieved.

8.3 A Laboratory/Quality Control Standard must be analyzed on a quarterly basis. The laboratory results for this standard should fall within the acceptance limits accompanying the standard. An acceptable alternative to this is to split samples with another laboratory. The RPD for split samples should be less than or equal to 20% to be acceptable.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Safety glasses, gloves, and lab coats should always be worn while processing samples.

9.2. Excess samples may be flushed down the sink unless they are determined to be hazardous waste.

10. REFERENCE

10.1 Standard Methods 21st edition, 2540 D.

APPENDIX

Chemical Hygiene Plan Template

GENERAL

Achievement of a safe and healthful workplace is the responsibility of the employer, the laboratory supervisory personnel and, finally, the laboratory personnel themselves. All laboratory employees must make every effort to protect themselves and their fellow workers by conscientiously adhering to the health and safety program that has been developed for the Wastewater Treatment Plant Laboratory. The goal is to reduce all hazards in the laboratory. The main objective of this text is (1) safety management (2) administration requirements (3) hazard identification/evaluation (4) protective equipment (5) emergency response (6) safe work practices.

Employer's responsibilities are as follows:

- To furnish to each employee a place of employment which is free from recognized hazards that are causing or likely to cause death or serious physical harm to employees.
- To provide medical services without cost to the employee, without loss of pay, and at a reasonable time and place to employees who work assignment requires medical surveillance, sustains injury on the job, or who are exposed to hazardous chemicals.

Wastewater Department's responsibilities are as follows:

- The Wastewater Department will provide a written Health and Safety Plan for its employees which will include hazard communication, chemical hygiene plan, confined space entry, emergency response plan, reporting and recording injuries, personal protective equipment, in-house accident/incident forms, lock out/tag out program, trenching and shoring policy, and a chlorine policy if chlorine is used for disinfection.
- The Wastewater Department will provide health and safety training for employees.
- The Wastewater Department will provide adequate safety equipment for its employees.

Laboratory Supervisor's responsibilities are as follows:

- Shall coordinate safety programs, and training requirements for laboratory personnel with the WWTP Safety Committee.
- Responsible for organizing, stimulating, and guiding the laboratory safety programs, as well as keeping up with changes in safety subject areas to provide knowledge and information, when required, that is current.
- Coordinates and oversees the Chemical Hygiene Plan.
- Monitor procurement, use and disposal of chemicals used in the laboratory.
- Develop safe work practices for new projects in the laboratory and ensure that they are practiced.
- Reports safety violations and hazards observed to the [person's title].

Safety Committee's responsibilities are as follows:

- The Safety Committee shall conduct periodic safety inspections.
- The Safety Committee shall report any violations of the safety rules to the Laboratory Supervisor.

- The Safety Committee will work with other employees to develop and implement safe work practices.
- The Safety Committee shall ensure that appropriate audits of safety equipment, emergency supplies and first aid supplies are made and that any discrepancies are corrected immediately.
- The Safety Committee shall know current requirements concerning safety regulations.
- Ensures that protective equipment such as gloves, goggles, laboratory coats, respirators, safety shoes, ear protection are properly used. Alerts the Laboratory Supervisor of any infractions.
- Reports any safety violations and hazards observed to the Laboratory Supervisor.
- Make monthly inspections of all equipment such as fire extinguishers, fire blankets, safety showers, for proper operation.
- Check monthly to ensure that the emergency supplies are complete.
- Reviews SDS inventory record at least once each quarter.

Hazardous Waste Coordinator's responsibilities are as follows;

- Maintains records for the types and amounts of hazardous wastes disposed.
- Coordinates the safe disposal of hazardous wastes by a qualified contractor.
- Provides information to employees regarding special disposal procedures.
- Ensures that storage area and containers for hazardous waste meets all OSHA, EPA and DOT regulations.

MEDICAL SERVICES

Any employee who sustains an injury on the job, requiring Emergency Aid, must be taken to the emergency room via ambulance for evaluation and/or treatment.
Any employee who sustains an injury on the job, requiring more than First-Aid yet does not require Emergency Aid, must be taken to the *Established* Health Center:
Address
Phone
Hours

FIRST-AID KITS

First-Aid kits are to be kept updated and are available at the following locations within the Wastewater Treatment Plant: [state locations].

RECORDS

An "Accident/Incident" report should be completed any time someone is injured on the job. This form is used to track accidents, make management aware of the incident, and for appropriate action to follow. This form should be given to your immediate supervisor.

Supervisors will record all work related injuries and illnesses on OSHA Form 200 within six working days after receiving an Accident/Incident report for injuries requiring more than First Aid. These forms should be given to the [person's title] to be forward to the [person's title].

Any occupational injury or illness which is fatal to one or more employee or which results in the hospitalization of five or more employees must be reported by phone (317-232-2693) or written document within 48 hours of the incident to:

IOSHA Area Director, Dept. of Labor
402 W. Washington,
Indianapolis, IN 46204-2751.

Form 200 must be kept current to a date within 45 calendar days. This form will be displayed on the bulletin board in the administration building. The [person's title] will be responsible for recording, reporting, and filing of all OSHA forms. He/She will also be responsible for sending the medical bills to the [state locations such as insurance office, etc.] and personnel file.

PERSONAL PROTECTIVE EQUIPMENT

The laboratory will have a sufficient supply of lab coats, eye protection, and gloves available for use as needed. Laboratory personnel are to wear lab coats while performing analyses in the lab, and wear eye protection when chemicals are handled. Wear appropriate gloves when the potential for contact with toxic chemicals or biological hazards exists. Use any additional protective equipment and/or apparel as required by the Standard Operating Procedure.

CHEMICAL HYGIENE PLAN

INTRODUCTION

The Occupational Safety and Health Administration's (OSHA's) regulation for "Occupational Exposures to Hazardous Chemicals in Laboratories", 29 CFR 1910.1450 *The Standard*, requires that each facility engaged in the laboratory use of hazardous chemicals develop and implement a written program known as a "Chemical Hygiene Plan" which sets forth procedures, equipment, personal protective equipment and safe work practices that will:

- Protect employees from the health hazards presented by hazardous chemicals used in that particular workplace
- Keep employee exposures to hazardous chemicals below the permissible exposure limits (PELs) specified in the standard

All laboratory personnel are responsible for familiarizing themselves with the WWTP's Chemical Hygiene Plan and adhering to it. Emphasis in the Chemical Hygiene Plan is placed first and foremost on protecting the employees.

SAFETY COMMITTEE

The Safety Committee is the overall coordinator of safety programs and requirements for all departments. In addition, the Safety Committee is responsible for organizing, stimulating, and guiding the Employer safety programs, as well as keeping up with changes in safety subject areas to provide knowledge and information, when required, that is current.
The [state department or person responsible] shall designate, in writing, a Safety Officer, a Safety Technician and a Hazardous Waste Coordinator; these names are listed beneath their prospective titles. These designated individuals are automatically members of the Safety Committee

Safety Officer: Person's Name

The Safety Officer has the overall responsibility for optimizing safety training, conducting inspections, safety equipment acquisition and updating safety information. If a problem arises, personnel may seek safety & health guidance from the Safety Officer, or Safety Technician.

The Safety Officer must see to it that protective equipment is available and used appropriately.
The Safety Officer is to make sure that periodic inspections (as specified) of emergency equipment are conducted; equipment such as fire extinguishers, safety showers, fire blanket, eye wash and safety supplies are to be inspected.
Further, the Safety Officer is to perform periodic inspections of the laboratory to uncover overlooked hazards, to ensure that lab personnel are following safety rules, and to remind workers to use safe work practices.
Any violations of the safety rules will be reported immediately to the Safety Officer. The Safety Officer will determine if the violation merits disciplinary action.
Other Responsibilities include:

a. Work with administrators and other employees to develop and implement appropriate chemical hygiene policies and safe work practices.
b. Monitor procurement, use, and disposal of chemicals used in the laboratory.
c. Maintains SDS Library
d. Develop safe work practices for new projects in the laboratory and ensure that they are practiced.
e. Know the current legal requirements concerning regulated substances.
f. Seek ways to improve the lab's Chemical Hygiene Program.

Safety Technician: Person's Name

a. Make monthly inspections of all equipment such as fire extinguishers, fire blankets, safety showers, and eyewash for proper operation. Submit inspection reports to the Safety Officer.
b. Ensure that appropriate audits of safety equipment, emergency supplies, and first aid supplies are made and that any discrepancies are corrected immediately.
c. Checks monthly to ensure that the emergency supplies are complete. Emergency supplies are to include the following:
    1) First aid supplies
    2) Acid and base neutralizer powder
    3) Spill absorbent powder
    4) Mercury spill tamer kit
    5) Fire blanket
    6) Dust pan
d. Report any deficiencies to the Safety Officer immediately.
e. Ensures that protective equipment such as gloves, goggles, laboratory coats or aprons are properly used. Alert the Safety Officer of any infractions.
f. Reports to the Safety Officer if any safety violations or hazards observed.

Hazardous Waste Coordinator: Person's Name

a. Maintains records for the types and amounts of hazardous wastes generated.
b. Coordinates for the safe disposal of hazardous wastes by a qualified contractor.
c. Provides technical information to WWTP employees regarding special disposal procedures.
d. Ensures that employees are wearing the proper personal protective equipment

when disposing of hazardous waste.

e. Ensures that storage containers for hazardous waste meets all OSHA, EPA, and DOT regulations.

Building Coordinator: Person's Name

a. Building Coordinator will work in concert with the Safety Officer to identify safety hazards and correct them.

b. The Safety Officer will request the Building Coordinator to order and arrange installation of safety equipment and correction of safety hazards.

c. The Building Coordinator will report all safety hazards or unsafe work conditions that come to his/her attention to the Safety Officer.

Note: When WWTP safety and health issues need to be addressed and decisions made, the afore mentioned persons will function as the laboratory's safety committee to make recommendations to upper management as required.

DEFINITION OF TERMS

1. ***Carcinogen***- a chemical is considered to be a carcinogen if:
   a. It has been evaluated by the Internal Agency for Research on Cancer (IARC) and found to be a carcinogen or potential carcinogen; or
   b. It is listed as a carcinogen or potential carcinogen in the Annual Report on Carcinogens published by the National Toxicology Program (NTP); or
   c. It is regulated by OSHA as a carcinogen.
2. ***Chemical***- any element, chemical compound or mixture of elements and/or compounds.
3. ***Chemical Exposure Limit List***- list of some common chemicals stored and used within the Cities Central Environmental Laboratory (CEL). The Permissible Exposure Limit (PEL), Threshold Limit Value (TLV), and Immediately Dangerous to Life and Health Limit (IDLHL) are listed where applicable.
4. ***Chemical Manufacturer***- an employer with a workplace where chemical(s) are produced for use or distribution.
5. ***Chemical Name***- is the scientific designations of a chemical in accordance with the nomenclature system developed by the International Union of Pure and Applied Chemistry (IUPAC) or the Chemical Abstracts Service (CAS) rules of nomenclature, or a name which will clearly identify the chemical for the purpose of conducting a hazard evaluation.
6. ***Combustible Liquid-*** any liquid having a flashpoint at or above 100^{o}F (37.8^{o}C), but below 200^{o}F (93.3^{o}C), except any mixture having components with flashpoints of 200^{o}F (93.3^{o}C) or higher, the total volume of which make up 99 percent or more of the total volume of the mixture.
7. ***Common Name***- designation or identification such as a code, name, code number, trade name, brand or generic name used to identify a chemical other than by its chemical name.
8. ***Compressed Gas***- defined as
   a. A gas or mixture of gases having, in a container, an absolute pressure exceeding 40 psi at 70^{o}F (21.1^{o}C); or
   b. A gas or mixture of gases having, in a container, an absolute pressure

exceeding 104 psi at 130°F (54.4°C), regardless of the pressure at 70°F (21.1°C); or

c. A liquid having a vapor pressure exceeding 40 psi at 100°F (37.8°C) as determined by ASTM Method D-323-72.

9. ***Corrosive***- a chemical that causes visible destruction of, or irreversible alterations in, living tissue by chemical action at the site of contact.
10. ***Explosive***- a chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.
11. ***Exposure***- occurs when an employee, in the course of employment, is subjected to a hazardous chemical through any route of entry (inhalation, ingestion, skin contact), and includes potential for exposure.
12. ***Flammable***- describes a chemical that falls into one of the following categories:
    a. Aerosol, flammable-an aerosol that, when tested by the method described in 16 CFR 1500.45, yields a flame projection exceeding 18 inches at full valve opening, or a flashback (a flame extending back to the valve) at any degree of valve opening.
    b. Gas, flammable-1) a gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of thirteen percent by volume or less; or 2) a gas that, at ambient temperature and pressure, forms a range of flammable mixtures with air more than twelve percent of volume, regardless of the lower limit.
    c. Liquid, flammable-any liquid having a flashpoint below 100°F (37.8°C), except any mixture having components with flashpoints of 100°F (37.8°C) or higher, the total of which make up 99 percent of the total volume of the mixture.
    d. Solid, flammable-a solid, other than a blasting agent or explosive as defined in Section 1910.109(a), that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard. A chemical shall be considered to be a flammable solid if, when tested by the method described in 16 CFR 1500.44, it ignites and burns with a self-sustained flame at a rate greater than one-tenth of an inch per second along its major axis.
13. ***Flashpoint***- the minimum temperature at which a liquid gives off a vapor in sufficient concentration to ignite.
14. ***Foreseeable emergency-*** any potential occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment which could result in an uncontrolled release of a hazardous chemical into the workplace.
15. ***Hazard warning-*** any symbols, pictures, or words or combination thereof appearing on a label or other appropriate form of warning which convey the hazards of the chemical(s) in the container(s).
16. ***Hazardous chemical***- any chemical which is a physical or health hazard
17. ***Health hazard***- a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. The term “health hazard" includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopoietic system, and agents which damage the lungs, skin, eyes, or mucous membrane.

18. ***Irritant***- a chemical, which is not corrosive, but which causes a reversible inflammatory effect on living tissue by chemical action at the site of contact.
19. ***Label***- any written, printed, or graphic material displayed on or affixed to containers of hazardous chemicals.
20. ***Laboratory***- a facility where the laboratory use of hazardous chemicals occurs. It is a workplace where relatively small quantities of hazardous chemicals are used on a non-production basis.
21. ***Laboratory scale***- is work with substances in which the containers used for reactions, transfers, and other handling of substances are designed to be easily and safely manipulated by one person. Laboratory scale excludes those workplaces whose function is to produce commercial quantities of materials.
22. ***Laboratory-type hood (Enclosed by-pass fume hood)***- a device located in a laboratory that is an enclosure on five sides with a moveable sash or fixed partial enclosure on the remaining side; constructed and maintained to draw air from the laboratory; and allows chemical manipulations to be conducted in the enclosure without insertion of any portion of the employee's body other than hands and arms.
23. ***Laboratory use***- means the handling or use of hazardous chemicals where all the following conditions are met:
    B. Chemical manipulations are performed on a laboratory scale.
    C. Multiple chemical procedures or chemicals are used.
    D. Procedures are not part of or simulate a production process.
    E. Protective laboratory practices and equipment are available and used commonly.
24. ***Safety Data Sheet (SDS)*** - is written or printed material concerning a hazardous chemical which is prepared in accordance with approved regulations.
25. ***Mixture***- any combination of two or more chemicals if the combination is not, in whole or part, the result of a chemical reaction.
26. ***Oxidizer***- a chemical other than a blasting agent or explosive that initiates or promotes combustion in other materials thereby causing fire either by itself or through the release of oxygen or other gases.
27. ***Permissible Exposure Limit***- means the maximum time-weighted average (TWA) concentration of a substance in air that an employee can be exposed to legally during an 8 hour shift.
28. ***Physical Hazard***- a chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable (reactive) or water-reactive.
29. ***Pyrophoric***- a chemical that will ignite spontaneously in air at a temperature of 130^{o}F (54.4^{o}C) or below.
30. ***Reproductive toxins***- chemicals which affect the reproductive capabilities including chromosomal damage (mutations) and effects on fetuses (teratogenesis).
31. ***Threshold Limit Value (TLV)***- a term used by the American Conference of Governmental Industrial Hygienists to express the airborne concentration of a material to which nearly all workers can be exposed day after day without adverse effects.
32. ***Unstable (reactive)***- a chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shock, pressure or temperature.
33. ***Water-reactive***- a chemical that reacts with water to release a substance that is either

flammable or presents a health hazard.

34. ***Work area***- a room or defined space in a workplace where hazardous chemicals are produced or used, and where employees are present.

CHEMICAL INVENTORY

Chemical inventory, or the SDS Library, is available in the [state location].
Although it is not stated that all chemicals are hazardous, all chemicals must be handled with CAUTION. Information regarding the potential hazard of a chemical can be found in the Safety Data Sheet (SDS) for the chemical. Other chemical information sources such as the *Merck Index* and the *Pocket Guide to Chemical Hazards* must also be consulted if other information is required. Consult the Safety Officer if information concerning a chemical cannot be found.

CHEMICAL HYGIENE AND SAFETY PLAN

General Rules

1. *Working Alone*

   Working alone in the laboratory will not be permitted when dealing with hazardous and corrosive chemicals or when the work involves a potentially dangerous procedure. Some areas of work that must be avoided when working alone are:

   a. Analysis involving highly flammable gas such as acetylene, nitrous oxide, methane or propane gas.
   b. Analysis involving the possible poisoning from toxic gas such as the analysis of cyanide or chlorine standard preparation involving bottled chlorine gas.
   c. Preparation of acid or base solutions which involve large amounts of the concentrated solution.

2. *Personal Apparel*

   a. Shoes must be worn at all times in the laboratory. Sandals, perforated shoes or open- toed shoes will not be worn in the laboratory.
   b. Shorts or torn clothing will not be allowed in laboratories. Use of full-length long sleeve lab coat is mandatory.

3. *Personal Housekeeping*

   All work areas must be kept clean and uncluttered with chemicals and equipment properly labeled and stored. The work area must be cleaned upon completion of a test or at the end of each working day.

4. *Horseplay*

   Avoid practical jokes or other behavior which might confuse, startle, or distract other workers especially in the presence of hazardous chemicals or when a potentially dangerous procedure is involved.

5. *Eating/Drinking/Smoking/Applying Cosmetics*

   Eating and applying cosmetics in areas where potentially hazardous chemicals are present will not be allowed. Eating, drinking and applying cosmetics in areas where chemicals are present is a potentially hazardous situation because of the possibility of contaminating the food, drink, or cosmetics with unknown substances. Eating should be restricted to designated break rooms and administrative areas. Drinking will be allowed on a limited basis within the laboratory. Employees may drink soda or coffee at desk areas, as long as hazardous chemicals are not present and/or not handled. Further, employees must use a closed container such as a soda can or covered cup with straw when drinking within the

laboratory. However, food, drink and cosmetics are not allowed on laboratory bench tops. Application of cosmetics is allowed in the administrative area and the restrooms. Smoking in any part of the laboratory (including stockrooms) is strictly prohibited.

6. *Unattended Operations*

   There will not be any unattended procedures involving hazardous substances or dangerous steps taken without prior approval from the Safety Officer. Appropriate warning signs outside areas where hazardous procedures are in progress must be clearly posted. All utility devices such as cooling water (secure connections), gases, etc., must be checked and secured (i.e., compressed gas cylinders 150 pounds and under are affixed to wall/ floor or bench).

7. *Glassware*

   Dispose of all broken or cracked glassware in proper broken glass disposal receptacles immediately. Chipped glassware may still be used if it can be polished to eliminate the sharp edges. Use caution when making rubber-to-glass connections. Support lengths of glass tubing while they are being inserted into rubber. Polish ends of glass tubing until smooth. Use a lubricant such as Water; NEVER USE GREASE OR OIL. Use gloves when making such connections and hold tubing as close as possible to the end being inserted to prevent bending and breaking. Never try to force rubber tubing or stoppers from glassware; cut rubber as necessary to remove.

8. *Disposal of Chemicals*

   No chemical or substance (except water or wastewater between pH 2 and 12.5) should ever be disposed of down the sink drain without first consulting the Hazardous Waste Coordinator or Safety Officer and reading the SDS.

Laboratory Standard Operating Procedures (SOPs)

Every SOP in the laboratory requires precautionary measures that must be taken in order to execute each procedure safely. The analyst must read and understand the procedure thoroughly (especially procedures involving toxic substances) before proceeding. Any questions regarding the safe execution of the procedure must be addressed before attempting to execute the procedure. Adherence to all safety work practices outlined below is a must to assure safety in the laboratory.

Use of Protective Equipment and Apparel

1. *Hoods and Ventilation*

   a. Any operation that might result in the release of toxic chemical vapor or dust must be performed under a properly functioning hood. Use a hood or other form of local ventilation when working with any appreciably volatile substances.
   b. Confirm adequate hood performance before each use.
   c. The hood should not be used for chemical storage. Acid, caustic, and flammable cabinets should be used as much as possible for storage.
   d. When a hood is not in use, keep the sash in the down position.
      The hood should be professionally certified yearly. Typically, a hood face velocity of 60-120 linear feet per minute is adequate. Also check to see if the hood inspection sticker is current. Do not use a malfunctioning hood and report any deficiency immediately to the Safety Officer or a Supervisor.

2. *Eye Protection*

The objective of the laboratory's Eye Protection Policy is to protect the eyes of WWTP employees and visitors. Moreover, the policy pertains to all employees, vender representatives (demonstrating, modifying, or repairing analytical or analytically-related equipment in the WWTP), and all personnel who closely observe analytical activities.
All personnel in the WWTP must wear WWTP-approved protective eyewear whenever they are involved in the following activities:

a. Any aspect of analyses except data manipulations
b. Labware preparation
c. Solution and sample preparation
d. Sample handling
e. Any close observation of an analytical process that requires protective eyewear

There are two general categories of lab situations in which different levels of eye protection are required. These categories are as follows:

General Protection

This is characterized by the involvement in analyses or support activities that require the use of materials, samples, or solutions that are not known to result in bodily damage upon contact but could be harmful if eye contact was made. These materials are further characterized as having a pH > 2.0 and < 12.0, and a normality < 2.0.
The following types of eyewear are approved for use according to the General Protection category:

a. Prescription glasses with shatter-proof lenses, equipped with side shields. The side shields will be provided by the WWTP.
b. General safety glasses that comply with the safety standard ANSI Z87.1- 1989. These are supplied by the WWTP.
c. General purpose face shields. These are also provided by the WWTP.

Enhanced Protection

This is a situation in which the analysis or support activity involves the use of a distinctly hazardous material. These materials are characterized with a pH < 2.0 or > 12.0, and/or a normality > 2.0. This level of protection is also required when the nature of materials is unknown.
The following types of eyewear are approved for use according to the Enhanced Protection category:

a. Safety goggles, chemical splash and impact resistant, indirectly vented; in compliance with safety standard ANSI Z87.l-1989. These are supplied by the WWTP.

When contact lenses are to be worn in the laboratory, protective chemical goggles providing protection from flying objects and chemical splash must be worn at all times when performing any type of analytical procedure, with the exception of data manipulations. Any WWTP personnel wearing contact lenses, and engaged in any aspect of analysis, must notify their supervisor of this vision correction method prior to analytical activity.
Contact lenses do not provide protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Corrosive chemicals trapped in this space cannot be washed off the surface of the cornea. If the

chemical in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible, to remove the lens.

Certain analytical areas and functions are currently considered not hazardous to eyes. An attempt will be made to accommodate these areas and associated personnel by exempting them from compliance with this policy as long as no obvious eye hazard exists. These areas include the office areas.

The success of the exemptions listed above depends on personnel and their ability to routinely remember to wear eye protection in the other areas and for other functions that require the protection.

3. Body Protection

Body protecting garments such as long length, long sleeved laboratory coats, must be worn at all times when performing any type of analytical procedure in the laboratory. Laboratory aprons can be worn under unusual circumstances, for example when the air conditioning is out of service. Laboratory aprons can also be worn over laboratory coats for added protection. Body protection will protect the body and clothing from chemical spills such as acids and bases.

4. Hand Protection

The danger of contact with corrosive or toxic chemicals must be minimized using protective rubber gloves. The following are guidelines to follow when selecting gloves:

Butyl Rubber (Long Length) Gloves

These gloves are heavy duty, usually green or black, have approximately a 22 mL thickness, and are typically 18-32 inches long. These gloves are used for working in acid baths because protection is provided to the upper arms and the gloves are acid resistant. Due to the thickness of the glove, better protection against rips and tears which may be caused by broken glass is provided.

Note: These gloves can be used when working in *any* acid bath at *any* concentration. Further, these are the only gloves acceptable for working in an acid bath.

Neoprene Rubber (Short Length) Gloves

These gloves are shorter than the long length gloves and are, henceforth, not acceptable for use in acid baths. Use Neoprene Rubber Gloves for the following:

a. Working with *any* acid *not* in an acid bath
b. Working with any alkalines not in an alkaline bath
c. Washing or rinsing of any glassware
d. Loading and unloading the glassware washers
e. Handling any soaps

Common Use Nitrile Gloves

Nitrile gloves may come in a variety of colors. This type glove is only 4-9 mL thick and only about 9.5 inches long. This type of glove is also acid permeable. Due to thinness, lack of arm protection, and acid permeability, these gloves would not protect the hand or arm against broken glass or chemical spills. These gloves are intended to be used when handling biological samples, such as Raw wastewater or Biosolids. *These gloves are not acceptable for use in the glassware section of the WWTP.*

Thermal Insulated Gloves

Gloves providing thermal insulation must be used at all times when transferring objects in

and out of an oven or furnace. The type of glove worn should be appropriate to the analysis being per-formed; when in doubt, consult the Safety Officer.

Chemical Storage and Handling

Many chemical substances used in a laboratory are toxic to humans when ingested or inhaled. Precautions must be taken with these substances to keep them out of the mouth, nose, eyes, and the skin. It is important to wear protective clothing, gloves, and safety glasses when handling toxic or corrosive chemicals. The following are specific laboratory rules and precautions that must be followed:

Chemical Storage

a. Never place beakers or unstopped (uncapped) vessels containing chemicals in a refrigerator. Volatile-containing samples should be stored in a special refrigerator designed to accommodate such materials, where available. These are explosion-proof refrigerators especially suited to this application.
b. Label all chemical containers correctly and clearly. The following information must be included in the chemical label:
    1. Date the chemical was prepared with initial of analyst who prepared the chemical.
    2. Date the chemical was received.
    3. Date the chemical was opened and expiration date.
    4. Appropriate hazard warning such as corrosive, flammable, carcinogen, or oxidizer (where applicable).
c. Store acids and bases separately in well ventilated areas and away from volatile organics and oxidizable materials. Do not store strong acids or bases on high shelves.
d. Store large reagent containers in the vented cabinets under the hoods. Ensure that flammable materials are stored in the flammable cabinets. Make sure that all containers used in the preparation of reagents or containers used to hold small amounts of reagents for short periods of time are clearly labeled.

Chemical Handling

a. Use fume hoods for all operations involving poisonous or offensive gases or fumes, as well as for operations involving highly flammable or potentially explosive materials.
b. Never heat an organic solvent in an open vessel over an open flame and keep open containers of organic solvents away from open flames or sources of sparks.
c. Avoid pointing the mouth of a vessel being heated towards any person, including yourself.
d. Never add anything to a concentrated acid, base, or strong oxidant; instead, add the acid, base or oxidant slowly and cautiously to the other ingredients, preferably no faster than it is consumed by reaction.
e. Never add solids (boiling chips, charcoal, etc.) to a hot liquid as the result may be violent boiling if the liquid happens to be superheated. Perform such additions when the liquid is still at room temperature.
f. Never pipette anything by mouth. Use an automatic pipettor or a conventional pipette with a rubber bulb.
g. Never pour anything back into a reagent bottle. Consult the Hazardous Waste Coordinator for the proper disposal of unused or contaminated chemical.
h. Prevent accidents such as splashes and container spills. Use a funnel if pouring strong

acids, bases or oxidants into a narrow-mouth vessel.

i. Wipe up small spills and bottle rings immediately. A large spill should be handled as indicated in Section 6 "Chemical Spills".

Note: Other chemicals, due to their high toxicity, must be handled with caution. For information regarding the hazard that the chemical might pose, consult the Safety Data Sheet (SDS) or other chemical reference materials discussed in this program. All necessary precautions outlined, such as the use of protective goggles, gloves, body protection, and fume hood must be followed when handling the chemical. Consult the Safety Officer if further assistance is needed. Some examples of these chemicals are:

1. Concentrated acids (hydrochloric, sulfuric, nitric, chromic, phosphoric, glacial acetic)
2. Concentrated bases (sodium, potassium, ammonium hydroxides)
3. Cyanide salts and phenols
4. Mercury and mercuric salts
5. Heavy metal compounds (Ba, Be, Pb, Cu, Ag, Zn, Cd, Cr, Ni, As, and others)
6. Oxidizing salts ($AgNO_3$, chromates) (potassium dichromates)

<u>Microbiological Hazards</u>

Potential dangers associated with this type of hazard involve hand-mouth contact while handling contaminated samples or laboratory equipment. Some safety rules that must be used are as follows:

a. Always use protective gloves when handling microbiological standards and samples.
b. Never pipette anything by mouth. Use an automatic pipettor or a conventional pipette with a rubber bulb.
c. Keep contaminated items (cultures, serological discards, etc.) in a separate waste container before sterilizing. Do <u>*not*</u> throw these in with the routine trash.
d. Sterilize or disinfect contaminated materials (cultures, used glassware, serological discards, etc.) by autoclaving before throwing them away or processing for reuse. Use a germicidal soap and hot water, or an autoclave, as appropriate for the type of analysis involved. All cell culture glassware is to be autoclaved.
e. Quickly clean up any spillage. Disinfect the area with bactericide, alcohol, or other type of disinfectant.
f. Frequently disinfect working surfaces with bactericide or germicide. Disinfect hands with dilute germicidal soap.

Chemical Spills

Small chemical spills and leaks must be cleaned up immediately to prevent a potential hazardous situation. Examples of Spill Tamer Kits that should be provided in the WWTP are: a Mercon Mercury Spill Kit and EM Science Caustic, Solvent, Acid Spill Kits. The kits are located [state location] and contain the following items:

EM Science Kit

1. Neutralizer solution for acids
2. Neutralizer solution for solvents
3. Neutralizer solution for caustics
4. Scraper
5. Bag

Mercon Kit

6. Mercury Spill Tamer Absorbent Solution – Mercon Vap
7. Air Spray
8. Wipes
9. Mercury Collector Container

Chemical Spill Control

Spill Control Measures for Acid or Base Solutions:

a. Concentrated acid spills should be cleaned up as follows: wearing gloves and goggles, add dry acid neutralizer to the liquid spill in excess. Cover all the affected areas repeatedly until all liquid is absorbed. Sweep/shovel the neutralized waste into a trash container. Wash down the cleaned area with copious amounts of water. Neutralize further if necessary, using sodium bicarbonate (baking soda), or commercial neutralizer. Sweep the neutralized wastewater from the spill into the floor drain or use absorbent toweling. It is important to neutralize the acid (or base) rather than trying to relocate in prior to clean-up.

b. Solutions should be contained and absorbed using the Spill Tamer Absorbent from the Spill-X Kit.

c. Add dry neutralizer for acids or neutralizer for alkalis until all reaction stops.

d. Sweep up the absorbent and dispose of properly. Consult the Safety Officer or Assistant Safety Officer for proper disposal procedure.

e. Rinse the spill area thoroughly with water.

f. Spills adjacent to a sink which can be washed into the sink safely should be handled in this manner. The area can be neutralized using cold water and dry neutralizer for acids or neutralizer for alkali's, or pH 6.9 solution.

Dry Alkali Spills

a. Dry, solids material should be swept together, using the dustpan and brush from the Spill-X Kit.

b. Dissolve the alkali in a beaker of water.

c. Flush the alkaline solution into the sink drain with generous amounts of cold water.

d. Rinse the spill area thoroughly with water, pH 6.9 solution, then water.

Mercury Spill

Any spill of mercury from a broken thermometer, manometer, etc., can create a serious toxicity problem if cleanup is not handled properly. The mercury will continue to vaporize causing a chronic exposure situation. Mercury poisoning can occur from chronic exposures as well as from a single exposure to large quantities of the metal.

a) Notify other lab personnel of the spill.
b) Turn off all air-circulating equipment: fume hood, vent fan, HVAC
c) Wearing rubber gloves use the Mercury Spill Kit located [state location].
d) Spray the air around the area with MerconSpray to eliminate mercury vapors
e) Saturate the spill area with MerconVap
f) Wipe up the mixture with MerconWipes, including all tools, cracks, clothes, shoes, etc.
g) Dispose the wipes in the MerconTainer and seal tightly
h) Take the container to a Hazardous Waste facility immediately

First Aid

First Aid Kits

First aid kits for minor injuries are located in the following areas in the WWTP:

- First Aid box in the laboratory is located [state location].
- First Aid box for the operators is located [state location].
- First Aid box for maintenance personnel is located [state location].
- First Aid box for collections personnel is located [state location].

Burn Emergency Kits

Burn Emergency Kits for minor burns are located in the following areas in the WWTP:

- First Aid box in the laboratory is located [state location].
- First Aid box for the operators is located [state location].
- First Aid box for maintenance personnel is located [state location].

Emergency Procedure for Chemical Burns

a. Flush the affected area with copious amounts of water using the emergency shower as needed for at least five minutes. Depending on the situation, get medical care immediately.
b. If first aid directions for burns caused by specific chemicals are available, follow these directions, after the initial flushing with water. First aid directions can usually be found by referring to the Safety Data Sheet.
c. ***Do not*** use oils, fats, salves, or ointments which could assist skin absorption.

Chemical Poisoning

Chemical poisoning can result from inhalation, ingestion or absorptions through the skin of a toxic chemical. If you suspect that you or any of your fellow employees have been exposed to any type of chemical poisoning, get help immediately. Proceed as follows:

Determine the source of the poison. It is possible that the whole area might be contaminated with poisonous gas or chemical. If it is determined that the whole area is contaminated, notify all other personnel and have the area evacuated. Secure the contaminated area. Call the poison control center and describe the situation. Do not attempt to enter the area unless you are wearing the proper personal protective equipment and breathing apparatus.

Ingestion of Chemical Solutions or Poisons:

a. Immediately notify another lab technician of the accident and type of material ingested. Obtain medical attention immediately.
b. Consult the SDS and the poison control center for immediate treatment until proper medical care can be obtained.
c. Do not induce vomiting if the solution swallowed was a strong base (alkali), strong acid, cyanide, strychnine, gasoline, kerosene, any hydrocarbon, or if the victim is having convulsions. Do not give liquids to an unconscious person.
d. For strong acids or bases, give the victim a glass of milk or large amounts of water unless the SDS designates otherwise.
e. Do not give oils, fats, or alcohol unless advised by a physician.
f. Prevent shock by keeping the victim warm.

SAFETY DATA SHEETS (SDS)

Any chemical manufacturer, distributor, or importer must provide Safety Data Sheets (SDSs) for hazardous chemicals to downstream users. The SDS includes information such as the properties of each chemical; the physical, health, and environmental health hazards; protective measures; and safety precautions for handling, storing, and transporting the chemical. The SDSs are required to be presented in a consistent, user-friendly, 16-section format. The SDS must be consistent with the UN Globally Harmonized System of Classification and Labeling of Chemicals (GHS), but OSHA will not enforce the content of these sections because they concern matters handled by other agencies. A description of all 16 sections of the SDS, along with their contents, is presented below:

Section 1 (Identification): This section identifies the chemical on the SDS as well as the recommended uses. It also provides the essential contact information of the supplier.

Section 2 (Hazard Identification): This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

Section 3 (Composition): This section identifies the ingredient(s) contained in the product indicated on the SDS, including impurities and stabilizing additives. This section includes information on substances, mixtures, and all chemicals where a trade secret is claimed.

Section 4 (First Aid Measures): This section describes the initial care that should be given by untrained responders to an individual who has been exposed to the chemical.

Section 5 (Fire Fighting Measures): This section provides recommendations for fighting a fire caused by the chemical.

Section 6 (Accidental Release Measures): This section provides recommendations on the appropriate response to spills, leaks, or releases, including containment and cleanup practices to prevent or minimize exposure to people, properties, or the environment. It may also include recommendations distinguishing between responses for large and small spills where the spill volume has a significant impact on the hazard.

Section 7 (Handling and Storage): This section provides guidance on the safe handling practices and conditions for safe storage of chemicals.

Section 8 Exposure Controls/Personal Protection): This section indicates the exposure limits, engineering controls, and personal protective measures that can be used to minimize worker exposure.

Section 9 Physical and Chemical Properties): This section identifies physical and chemical properties associated with the substance or mixture.

Section 10 (Stability and Reactivity): This section describes the reactivity hazards of the chemical and the chemical stability information. This section is broken into three parts: reactivity, chemical stability, and other.

Section 11 (Toxicological Information): This section identifies toxicological and health effects information or indicates that such data are not available.

Section 12 (Ecological information): This section provides information to evaluate

the environmental impact of the chemical(s) if it were released to the environment.

Section 13 (Disposal Considerations): This section provides guidance on proper disposal practices, recycling or reclamation of the chemical(s) or its container, and safe handling practices. To minimize exposure, this section should also refer the reader to Section 8 (Exposure Controls/Personal Protection) of the SDS.

Section 14 (Transport Information): This section provides guidance on classification information for shipping and transporting of hazardous chemical(s) by road, air, rail, or sea.

Section 15 (Regulatory Information): This section identifies the safety, health, and environmental regulations specific for the product that is not indicated anywhere else on the SDS.

Section 16 (Other Information): This section indicates when the SDS was prepared or when the last known revision was made. The SDS may also state where the changes have been made to the previous version. You may wish to contact the supplier for an explanation of the changes. Other useful information also may be included here.

Safety Data Sheets for the WWTP are located [state location].

The SDS library is maintained by the SDS Coordinator/Safety Officer in [state location]. Employee supervisors are responsible for ensuring that all applicable SDSs are available at all times for chemicals at the respective work centers and that chemicals are not used unless this information is available.
All chemicals used in the laboratory facility are purchased chemicals and all SDSs are supplied by the chemical manufacturer. If the information on the SDS is not complete or additional information is required, the Safety Officer must be notified before any further use of the chemical. The Safety Officer will then contact the chemical manufacturer and request the missing information.

CONTAINER LABELING

Chemical container labeling is the primary initial source of warning for employees. This requirement is considered one of the most important sections of this program in ensuring the overall effectiveness of the Chemical Hygiene Plan.
As discussed previously, chemicals used in the WWTP are purchased chemicals. Thus, proper labeling of all these chemicals is the responsibility of the chemical manufacturer. Any chemical delivered to the WWTP will be inspected upon receipt for proper labeling and completeness of information (SDS included). If any chemical is not properly labeled and not accompanied by SDS, the delivery will be rejected or sent back to source of delivery.
The Safety Officer or Safety Technician will oversee this chemical delivery process. WWTP personnel receiving chemicals need to ensure that the following information appears on all incoming chemicals:

A. the identity of the chemical
B. appropriate hazard warning
C. the name of the chemical manufacturer
D. the chemical label in good condition and readable
E. the chemical container in good condition

F. shipping papers in order

If any discrepancy is found on the chemical labeling, packaging or shipping papers, WWTP personnel should not accept the delivery.

Secondary containers, or containers that hold a smaller portion of a stock-chemical (i.e., a small gas can filled from a large cylinder or a 500 mL titration bottle filled from a 1-gallon sulfuric acid bottle) must be labeled with that same chemical hazard information as its stock solution.

All secondary containers stored in the WWTP work area or counter-top MUST be clearly labeled with the applicable information and appropriate hazard warning. If a reagent contains several hazardous materials, the name of the reagent must be clearly labeled on the container. The WWTP Safety Officer or Safety Technician will be responsible for ensuring that this procedure is followed.

HAZARDOUS MATERIAL AWARENESS, HANDLING AND DISPOSAL

Administration

The aim of appropriate handling and proper disposal of hazardous materials is to assure minimal harm to people, as well as to the environment. Hazardous waste abatement minimizes the potential for exposure to terrestrial and aquatic life, and it minimizes the cost of clean-up and disposal activities to the City. Awareness is one of the key factors in minimizing exposure to hazardous chemicals. Therefore, all WWTP lab personnel are required to attend Hazardous Material Safety Training. The Hazardous Waste Coordinator is responsible to insure the safe disposal of wastes by qualified hazardous waste disposal professionals.

Identification of the Waste

Each employee must be familiar with the variety of hazardous materials which they might come into contact with in the WWTP. For every analytical procedure, one or maybe all the chemicals involved might be hazardous. Any chemical (even if it poses minimum threat or harm), when not handled safely or correctly, can cause severe health damage and/or death. When performing any analytical test, the following procedures must be observed:

1) Familiarize yourself with all the chemicals involved.
2) If information is needed concerning the nature of the chemicals, the employee must consult the SAFETY DATA SHEET (SDS) or other available reference manual.
3) Consult the Safety Officer of Hazardous Waste Coordinator if necessary.
4) Follow the proper use of protective equipment described in Section V of this program.
5) If the chemical requires special disposal procedures, consult the Hazardous Waste Coordinator.
6) Maintain familiarity with the chemicals as the test may be repeated, or for future reference.

Minimizing Hazardous Waste

Minimizing the potential for exposure of humans to hazardous wastes is accomplished by minimizing the wastes.

The volumes of hazardous wastes generated are dependent on the type and number of analyses. Due to these variables, the rate at which waste will accumulate fluctuates. All hazardous waste is sent to the Household Hazardous Waste Program. Here there is a log book of all hazardous waste.

The Hazardous Waste Coordinator will be in charge of maintaining all hazardous waste log books. The Hazardous Waste Log Book will be kept in the hazardous waste storage area.

Handling and Disposal

Handling

Proper handling of chemical waste generated in each lab section will be the responsibility of the analyst at the time the waste is generated.

Disposal

Any waste deemed Hazardous needs to be taken to the Household Hazardous Waste site. Care must be taken by the employee to use common laboratory safety procedures when handling hazardous materials. Always be prepared for emergency spills or splattering. Wear appropriate protective equipment. Moreover, when handling chemicals wear the following safety equipment:

A. goggles
B. lab coat
C. gloves
D. half-mask respirator with appropriate cartridges

Waste will be moved from the general laboratory work area at regular intervals to the Household Hazardous Waste site to minimize volumes of hazardous materials accumulating in the laboratory.

The following is a list of hazardous wastes that could be generated by the WWTP which will be subject to special disposal procedures:

A. phenol
B. mercury and compounds-
C. highly concentrated acids and bases
D. highly concentrated metal compounds

FUME HOOD MANAGEMENT

General Information

Fume hoods and other associated protective equipment should be maintained in satisfactory operating condition at all times. Monitoring of performance and any scheduled preventive maintenance should be done in accordance with the manufacturer's suggested guidance. The OSHA Laboratory Standard states that fume hoods need to have a hood velocity of 60 to 100 linear feet per minute to be considered as adequate.

In order to ensure that lab fume hoods continue to meet this standard the Safety Technician arranges yearly professional fume hood inspections. Moreover, during each inspection, fume hood velocity is checked. For each hood inspected, an individual inspection sheet is used and all entries resulting from the inspection must be made here. Hoods passing inspection receive an approval sticker.

Hoods should also be closed at all times except when work is occurring in them or adjustments need to be made. To prevent restriction of air flow, equipment used in the hood should be kept to a minimum and should not block vents or air flow. If at any time an employee feels that the fume hood is not functioning properly, he/she should notify the Safety Officer or the Safety Technician at once.

EMPLOYEE TRAINING AND INFORMATION-HAZARD AWARENESS

General Information

It will be the responsibility of the Safety Officer to inform employees of the hazards that they may exposed to in the workplace. The primary goal of the Chemical Hygiene Plan is to protect employees from hazards through an extensive training program which will provide the employee the necessary tools required to minimize the risk of exposure in the laboratory.

Training and Information Program

In order for employees to be properly informed about hazardous materials in the workplace or specific work area, a training and information program will be conducted on a quarterly basis. Discussion will include the requirements of the Chemical Hygiene Plan and specific problem areas (if any) as well as suggestions from all employees to improve the safety program of the laboratory. The Safety Officer will coordinate safety (training) meetings for the laboratory.

Key Provisions of Training

1. The requirements of the Chemical Hygiene Plan, its importance and primary goals shall be transmitted to all employees.
2. Information on operations of all work areas where an employee may be exposed to hazardous materials will be discussed in detail. Proper handling and precautionary measures that must be taken to abate the hazard shall be emphasized.
3. The employees shall be informed of the location of the hazard communication materials such as the Safety Data Sheets, Hazard Communication Book, other safety references and emergency safety supplies.
4. Location and lists of hazardous materials that the WWTP generates shall be made available to all employees.
5. Methods and observations to detect the presence of a hazardous material.
6. Use of proper protective equipment.
7. In the event that a new hazardous material is introduced to the workplace, information about the material shall be immediately transmitted to all employees who will be working with that material.
8. Ensure that all lab employees are given training on emergency and evacuation procedures.

New Employee Training

All new WWTP employees shall attend General Safety Orientation Training given by the Safety Committee every three months. While waiting for New Employee Safety Orientation training, all new employees will undergo fundamental safety training which will allow them to start work in the WWTP. The new employee shall be indoctrinated on OSHA's Hazard Communication and its Laboratory Standards. Each employee will be informed of his or her role and responsibilities within the program. The employee will be required to sign a form acknowledging that he or she has been informed of the existence of the City’s Chemical Hygiene Plan. A record of the employee's acknowledgement shall be kept in the laboratory's training file, maintained by the Safety Committee.

General Safety Training for All WWTP Employees

All WWTP employees shall be trained on the following safety procedures on a continuing basis.

1. Emergency evacuation
2. Use of fume hood
3. Use of Personal Protective Equipment
4. Use of safety eyewash/shower
5. Use of fire blanket
6. Emergency response procedures for acid or caustic spills, inhalation or ingestion of poison, medical emergencies, and fire or explosion
7. Use of different types of fire extinguishers
8. Use of the SDS and other safety reference materials

Safety Supplies/Equipment

Laboratory safety supplies and equipment such as protective gloves, goggles and aprons or protective apparel, fire extinguishers, emergency eyewash and showers, fire blanket, spill tamer kit, and first aid kit shall be inspected *EACH MONTH* by the Safety Technician for completeness and proper operation.

The Safety Technician will be responsible for keeping safety supplies and equipment in stock and functioning properly.

ALL SAFETY TRAINING MUST BE DOCUMENTED!

EMPLOYEE EXPOSURE EVALUATION AND MEDICAL CONSULTATION

This section of the program addresses the need for performing a formal evaluation of a suspected employee exposure to a hazardous chemical in the laboratory.

Contributing factors for exposure to hazardous chemicals such as failure to use proper protective apparel and equipment or failure to follow outlined safe work practices will be evaluated. Any deficiency found shall be corrected immediately to avoid the same problem in the future.

Criteria for Reasonable Suspicion of Exposure

1. The employee had direct skin or eye contact with the chemical.
2. Manifestation of health hazard symptoms such as headache, rash, nausea, irritation or nose or throat, coughing, dizziness, tearing, irritation or redness of the eyes, etc.
3. Some or all the symptoms disappear when the employee is away from the work area and into fresh air.
4. Symptoms reappear as soon as the employee returns to the work area.
5. Complaints are received from more than one employee.
6. An unusual odor or smell is noticed in the work area.

Exposure Evaluation

The following steps shall be taken to determine the employee's exposure and necessary medical attention needed.

Note: If it is necessary for the employee to receive immediate medical attention, appropriate action shall be taken before the following steps are taken.

1. Interview the employee or the victim initiating the complaint.
2. Gathering of essential information regarding the complaint such as (1) suspected chemical, (2) chemicals present in the work area, (3) symptoms, (4) were control

measures such as protective apparel and equipment used; (5) duration of exposure, route of exposure, concentration of the substance in its container; (6) area of the room where exposure occurred; (7) severity and type of symptoms experienced; and (8) are all important factors properly recorded.

3. In cases where there is concern about over exposure to a chemical or chemicals, personal air sampling/environmental air sampling shall be performed by the Safety Division in order to determine the level of exposure.
4. The decision must be made as to the need to send the employee to medical consultation.

A memorandum documenting the complaint and outcome of the investigation regarding the complaint shall be written.

Medical Consultation

Employees known to have been overexposed to hazardous chemicals shall receive prompt medical attention. The employee will be sent to [location, including address and phone number]. In the event of an emergency, the employee will be taken to the nearest emergency room via ambulance.

Additionally, a first report of accident must be completed by the employee's supervisor and a copy must be sent to the Human Resource and the Safety Committee. The employee will be notified of the outcome of the medical examination.

Exposure/Medical Records

Records of (1) employee exposure to hazardous chemicals, (2) investigation conducted and outcome, (3) medical attention required and (4) results shall be kept on file and made available to affected employees upon their request.

TRAINING RECORDS/DOCUMENTATION

Employee safety training efforts shall be documented in compliance with the Hazard Communication and Laboratory Standards information and training requirements. The documentation shall include the following information:

1. List of employees who attend the training
2. Type of training received
3. Person/agency who provided the training
4. Date
5. Employee acknowledgement

All employee safety training records shall be kept in a binder titled "Safety Training."

ACCIDENT REPORTING PROCEDURE

1. All accidents and injuries are to be reported immediately.
2. A standard form will be used to report on-the-job injuries regardless of severity.
3. The supervisor will complete the injury report form with all the necessary information regarding the exact nature of the injury.
4. The supervisor will then submit the report to the Human Resource and the Safety Committee.

Reference:

Spellman, Frank R. Ph.D. *Safe Work Practices for the Environmental Laboratory.* Section 7.3 "Chemical Hygiene Plan: An Example", pp 46 – 65. Technomic Publishing Co., Inc. Lancaster, PA. 1998.

EMERGENCY NUMBERS

FIRE……9-911

POLICE……9-911

AMBULANCE……9-911

FIRST AID……9-911

POISON CONTROL CENTER……9- 1 (800) 222-1222

SAFETY HOTLINES

CHEMTREC (Chemical Transport
Emergency Center) 24-Hour……9-1-800-424-9300

EPA (Environmental Protection Agency)……9-1-800-368-5888

NIOSH (National Institute of
Occupational Safety and Health)……9-1-800-356-4674

OSHA (Occupational Safety & Health
Administration)……9-1-202-523-7075

Hazard Communication Program Template
(29 CFR 1910.1200)

SCOPE
A hazardous material is any item or agent (biological, chemical, or physical) that has the potential to cause harm to humans, animals, or the environment, either by itself or through the interaction with other factors, such as materials or processes. Hazardous materials are defined and regulated by laws and regulations administered by the U.S.EPA, OSHA, U.S.DOT, and the Nuclear Regulatory Commission (NRC). Each agency defines hazardous material according to the scope and purpose of the governing law:

- OSHA defines *hazardous material* as "any substance or chemical that is a health or physical hazard."
- The U.S.EPA incorporated OSHA's definition adding, "any item or chemical which can cause harm to people, plants, or animals when released by spilling, leaking, pumping, dumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, or disposing into the environment."
- The U.S.DOT defines a hazardous material as "any item or chemical which, when being transported or moved, is a risk to public safety or the environment."
- The NRC regulates items or chemicals that are "special nuclear source" or byproduct materials or radioactive substances.

APPLICATION
The Chemical Hazard Program is applicable to all **Facility Name** personnel. The Program will be available to all personnel and reviewed/updated annually.

OBJECTIVE
The objective of this program is to set forth policies and procedures concerning Hazard Communication which will enhance the safety and well-being of ***Facility Name*** employees. Furthermore, execution of this program is designed to help ***Facility Name*** comply with the Occupational Safety and Health Administration's (OSHA) Hazard Communication Standard.

HANDLING
Engineering Controls

Engineering controls must be used to reduce employee exposure to hazardous materials whenever feasible. The two most common controls are local exhaust and general ventilation, which limit an employee's exposure to airborne contaminants. When engineering controls aren't available or fail to adequately reduce hazards, PPE is required.

PPE

PPE must be provided and worn in accordance with the manufacturer's recommendations indicated on each, individual chemical's Safety Data Sheet.

Transporting

Shipment of hazardous materials should always be inspected for evidence of leakage prior to accepting the shipment. In the event that package/container is damaged or leaking, the shipper should be notified, and the material returned to the supplier for replacement. If re-packaging of the material is not optional, place the leaking container in a larger, leak-proof container for secondary containment until the item can be properly contained in a suitable USDOT shipping device.

Deliveries of bulk chemicals must follow precautionary measures when dispensing the hazardous material, so it is not spilled on to the facility ground. If catch-basin or other storm water drainage systems are nearby, it is important to ascertain the systems are returned to the treatment plant and not discharged to the surface waters.

When transporting hazardous chemicals, follow these precautionary guidelines:

- Materials should be transported in secondary containment of sufficient size to hold the entire contents in the event of a spill or leakage
- Routes for transportation should be planned to minimize exposure to personnel in the event of a spill
- Spill prevention kits should be placed at strategic locations in anticipation of a spill or leakage
- Only personnel trained in spill response should be allowed to clean up small chemical spills. Larger chemical spills (greater than the capacity of the spill kit or beyond the competency of personnel) should be contained by the **Facility Name** Fire Department.

Dropping even a gallon container of acutely toxic chemicals, such as concentrated acid, can result in devastating injuries and/or cause serious health issues. When carrying any container of acutely toxic chemicals, extreme care must be taken to not drop, spill, or damage the container. Employees should limit the number of containers they carry; keep walkways clear of debris, water, obstructions, or other slip/trip hazards; and pay careful attention to where they are walking. A bottler carrier, polypropylene tray, or utility cart may be utilized to assist in safely transporting smaller quantities of toxic chemicals.

HAZARDOUS MATERIAL SPILLS

Any chemical spill, regardless of its size, should be immediately neutralized and cleaned up using spill kit materials that are designed for that purpose. **Facility Name** employs spill kits for neutralizing and/or cleaning up acid spills, base spills, mercury spills, and flammable liquids spills. Each spill kit shall be accompanied by a Quick Reference Guide that explains the procedure in the simplest terms. Employees should never attempt to clean up a spill that is beyond their training and if the proper resources are not available. If a spill or release occurs, it may be necessary to evacuate the area and contact the **Facility Name** Fire Department Emergency Response Team.

Mercury Spill Procedure

Mercury is toxic and can cause nerve and organ damage if ingested. Any mercury spill calls for quick action to clear the area and careful cleanup. In order to properly clean a mercury spill, a specified spill kit is necessary.

What to Avoid

1) Do not use a vacuum cleaner.
    - It will be contaminated with mercury and will blow vapor into the room.

2) Do not use a broom.
    - It will break the mercury into smaller, harder-to-find beads.
3) Do not wash contaminated clothing.
    - Clothes shall be bagged and discarded.

Small Spill

1) Spills of a few grams (Such as a broken thermometer) require that you remove all people and animals from the room.
2) Use gloves, apron, and protective eyewear.
3) Use your in-lab Mercury Spill Kit according to its instructions.
4) Dispose of any PPE and used materials into a Ziploc-style bag and take to local Hazardous Waste site.

Larger Spill

1) Spills of more than a few grams require that you remove people from the room.
2) Use gloves, disposable suit, protective eyewear, shoe covers, and a respirator.
3) Ventilate the room by opening a window.
4) Turn off or block vents for central air/heat until the spill is cleaned.
5) Close and lock the door. Post a warning sign on the door.
6) Call your local health or environmental agency.

***** For spills larger than 2 tablespoons, call the National Response Center at (800) 424-8802!

Broken Fluorescent Lamp

1) Ventilate the room by opening the window.
2) Wear gloves and protective eyewear.
3) Remove people from the room.
4) Using a piece of cardboard, scoop up broken glass and other debris.
5) Use sticky tape to pick up small glass particles.
6) Dispose of gloves, glass, tape and any other materials into a Ziploc-style bag and take to local Hazardous Waste site.

<u>Chemical Spill Procedure</u>

1) Assess the Danger
    - If it's a major spill that poses an immediate health threat, evacuate the area and alert Emergency Personnel.
    - If it's a minor threat, alert surrounding personnel.
    - Remove anything that might cause a fire from the spill (Any oxidizing materials).
2) Attend to anyone who may need medical attention.
    - Remove any directly exposed clothing and douse chemical burns with water for at least 15 minutes.
3) Identify the chemical
4) Refer to the SDS to determine chemical's properties and PPE required
5) Protect yourself with proper safety gear.
    - Often includes gloves, glasses, lab coat or apron, and possibly a respirator.

Neutralize the Spill

1) Place barrier agent around the area of the spill.
    - Baking Soda (or relevant spill kit agent) for an acid spill
    - Vinegar (or relevant spill kit agent) for an alkaline spill
2) Move agent onto your spill using an inward motion.

- For an acid spill, the reaction will produce a small amount of foam as carbon dioxide evolves. When there is no more fizzing, the acid has been neutralized.
- For an alkaline spill, pour the vinegar directly onto the alkaline. Test with pH paper to determine neutrality.

Clean up Spill

1) Scoop up the material with a dust pan
   - Material will be solid for an acid spill and may be swept with a brush.
   - Material will be liquid for an alkaline spill and may be mopped up with paper towels.
2) Dispose of material in a Ziploc-type container
3) Wipe the area with additional towels, using soap and plenty of water.

Dispose of Materials

1) Place disposable PPE, solid material, used towels, and other disposable materials into a bucket and securely fasten the lid.
2) Apply a warning label
3) Take to a local Hazardous Waste Disposal Site

<u>Flammable Liquids Spill Procedure</u>

Spill kits will be available in most buildings of the **Facility Name** and in **Facility Name** vehicle(s) used by personnel. The kits located at the **Facility Name** building(s) will contain the necessary PPE (gloves and goggles) for cleaning a chemical spill. The kits in the vehicle(s) however, are smaller in size and do not contain PPE. The PPE that is required to clean/contain a chemical spill (gloves and goggles) are regularly available in each vehicle. Each kit, regardless of size, contains the following items: chemical/hazmat socks; universal pads; universal sorbent; and disposal bags with ties. Follow the direction on the back of the kit for proper application and clean-up/containment up a flammable liquids spill.

STORAGE

All hazardous materials must be properly labeled with their exact contents, hazardous properties, date of receipt, and, if applicable, date of expiration. Hazardous substances should be stored in the original containers in which they were packaged at the manufacturer's plant. If this is not practical, the substance should be transferred into a container that is constructed to withstand the effect of the substance over the maximum storage time according to the manufacturer's recommendations. Store chemicals in an orderly manner with older products being most accessible and newer products being less accessible.

Chemical storage should provide secondary containment to keep spills from contaminating surrounding surfaces, which could cause injury to an unsuspecting employee. A rubber tub or polypropylene tray may be used as secondary containment as long as it is compatible with the material being stored.

Hazardous materials must be stored based on their compatibility; incompatible materials must not be stored in a manner that they may come in contact with each other. Storing incompatible materials together may result in heat or pressure; fire or explosion; violent reactions; toxic dust, mist, vapor, or gases; and flammable vapor or gases.

- Flammable materials in container larger than one gallon should be stored in a ventilated National Fire protection association (NFPA)- approved flammable storage cabinet. The cabinet should be installed per the manufacturer's recommendations.

- Acids and bases should not be stored in flammable storage cabinets or other areas that are next to combustible, flammable, or other hazardous materials that could violently react with acid or base.
- Acids and bases must be segregated from each other.

FIRST AID

Facility Name personnel are at risk of serious chemical-related injuries and illnesses because of the concentrations of chemicals being used in the workplace. When working with chemicals, health effect can be chronic and may not be apparent for many years or they can be acute and cause immediate and serious damage to the body upon exposure. The most common chemical exposures are to the hands, arms, and eyes. Proper PPE should always be utilized when working near chemicals. The use and type of PPE will depend on the type and concentration of the chemicals being used; refer to the chemical's SDS for PPE prescription.

Chemical Ingestion

If ingestion occurs, consult the chemical's Safety Data Sheet and call the Poison Control Information center immediately! (800) 222-1222

Chemical Splash in Eye

Chemicals splashed into the eye can lead to injuries that cause loss of vision. Therefore, it is imperative that chemicals are immediately flushed from the eye.

1) Flush eye with water.
   - Use clean, lukewarm tap water for at least 20 minutes.
2) Step into safety shower.
   - Aim a gentle stream on your forehead over the affected eye, while holding the affected eye open.
3) Wash hands with soap & water.
   - Wash thoroughly so no chemical or soap resides.
4) Remove contact lenses (If applicable).
5) Seek emergency medical assistance.
   - Take the container with you to the ER so they can assess the danger.
   - Wear sunglasses due to light sensitivity.

Caution:

- Do not rub the eye!
- Do not use anything other than water or saline rinse on the eye (No eye drops)!

Chemical Burn

Chemical burns require large amounts of water to flush the chemicals form the skin. Do not use bare hands to remove chemicals from the skin. Ensure run-off water does not flow over unaffected skin or onto the rescuer.

1) Remove the chemical.
   - Brush off any dry chemical then rinse the skin with cool, gentle running water for 20 minutes.
2) Remove clothing or jewelry that has been contaminated.
3) Wrap the burn area.

- Use a dry, sterile dressing or clean cloth if dressing not available.

4) Rewash the burned area.
   - Using clean water, rewash the area for several more minutes if the person experiences increased burning.
5) Take an over-the-counter pain reliever.
6) Get a tetanus shot.
   - All burns are susceptible to tetanus.

Seek Emergency Assistance if:

- The person shows sign of shock, fainting, or shallow breathing.
- The burn penetrates to the second layer of skin and the resulting second-degree burn is more than 3 inches in diameter.
- Burn occurred over the eye, hand, foot, face, groin, buttocks, or a major joint.
- The person experiences pain that an over-the-counter reliever can't control.

Chemical Inhalation

Inhalation hazards include pesticides, fumigants, chemical fumes, vapors, and gases. Signs and symptoms vary with each type of exposure; some cause eye irritation while other cause irritation in the respiratory tract. Additional symptoms may include: pale or bluish skin color; chest pain or tightness; dizziness; headache; confusion; irritability; nausea; or vomiting.

1) Call for emergency medical help.
   - Never attempt to rescue a person without notifying others first.
2) Remove the person from the affected air.
   - Take several deep breaths of fresh air and hold your breath as you enter the room, holding a wet cloth over your nose & mouth.
3) Open windows and doors to remove the fumes.
4) After rescuing the person from danger, check and monitor the person's airway, breathing & pulse.
   - Administer CPR if necessary (And if you are properly certified).
   - If able, administer first aid to the eyes.
   - If person vomits, put on gloves and clear the person's airway.
   - Even if person seems fine, call for emergency medical help!

Do Not:

1) Give the person anything by mouth.
2) Induce vomiting unless told to do so by Poison Control
3) Try to neutralize the poison unless instructed to do so by Poison Control
4) Wait for symptoms to develop. Call for emergency medical help immediately!

Fire

If clothing is on fire, the flame should be extinguished (STOP, DROP, and ROLL).

1) Remove the person from the environment, if possible.
2) Immediately call for medical assistance.
3) If a shower is immediately available, cool water will help stop the spread of the burn.
   - If the person shivers, discontinue to cooling process; hypothermia is a risk for burn victims.
   - Remove any clothing or jewelry from unburned skin.

Do Not:

1) Use a salve or cream for the initial treatment of burned skin; they will retain the heat and continue to burn the skin.

Shock

Shock may result from chemical inhalation or poisoning, severe chemical burn, or other causes. When unattended, the victim's organs will suffer from blood and oxygen loss that can lead to permanent organ damage or death.

1) Call 911
2) Have the person lie down.
   - Ly them on their back with their feet about one foot higher than their head.
   - If the elevation of the feet causes pain or injury, keep the person flat & still.
3) Check for signs of circulation (Breathing, Coughing, or Movement)
   - If absent, give the person CPR immediately!
4) Keep the person warm and comfortable.
   - Loosen belt & tight clothing.
   - Cover the person with a blanket.
5) Turn the person on their side.
   - Prevents choking from vomit or bleeding.

Do Not:

Give the person anything by mouth.

Signs/Symptoms of Shock May Include:

1) The skin is cool and clammy and may appear pale or grey.
2) The pulse is weak and rapid.
3) Breathing may be slow and shallow, or hyperventilation may occur.
4) Blood pressure is below normal.
5) Person may be nauseated.
6) The eyes lack luster and seem to stare.
   - Sometimes the pupils are dilated.
7) The person may be conscious or unconscious.
   - If conscious, they may feel faint, weak, or confused.
   - Person may seem to be overly excited and anxious.

POISON CONTROL CENTER: (800) 222-1222

Safety Data Sheets (SDS)

Hazardous Chemical List

A list of the hazardous materials and chemicals used in the course of ***Facility Name*** activities will be maintained and updated by a designated member of *the* ***Facility Name*** *Safety Committee.* This list is to include all substances that require a Safety Data Sheet (SDS).

One copy of this list will be kept in the front of each SDS book. For each chemical used in the workplace, an SDS sheet must be available on that jobsite.

SDSs

A member of the **Facility Name** Safety Committee will keep all SDSs in an organized fashion and will place them in *a binder* for all employees to view at will. A duplicate set of SDS information will be maintained by the designated member of the Safety Committee **(insert title)**.

SDS books and the Hazardous Chemical List will be maintained and kept up to date by *the SDS **(insert title)***. As they become obsolete, SDSs are replaced by updated copies. Obsolete SDSs will be retained for 30 years.

The Safety Committee will verify, annually, that SDSs correctly reflect chemical reformulations, improvements, or updates.

If a hazardous chemical or substance is received without a proper SDS, the receiving person will immediately notify the SDS **(insert title)**. *That person w*ill immediately contact the manufacturer or distributor of the product. If the manufacturer or distributor is unable to produce an SDS, the Safety Committee will return the product to the supplier.

Note: Material Safety Data Sheets (MSDSs) cannot be used after June 1, 2015.

SECTIONS ON A SAFETY DATA SHEET

The Hazard Communication Standard (HCS) requires chemical manufacturers, distributors, or importers to provide Safety Data Sheets (SDSs) (formerly known as Material Safety Data Sheets or MSDSs) to communicate the hazards of chemical products. As of June 1, 2015, the HCS will require new SDSs to be in a uniform format that must include the section numbers, headings, and associated information listed below:

***Section 1*: Identification**

Product identifier

- Manufacturer or distributor name
- Address
- Phone number
- Emergency phone number
- Recommended use
- Restrictions on use

***Section 2*: Hazard(s) Identification**

Includes all hazards regarding the chemical and includes the required label elements

***Section 3*: Composition/Information on Ingredients**

Includes information on chemical ingredients and trade secret claims

***Section 4*: First-Aid Measures**

Includes important symptoms/effects, including acute or delayed symptoms and required treatment

***Section 5*: Fire-Fighting Measures**

- Lists suitable extinguishing techniques and equipment and lists chemical hazards from fire

***Section 6*: Accidental Release Measures**
Lists emergency procedures, protective equipment, proper methods of containment, and cleanup

***Section 7*: Handling and Storage**
Lists precautions for safe handling and storage, including incompatibilities

***Section 8*: Exposure Controls/Personal Protection** Lists OSHA's Permissible Exposure Limits (PELs); Threshold Limit Values (TLVs); appropriate engineering controls; and personal protective equipment.

***Section 9*: Physical and Chemical Properties**
Lists the chemical's characteristics

***Section 10*: Stability and Reactivity**
Lists chemical stability and possibility the
of hazardous reactions

***Section 11*: Toxicological Information**
Includes routes of exposure, related symptoms including acute and chronic effects, and numerical measures of toxicity

***Section 12*: Ecological Information**

***Section 13*: Disposal Considerations**

***Section 14*: Transport Information**

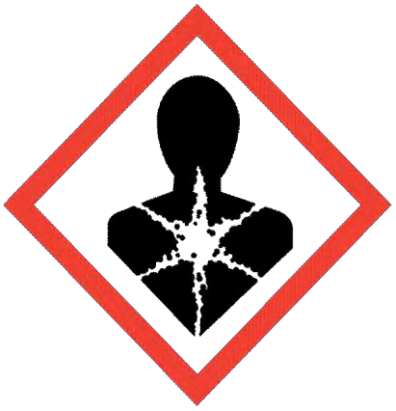
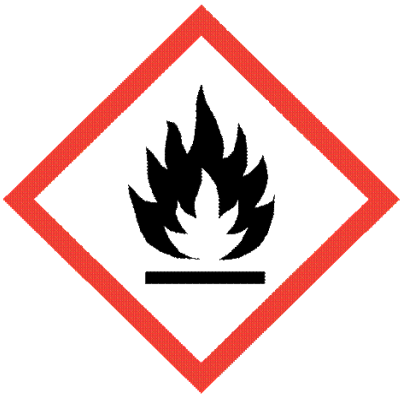
***Section 15*: Regulatory Information**

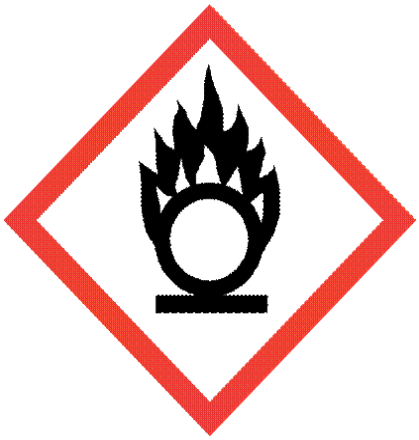
***Section 16*: Other Information**
Includes the date of preparation or last revision

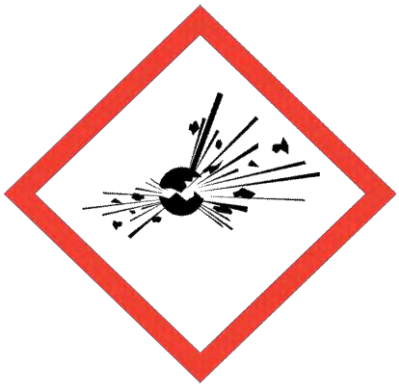
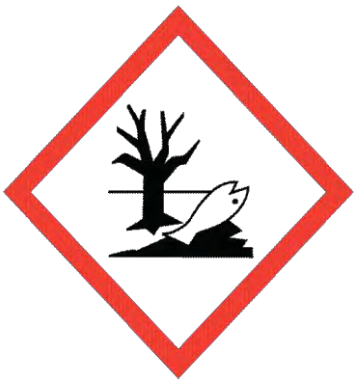
Labeling and Pictograms

Each container of a hazardous chemical must be properly labeled with the identity of the hazardous material, the appropriate hazard warnings, pictogram(s), signal word(s), and the name and address of the manufacturer. Appropriate labels must be on all containers, regardless of size. Containers must be approved and recommended for storage and/or dispensing of the particular hazardous chemicals contained in them.
Worn and torn labels must be replaced. It is the responsibility of employees to report inappropriate labels to their supervisor. It is the responsibility of the Safety Committee to ensure that appropriate labels are in place and that replacement labels are available.
Portable containers of hazardous materials do not require labeling if the materials are transferred from labeled containers and are intended for immediate use by the employee who performs the transfer. Portable containers not immediately used will be emptied (and cleaned when necessary) within 24 hours.

Pictograms and Hazards

HEALTH HAZARD	FLAME	GAS CYLINDER
		
Carcinogen Mutagenicity Reproductive Toxicity Respiratory Sensitizer Target Organ Toxicity Aspiration Toxicity	Flammable Pyrophorics Self-Heating Emits Flammable Gas Self-Peroxides	Gases Under Pressure

CORROSION	EXCLAMATION MARK	FLAME OVER CIRCLE
		
Skin Corrosion/Burns Eye Damage Corrosive to Metals	Irritant (skin and eye) Skin Sensitizer Acute Toxicity Narcotic Effects Respiratory Tract Irritant Hazardous to Ozone Layer	Oxidizers

EXPLODING BOMB	SKULL AND CROSSBONES	ENVIRONMENT
		
Explosives Self-Reactives Organic Peroxides	Acute Toxicity (fatal or toxic)	Aquatic Toxicity *(Non-Mandatory)

HAZARDOUS MATERIAL LABEL

PRODUCT IDENTIFIER

CODE

Product Name

SUPPLIER IDENTIFICATION

Company Name

Address

Emergency Phone Number

PRECAUTIONARY STATEMENTS

Keep container tightly closed

Store in cool, well ventilated place that is locked

Keep away from heat/sparks/open flame; No smoking

Only use non-sparking tools

Use explosion-proof electrical equipment

Take precautionary measure against static discharge

Ground and bond container and receiving equipment

Do not breathe vapors

Wear protective gloves

Do not eat, drink or smoke when using this product

Wash hands thoroughly after handling

Dispose of in accordance with local, regional, national, international regulations as specified

In Case of Fire: Use dry chemical (BC) or carbon dioxide (CO_2) fire extinguisher to extinguish

First Aid: If exposed call Poison Center

If on skin (or hair): Take off immediately any contaminated clothing; Rinse skin with water

HAZARD PICTOGRAMS

SIGNAL WORD

DANGER

HAZARD STATEMENT

Highly flammable liquid and vapor!
May cause liver and kidney damage!

SUPPLEMENTAL INFORMATION

Directions for Use:
Fill weight: Lot Number:
Gross weight: Fill Date:
Expiration Date:

Hazardous Waste Disposal

Daily operation of the wastewater treatment process generates various hazardous, chemical wastes. Some of these wastes include cleaners, spent oils, paints, and solvents necessary for **Facility Name** maintenance. Correctly determining whether a waste meets the definition of hazardous waste is essential in determining how the waste should be managed. The Federal Hazardous Substance act defines hazardous substance as:

> "Any substance or mixture of substances that is toxic, corrosive, is an irritant, is a strong sensitizer, is flammable or combustible, or generates pressure through decomposition, heat, or other means."
>
> "Any substance or mixture of substances that may cause substantial personal injury or illness during or as a proximate result of any customary or foreseeable handling."

Ignitable Waste

Ignitable wastes create fire under certain conditions, are spontaneously combustible, or have a flashpoint below 140°F. Examples include spent oils and used solvents.

Corrosive Waste

Corrosive wastes are acids or bases that are capable of corroding metal containers. Examples include battery acid, sulfuric acid, hydrochloric acid, muriatic acid, and sodium hydroxide.

Reactive Waste

Reactive wastes are unstable under normal, ambient conditions. They can cause explosions or generate toxic fumes, gases, or vapors when heated or compressed or mixed with water. Examples include concentrated acids and bases located in the wastewater laboratory.

Toxic Waste

Toxic wastes are harmful or fatal when ingested or absorbed. Examples include lead and mercury.

Universal Waste

Universal waste is a group of commonly-occurring hazardous waste the EPA has set forth streamlined standards of regulation. The regulations were developed to govern proper collection and management of these common wastes. Universal waste items include batteries, pesticides, mercury-containing equipment, and fluorescent lamp bulbs.

Used Oil Waste

Used oil is any oil that (1) has been refined from crude oil or (2) any synthetic oil that has been used and, as a result of such use, is contaminated by physical or chemical impurities. Used motor oil can contain toxic chemicals and heavy metals.

Used Oil Containers

Used oil containers should be inspected regularly for condition, accumulation, secondary containment, and labeling. Maintenance and inspection records of used oil containers must be kept permanently.

Used oil containers must have labels that include the following information:

- Accumulation start date
- Label or stenciled lettering with the word "hazardous waste"
- Physical state of the waste, it contents, and composition
- Hazardous properties of the waste's characteristics
- Name/Address of **Facility Name**

Training

All full-time and part-time employees, new hires, and contractors of ***Facility Name*** are required to be trained on the following:

- Label elements
- Pictograms
- SDS format to facilitate recognition and understanding of the product, its required personal protective equipment (PPE), and first aid requirements
- Chemicals they will be exposed to
- Locations of SDSs
- Chemicals in supply lines within the site

Each affected employee working for or associated with ***Facility Name*** is required to review the Hazard Communication Program with either the Safety Committee or the employee's supervisor and sign the acknowledgment form. This training is to be done during the new employee orientation process before the new employee assumes status as an active employee. Existing employees must read and sign the acknowledgement that they understand the components of the Hazard Communication Program.

Employees will receive training on any new hazardous chemical/material introduced into the workplace before the chemical/material is used or when changes are made to the program.

Non-Routine Tasks

Before any non-routine task is performed, employees shall be advised and/or they must contact *their supervisor* for special precautions to follow and *supervisors* shall inform any personnel who could be exposed to hazardous materials.

If a non-routine task is necessary, *the employee's supervisor* will provide the affected employees with information about the activity as it relates to the specific chemicals expected to be encountered:

- Specific chemical name(s) and hazard(s)
- Personal protective equipment required and safety measures to be taken
- Measures that have been taken to lessen the hazards including ventilation, respirators and emergency procedures.
-

*The **insert title*** will contact each contractor before work starts to gather and disseminate any information concerning chemical hazards the contractor is bringing into the workplace.

Reporting

The Emergency Planning and Community Right-to-Know Act (EPCRA) was authorized by Title III of the Superfund Amendments and Reauthorization Act (SARA) as national legislation on community safety. The Act was developed to impose requirements on states and regulated facilities to minimize environmental and safety hazards posed by the storage and handling of toxic chemicals. To implement EPCRS, Congress requires each state to appoint a State Emergency Response Commission (SERC) that will be further divided into Local Emergency Planning Committees (LEPC). Indiana's SERC is under the direction of the Indiana Department of Homeland Security. Likewise, the **Facility Name** LEPC is operated by the **insert name of LEPC.**

Under EPCRA, facilities must report the storage, use, and release of certain hazardous chemicals. Facilities with an extremely hazardous substance (EHS) on-site, greater than the threshold planning quantities (TPC), are subject to EPCRA emergency planning requirements.
Facility Name at any point in time, may have the **insert name(s) of EHS** on-site. The TPC for **insert name(s)** is **insert quantity**. Therefore, the **insert name(s) of EHS** that may be located on-site at **Facility Name** is considered and extremely hazardous substance that is subject to EPCRA emergency planning requirements. Reference the **Facility Name EHS** Handling Program to view the EPCRA Emergency Plan.
SARA Title III reporting requirements for chlorine are the completion and submission of forms:

- Tier II Hazmat Report
- Form 311
- Form 312
- Form 302

Forms shall be completed by **insert person's name and title** and submitted to the IDHS SERC via Tier II Manager no later than March 1st of each year.

Reference

1) Water Environment Federation. *Safety, Health, and Security in Wastewater Systems.* Sixth Edition: 2012.

LABORATORY REAGENT WATER

SCOPE

The American Society for Testing and Materials (ASTM) has established the following technical guidelines for laboratory reagent water standards:

Measurement	Type I	Type II	Type III	Type IV
Resistivity (MΩ-cm)	> 18	> 1	> 0.4	> 0.2
Conductivity (µS/cm)	< 0.056	< 1	< 2.5	< 5
pH (s.u.)	N/A	N/A	N/A	5.0-8.0
TOC µg/L (ppb)	< 50	< 50	< 200	N/A
Sodium µg/L (ppb)	< 1	< 5	< 10	< 50
Chloride µg/L (ppb)	< 1	< 5	< 10	< 50
Silica µg/L (ppb)	< 3	< 3	< 500	N/A

Sub-standards have also been established by the ASTM for heterotrophic bacteria count (HBC) and endotoxins. For the purpose of wastewater analysis, HBC should be limited to the Type II standard of < 10 CFU/mL, but endotoxins are not a primary concern in the wastewater laboratory. Likewise, the International Organization for Standardization (ISO) and the Clinical Laboratory Standards Institute (CLSI) have established technical standards for Clinical Laboratory Reagent Water (CLRW).

Typical usage for laboratory reagent water is outlined below:

> **Type I:** Used for clinical laboratory applications, preparation of buffers and media for cell culture and IVF, production of reagents for molecular biology applications (DNA, PCR), and preparation of solutions for electrophoresis and blotting.
>
> **Type II:** Used for buffers, pH solutions, and microbiological culture media preparation and preparation of reagents for chemical analysis.
>
> **Type III:** Used for glassware rinsing, filling autoclaves, heating baths, and humidifiers. Also used as feed water to Type I & Type II systems.

Source: Puretec Industrial Water. Laboratory Water Quality Standards. 2017.

APPLICATION

Type II laboratory reagent water is appropriate for ALL chemical and biological analyses in the WWTP laboratory. Only freshly prepared laboratory reagent water is used for ammonia analysis to prevent contamination from ammonia in the air. Dilution water for BOD_5 analysis is stored in light shielded containers, sealed in such a manner to allow a free exchange of air without contamination. (Examples: cotton plugs, loose fitting container lids) Laboratory reagent water for other laboratory tests is stored in tightly stoppered glass containers or withdrawn fresh from the water purification unit.

In addition, if the laboratory reagent water is used for bacteriological analysis, it must meet the following additional specifications:

Chemical Tests:	
Conductivity, > 0.5 megohms resistance or < 2µmhos/cm at 25°C	Monitored Continuously
pH = 5.5-7.5 s.u.	Monitored with each use
Total organic carbon = < 1.0 mg/L	Monitored Monthly
Heavy metals, single (Cd, Cr, Cu, Ni, Pb and Zn) = < 0.05 mg/L	Monitored Annually
Heavy metals, total = < 0.10 mg/L	Monitored Annually
Ammonia/organic nitrogen = < 0.10 mg/L	Monitored Monthly
Total chlorine residual = < 0.01 mg/L	Monitored with each use
Bacteriological Tests:	
Heterotrophic plate count = < 1000 CFU/mL	Monitored Monthly
Use test (SM 21st ed. 9020 B. 4 *e.*) Student's t ≤ 2.78	Quarterly and for a new source

One laboratory that performs heavy metal analysis is Element Materials, Inc.:

Element Materials Inc.
328 Ley Road
Ft. Wayne, IN 46825
(260) 222-2136

SUITABILITY

A suitability test must be run annually to determine that the Type II laboratory reagent water being used is suitable for microbiological analysis before the start of each disinfection (*E. coli* testing) season. The suitability test will determine the ratio of bacT growth-inhibiting substances, the ratio of nitrogen and carbon sources promoting bacT growth, the ratio of only nitrogen sources promoting bacT growth, and the ratio of only carbon sources promoting bacT growth.

One laboratory that performs this analysis is Daily Laboratories:

Daily Laboratories
2200 West Altorfer Drive, Suite B
Peoria, IL 61615
(309) 691-4513

If the laboratory purchases a batch lot (all containers have the same lot number) of laboratory reagent water, it must ask the supplier to submit a copy of test results for the files. This water must be tested for the parameters shown above for Type II laboratory reagent water in addition to those listed above if bacteriological analysis is performed in the laboratory. Suppliers typically perform analytical testing annually and the laboratory must have the most recent analytical record on file.

REFERENCE: Standard Methods 21st Edition. Method 9020 B. Page 9-7.

LABORATORY SAMPLE AND WASTE DISPOSAL

It is the policy of the **Facility Name** to minimize the amount of waste generated from lab operations and to comply with all regulations concerning its safe and proper handling and disposal. Chemicals, reagents, standards, and stock solutions are purchased in quantities that minimize the amount remaining after expiration. Samples and waste products are segregated by waste type, characteristics, and compatibility to minimize the quantity of hazardous waste generated and these wastes are handling as outlined below.

Sample Disposal:

Samples are held until analysis is complete and all data recorded. Sample and waste disposal are handled two (2) ways: on-site disposal, and off-site disposal.

1. On-site Sample Disposal - Non-hazardous water samples that can be discharged to the **Facility Name** Treatment Plant are poured down the drain. This includes small quantities of dilute < 10% acidic, caustic, and neutral water samples that contain non-hazardous levels of metals, other inorganics, and/or organics. Used samples containers, digestion tubes/vials, storage tubes/vials, etc. used for these non-hazardous samples are emptied, rinsed, and disposed in the trash. Rinsed plastic and glass containers may be saved for off-site recycling.

2. Off-site Sample Disposal - All hazardous liquid samples, reagents, standards, and solutions with volumes > 500 mL in addition to all hazardous solid samples and solid chemicals are to be segregated by hazard class and waste type. They are then combined or stored in their original container for lab packing. An approved contractor is used to transport and dispose of the lab packed wastes. Documentation of off-site disposal consists of a manifest listing all the samples, reagents, solids, etc. in addition to completion of any other requirements set forth by the disposal contractor. All manifests and associated paperwork are to be kept on file.

Laboratory Waste Disposal:

In addition to samples, several individual waste streams consisting of reagents, quality control standards, solutions, contaminated materials, and spill cleanup waste are the result of normal analytical testing and each is disposed of appropriately.

1. General sample processing waste - this category includes disposable pipet tips, weigh boats, spill cleanup materials, gloves, absorbents, and any other disposal materials which may be contaminated with a hazardous sample or chemical. Each item is evaluated to determine if contact with a hazardous sample or chemical had occurred. If it is deemed an item is potentially hazardous, it is segregated for lab packing and off-site disposal.

2. Concentrated Acid/Alkaline wastes - Sample processing and spill cleanup waste, samples, standards, reagents, and stock solutions containing more than > 10% concentrated acids or caustics are to be segregated by hazard class, waste type, and compatibility and combined or lab packed for off-site disposal. When appropriate, they

may be neutralized to an acceptable pH followed by disposal down the drain for non-hazardous liquids or combined with the trash for non-hazardous solids. Absorbent papers used in the fume exhaust hood contaminated with > 10% concentrated acids are to be air dried with the fume exhaust hoods functioning than disposed of in the Biohazard container in the lab.

3. Mercury wastes - Broken mercury thermometers, used UV bulbs, sample processing and spill cleanup waste, samples, standards, reagents, and stock solutions containing or contaminated with mercury at > 260 mg/L are to be segregated and lab packed for off-site disposal.

4. Routine lab trash - paper towels, weigh boats, pipet tips, and gloves which have not been contaminated with a hazardous chemical or sample are disposed of in the trash. All recyclable paper, glass, and plastic are to be disposed of in their respective recycling containers.

Method Detection Limit Condensed Procedure for the Determination of TNTplus NH_3-N and PO_4^{3-}-P Using a Single Spectrophotometer

I. PROCEDURE

Section 1. Estimate the initial MDL as follows:

A. The mean concentration plus three (3) times the standard deviation of 10 laboratory reagent water [(1)]method blanks when using the TNTplus 830 for ammonia-N and TNTplus 843 for total phosphorus.

[(1)] Method Blank - a clean sample processed simultaneously with and under the same conditions as samples containing the analyte of interest through all steps of the analytical procedure

Table 1
Example Spreadsheet for Estimating the Initial MDL

Estimate of Initial MDL Study **Analysis of 10 Laboratory Reagent Water Method Blanks**					
Hach Method 10205			**Hach Method 10209**		
NH_3-N using TNTplus 830 vials			**PO_4^{3-}-P using TNTplus 843 vials**		
Date	Analyst	Result, mg/L	Date	Analyst	Result, mg/L
MM/DD/YYYY		0.002	MM/DD/YYYY		0.002
MM/DD/YYYY		0.003	MM/DD/YYYY		0.004
MM/DD/YYYY		-0.002	MM/DD/YYYY		-0.003
MM/DD/YYYY		0.001	MM/DD/YYYY		0.003
MM/DD/YYYY		0.003	MM/DD/YYYY		-0.001
MM/DD/YYYY		0.000	MM/DD/YYYY		0.004
MM/DD/YYYY		-0.003	MM/DD/YYYY		0.002
MM/DD/YYYY		0.002	MM/DD/YYYY		-0.002
MM/DD/YYYY		0.001	MM/DD/YYYY		0.000
MM/DD/YYYY		-0.002	MM/DD/YYYY		0.003
	Mean (X) =	0.00050		Mean (X) =	0.00120
Standard Deviation (S) =		0.00217	Standard Deviation (S) =		0.00253
X + 3S = 0.00050 + 0.00651 = Initial MDL Estimate = 0.0070 mg/L			X + 3S = 0.00120 + 0.00759 = Initial MDL Estimate = 0.00879 mg/L		

Section 2. Determine the initial MDL as follows:

A. Select a spiking level, typically between 2 – 10 times the estimated MDL from **Table 1.**

1. NH_3-N Initial MDL Estimate = 0.0070 mg/L

2. 0.0070 mg/L X 2 = **0.014 mg/L** *and* 0.0070 mg/L X 10 = **0.070 mg/L**

3. Concentration of Spiked Samples chosen for Initial NH_3-N MDL determination
= 0.050 mg/L

4. PO_4^{3-}-P Initial MDL Estimate = 0.00879 mg/L

5. 0.00879 mg/L X 2 = **0.018 mg/L** *and* 0.00879 mg/L X 10 = **0.088 mg/L**

6. Concentration of Spiked Samples chosen for Initial PO_4^{3-}-P MDL determination
= 0.075 mg/L

B. Process a minimum of seven (7) spiked samples and seven (7) method blank samples through all steps of the method. The samples used for the MDL must be analyzed in at least three batches on three separate calendar dates. (Preparation and analysis may be on the same day.) Existing data may be used, if compliant with the requirements for at least three batches, and generated within the last twenty four months. The most recent available data for method blank samples and spiked samples must be used. **Table 2 and Table 3** below are spreadsheets examples for NH_3-N and PO_4^{3-}-P initial MDL determinations.

C. Make all calculations as specified below and record the results as mg/L. **Note:** If blank subtraction is routinely used, add (if negative) or subtract (if positive) the average of the method blank results from the spiked samples results in the same run.

1. Calculate the mean (X) and standard deviation (S) of the seven (7) replicate method blank measurements.

2. Calculate the mean (X) and standard deviation (S) of the seven (7) replicate spiked sample measurements.

D. Calculate the MDL_b (the MDL based on method blanks as follows):

1. If none of the method blanks gives a numerical result, the MDL_b does not apply. A numerical result includes both positive and negative results.

2. If some (but not all) of the method blanks give a numerical result, set the MDL_b to the highest blank result.

3. If all the method blanks give numerical results, the calculate the MDL_b as:

$$MDL_b = X + t_{(n-1,\ 1-\alpha=0.99)}S_b$$

Where:
MDL_b = the method detection limit based on method blanks

X = mean of the method blank results (use zero "0" in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha= 0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate of n-1 degrees of freedom. See **Table 4** below.

S_b = sample standard deviation of the seven (7) replicate method blanks results.

E. Calculate the MDL_s (the MDL based on the spiked samples) as follows:

$MDL_S = t_{(n-1, 1-\alpha= 0.99)}S_s$

Where:

MDL_s = the method detection limit based on the spiked samples

$t_{(n-1, 1-\alpha= 0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate of n-1 degrees of freedom. See **Table 4** below.

S_s = sample standard deviation of the seven (7) replicate spiked samples.

F. Select the <u>greater</u> of MDL_b or MDL_s as the initial MDL.

Table 2
Spreadsheet Example for Determining the NH_3-N Initial MDL

Analyst	Date	Method Blanks, mg/L	Spiked Samples, mg/L
	MM/DD/YYYY	0.002	0.052
	MM/DD/YYYY	0.003	0.047
	MM/DD/YYYY	0.000	0.053
	MM/DD/YYYY	-0.002	0.055
	MM/DD/YYYY	0.001	0.046
	MM/DD/YYYY	0.003	0.049
	MM/DD/YYYY	0.002	0.046
	Mean (X) =	0.00129	0.04971
	Standard Deviation (S) =	0.00180	0.00364
	[(2)] % RSD =	NA	7.3%

[(2)] % RSD (Percentage Relative Standard Deviation) = Mean ÷ Standard Deviation (X/S)

= [Mean (X) + (Student's t-value for seven (7) replicates X Standard Deviation (S)]
= [0.00129 + (3.143 x 0.00180)] = 0.007 mg/L

However, in reviewing the method blank data again, it was observed that one of the results = 0.000 mg/L, so based on the 2. D.2. above, the MDL_b is set at the highest method blank result of 0.003 mg/L.

MDL_s = Student's t-value for seven (7) replicates X Standard Deviation (S)
= 3.143 x 0.00364 = 0.011 mg/L

Selecting the greater of MDL_b or MDL_s as the initial MDL, the MDL_s value is used, and the initial MDL is set at 0.011 mg/L.

Checking the MDL ("4-point" Check, refer to **Section 3**):

Calculated MDL < **Spike Level** < 10 times the calculated MDL
0.011 mg/L < 0.050 mg/L < 0.11 mg/L = **Yes** No

Mean result (mg/L) of the 7 replicates = Prepared concentration (mg/L) ± 50%
0.049 mg/L = 0.050 ± 0.025 mg/L = **Yes** No

Is % RSD for spiked samples ≤ 20%
7.3% ≤ 20% **Yes** No

MDL > discharge permit limits Yes No
Note: Permit limits are facility specific

Table 3
Spreadsheet Example for Determining the PO_4^{3-}-P Initial MDL

Analyst	Date	Method Blanks, mg/L	Spiked Samples, mg/L
	MM/DD/YYYY	0.003	0.080
	MM/DD/YYYY	0.004	0.073
	MM/DD/YYYY	-0.002	0.081
	MM/DD/YYYY	-0.003	0.078
	MM/DD/YYYY	0.005	0.083
	MM/DD/YYYY	0.002	0.081
	MM/DD/YYYY	0.001	0.068
	Mean (X) =	0.00143	0.07771
	Standard Deviation (S) =	0.00299	0.00535
	[(2)] % RSD =	NA	6.9%

[(2)] % RSD (Percentage Relative Standard Deviation) = Mean ÷ Standard Deviation (X/S)

MDL_b = [Mean (X) + (Student's t-value for Seven (7) replicates X Standard Deviation (S)]
= 0.00143 + (3.143 X 0.00299) = 0.011 mg/L

MDL_s = Student's t-value X Standard Deviation (S) = 3.143 X 0.00535 = 0.017 mg/L

Selecting the greater of MDL_b or MDL_s as the initial MDL, the MDL_s value is used, and the initial MDL is set at 0.017 mg/L.

Checking the MDL:

Calculated MDL < **Spike Level** < 10 times the calculated MDL
0.017 mg/L < 0.075 mg/L > 0.17 mg/L = **Yes** No
Mean result (mg/L) of the 7 replicates = Prepared concentration (mg/L) ± 50%
0.078 mg/L = 0.075 ± 0.0375 mg/L = **Yes** No

Is % RSD for spiked samples ≤ 20%
6.9% ≤ 20% = **Yes** No

MDL > discharge permit limits Yes No
Note: Permit limits are facility specific

Table 4 – Single-Tailed 99th Percentile t Statistic

Number of replicates	Degrees of freedom (n−1)	$t_{(n-1,\ 1-\alpha=\ 0.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
32	31	2.453
48	47	2.408
50	49	2.405
61	60	2.390
64	63	2.387
80	79	2.374

Number of replicates	Degrees of freedom (n−1)	$t_{(n-1,\ 1-\alpha=\ 0.99)}$
96	95	2.366
100	99	2.365

Section 3. Checking your Method Detection Limit (MDL) Determination ("4-Point" Check): the MDL calculated in the laboratory can be checked using the following criteria:

A. Was the concentration of the replicate samples analyzed to determine the MDL greater than 10 times the calculated MDL? If yes, the replicate sample concentrations were too high and a lower sample concentration needs to be prepared and the MDL determined again;

B. Is the calculated MDL higher than the concentration of the replicate samples used in the determination of the MDL? If yes, the replicate sample concentrations were too low and a higher sample concentration needs to be prepared and the MDL determined again;

C. Is the calculated MDL greater than the discharge permit limits specified in your wastewater permit? Even the lowest discharge permit limits should be significantly greater than the MDLs calculated in the wastewater laboratory. If the MDLs calculated in your laboratory are higher than your discharge permit limits, you need to evaluate the entire analytical procedure and correct any problems causing high MDL values;

D. Are the results obtained from the analysis of the replicate samples analyzed to determine the MDL reasonable when compared to the actual concentration of the replicates analyzed? For example, if the concentration of seven spiked sample replicates used to determine the MDL for ammonia-nitrogen was 0.15 mg/L and the calculated mean (average) of the seven replicates was 0.63 mg/L, the results are questionable. Standard Methods 21st ed. 1020 B. 3. States "Calculate recoveries for MDL samples. Recoveries should be between 50 and 150% and %RSD values ≤ 20% or repeat the MDL determination." Since the concentration of the replicates used for MDL determinations is at the lower end of the useable concentration range in the wastewater laboratory, any reference made in the Standard Operating Procedure as to the treatment of method blanks during calculations should be followed.

The following equations/statement are useful when evaluating the calculated MDL:

Calculated MDL < **Spike Level** < 10 times the calculated MDL

Mean result (mg/L) of the 7 replicates = Prepared concentration (mg/L) ± 50%
% RSD values for spiked samples ≤ 20%

Calculated MDL < Permit Discharge Limit

Section 4. Ongoing Data Collection:

A. During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked samples in separate batches, using the same spiking concentration used in **Section 2.** If any analytes are repeatedly not detected in the quarterly spiked sample analyses, or do not meet the qualitative identification criteria of the method, then this is an indication that the spiking level is not high enough and should be adjusted upward. Note that it is not necessary to analyze additional method blanks together with the spiked samples, the method blank population should include all the routine method blanks analyzed with each batch during the course of sample analysis.

B. Ensure that at least seven spiked samples and seven method blanks are completed for the annual verification. If only one instrument is in use, a minimum of seven spikes samples are still required, but they may be drawn from the last two years of data collection.

C. At least once per year, re-evaluate the spiking level.

1. If more than 5% of the spiked samples do not return positive numerical results that meet all method qualitative identification criteria, then the spiking level must be increased, and the initial MDL re-determined following the procedure in **Section 2.**

D. If the method is altered in a way that can be reasonably expected to change its sensitivity, then re-determine the initial MDL according to **Section 2**, and the restart the ongoing data collection.

Example Spreadsheet for Ongoing Data Collection

Minimum of two (2) Spiked Samples of Laboratory Reagent Water Required per Quarter **Spiked Samples must have the same concentration as used in Initial MDL Determination				
Ammonia-N Method 10205 using TNTplus 830 Vials				
1st Quarter				
		Sample	**Spiked Sample	Percent
Date	Analyst	Result, mg/L	Analyzed, mg/L	Recovery
MM/DD/YYYY		0.047	0.050	94.0%
MM/DD/YYYY		0.055	0.050	110.0%
2nd Quarter				
		Sample	**Spiked Sample	Percent
Date	Analyst	Result, mg/L	Analyzed, mg/L	Recovery
MM/DD/YYYY		0.049	0.050	98.0%
MM/DD/YYYY		0.052	0.050	104.0%
3rd Quarter				
		Sample	**Spiked Sample	Percent
Date	Analyst	Result, mg/L	Analyzed, mg/L	Recovery
MM/DD/YYYY		0.045	0.050	90.0%
MM/DD/YYYY		0.053	0.050	106.0%
4th Quarter				
		Sample	**Spiked Sample	Percent
Date	Analyst	Result, mg/L	Analyzed, mg/L	Recovery
MM/DD/YYYY		0.051	0.050	102.0%
MM/DD/YYYY		0.047	0.050	94.0%

Section 5. Ongoing Annual Verification:

A. At least once every thirteen months, re-calculate MDL_s and MDL_b from the collected spiked samples and method blank results using the equations in **Section 2.**

B. Include data generated within the last twenty four months, but only data with the same sample spiking level. Only documented instances of gross failures (*e.g.,* instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations. (The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.) If the laboratory believes the sensitivity of the method has changed significantly, then the most recent data available may be used, maintaining compliance with the requirement for at least seven replicates in three separate batches on three separate days (see **Section 2.B.).**

C. Include the initial MDL spiked samples, if the data were generated within twenty four months.

D. Only use data associated with acceptable calibrations and batch QC. Include all routine data, with the exception of batches that are rejected and the associated samples reanalyzed. If the method has been altered in a way that can be reasonably expected to change its sensitivity, then use only data collected after the change.

E. Ideally, use all method blank results from the last 24 months for the MDL_b calculation. The laboratory has the option to use only the last six months of method blank data or the fifty most recent method blanks, whichever criteria yields the greater number of method blanks.

F. The verified MDL is the greater of the MDL_s or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

II. DOCUMENTATION

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting limits. Data and calculations used to establish the MDL must be able to be reconstructed upon request. The sample matrix used to determine the MDL must also be identified with the MDL value. Document the mean spiked and recovered analyte levels with the MDL. The rationale for removal of outlier results, if any, must be documented and maintained on file with the results of the MDL determination.

III. REFERENCES

A. EPA821-R-16-006
B. SM 21st ed. 1020 B. 3.

PIPETTOR CALIBRATION

A) GENERAL

To ensure measurement accuracy, all Pipettors should be checked daily for dust and dirt with specific attention being paid to the tip cone. Use only 70% ethanol to clean the outsides of the Pipettors. Pipettor calibration must be performed monthly. Each Pipettor will be assigned a specific volume and is to be calibrated only for the volume in which it will deliver. Any changes made to the Pipettor's volume deliverance must be accompanied by recalibration.

B) RECORD KEEPING

All maintenance and calibration of Pipettors is to be recorded in the lab Equipment Manual. Technician must include the Pipettor, procedure performed, and the date of the performance in all records.

C) PERSONAL PROTECTIVE EQUIPMENT

None required for this procedure

D) AUTOMATIC PIPETTOR OPERATION – The Pipettors listed below are those used in the facility submitting the Pipettor Calibration document and included for reference only. Please follow the manufacturer's instructions for Pipettor(s) operation in your facility.

<u>$CBOD_5$ / TSS / CL^-</u>

<u>0.3 mL Finnpipette F2</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>3.0 mL Finnpipette F2</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>5.0 mL Finnpipette II</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>6.0 mL Finnpipette II</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>NH_3-N & PO_4^{3-}-P</u>

<u>0.2 mL Eppendorf</u>

1) Push down to 1st notch
2) Withdraw aliquot (Release)
3) Push down to 2nd notch to dispel aliquot

<u>0.4 mL Finnpipette F2</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>0.5 mL Finnpipette F2</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

<u>1.0 mL Finnpipettes F2</u>

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

5.0 mL Finnpipette II

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

10.0 mL Finnpipette F2

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

1.0 mL Oxford MacroSet (Chloride Only)

1) Push all the way down
2) Withdraw aliquot (Release)
3) Push all the way down to dispel aliquot

2.0 mL Finnpipettes F2

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

4.5 mL Finnpipettes F2

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

5.0 mL Finnpipette F2

1) Push all the way down to 2nd notch
2) Withdraw aliquot (Release)
3) Push down to 1st notch to dispel aliquot

E) MATERIALS NEEDED:

Analytical balance
Plastic weigh boat
> 100 mL laboratory reagent water
Pre-wetted Pipettor tip
250 mL beaker
Masking Tape
Pipettor

F) PROCEDURE:

1. Using the 250 mL beaker containing > 100 mL laboratory reagent water, pre-wet the Pipettor tip by withdrawing an aliquot and dispensing it back into the beaker.
2. Tare the plastic weigh boat by placing it on the balance. Wait until the reading is stable and press "Zero."
3. Via manufacturer's instructions, set the Pipettor to the desired volume.
4. Withdraw an aliquot from the 250 mL beaker and dispense the water into the plastic weigh boat. The weight of the aliquot should be within 2.0% of the Pipettor's assigned volume (See below QA/QC chart for acceptance brackets).
5. Repeat steps 2 thru 5 until five consecutive aliquots are within 2.0% of the Pipettor's assigned volume.
6. Remove the tape that has the previous month's calibration record from the Pipettor. On a new piece of tape, record the calibration volume and date along with the initials of the technician performing the calibration. Place the tape on the Pipettor.

G) CALCULATION:

1.0 mL laboratory reagent water = 1.0 gram @ 20°C

H) QUALITY ASSURANCE/QUALITY CONTROL

1. To ascertain calibration accuracy, it is required that five consecutive aliquots be within 2.0% of the Pipettor's assigned value before the Pipettor is properly calibrated.

2% Tolerance Limit Chart

0.1 mL	0.0980 g to 0.1020 g
0.2 mL	0.1960 g to 0.2040 g
0.3 mL	0.2940 g to 0.3060 g
0.4 mL	0.3920 g to 0.4080 g
0.5 mL	0.4900 g to 0.5100 g
1.0 mL	0.9800 g to 1.0200 g
2.0 mL	1.9600 g to 2.0400 g
3.0 mL	2.9400 g to 3.0600 g
4.0 mL	3.9200 g to 4.0800 g
5.0 mL	4.9000 g to 5.1000 g
6.0 mL	5.8800 g to 6.1200 g
7.0 mL	6.8600 g to 7.1400 g
8.0 mL	7.8400 g to 8.1600 g
9.0 mL	8.8200 g to 9.1800 g
10.0 mL	9.8000 g to 10.2000 g

REFERENCES:

1) Fisher Scientific Fisherbrand Finnpipette II Digital. Instructions for Use Booklet.
2) Oxford Macro-Set Transfer Pipetting System. Instrument Usage Booklet.
3) Eppendorf Autoclavable Pipette Model 4810. Instruction Manual.

PREPARATION OF ACIDS and ALKALINES COMMONLY USED in the WASTEWATER LABORATORY

A) GENERAL SCOPE & APPLICATION

Acid solutions are used to preserve wastewater samples and to clean laboratory glassware. Always prepare acid solutions by cautiously adding the required amount of concentrated acid to the designated volume of laboratory reagent water.

Alkaline solutions are most commonly used to adjust sample pH after acid preservation. Prepare solutions by dissolving the required amount of solid sodium hydroxide (NaOH) into the designated volume of laboratory reagent water. Alternatively, prepare solutions by diluting the required amount of concentrated ammonium hydroxide (NH_4OH) into the designated volume of laboratory reagent water.

B) PREPARATION

Hydrochloric Acid (HCl)
Concentrated = 36% or 12*N*

Volume of Concentrated, 12*N* HCl to prepare 1 L of
6*N* Solution = 500 mL
1*N* Solution = 83 mL
0.1N Solution = 8.3 mL

Volume of 6*N* HCl reagent to prepare 1 L of
0.1*N* Solution = 17 mL

Volume of 1*N* HCl reagent to prepare 1 L of
0.02*N* Solution = 20 mL

Sulfuric Acid (H_2SO_4)
Concentrated = 96% or 36*N*

Volume of Concentrated, 36*N* H_2SO_4 to prepare 1 L of
18*N* Solution = 500 mL
6*N* Solution = 167 mL
1*N* Solution = 28 mL
0.1*N* Solution = 2.8 mL

Volume of 6*N* H_2SO_4 reagent to prepare 1 L of
0.1N Solution = 17 mL

Volume of 1*N* H_2SO_4 reagent to prepare 1 L of
0.02*N* Solution = 20 mL

Nitric Acid (HNO_3)
Concentrated = 69% or 15.8*N*

Volume of Concentrated, 15.8*N* HNO_3 to prepare 1 L of
6*N* Solution = 380 mL
1*N* Solution = 64 mL
0.1N Solution = 6.4 mL

Volume of 6*N* HNO_3 reagent to prepare 1 L of
0.1N Solution = 17 mL

Volume of 1*N* HNO_3 reagent to prepare 1 L of
0.02*N* Solution = 20 mL

Acetic Acid (CH_3CO_2H)
Concentrated = 99.8% or 17.4*N*

Sodium Hydroxide (NaOH)
Concentrated = Solid NaOH

15*N* Stock NaOH Solution Preparation:
Dissolve 625 g of solid NaOH in 800 mL of laboratory reagent water to form 1 L of stock solution – CAUTION – this preparation generates a lot of heat and the solution must be cooled to room temperature before bringing up to 1 L with laboratory reagent water. Refer to SM 21st ed. for additional solution preparation instructions.

Required Volume of 15*N* Stock NaOH Solution to prepare 1 L of
6*N* Solution = 400 mL
1*N* Solution = 67 mL
0.1*N* Solution = 6.7 mL

Required Weight of Concentrated (Solid NaOH) to prepare 1 L of
6*N* Solution = 240 g
1*N* Solution = 40 g
0.1N Solution = 4.0 g

Ammonium Hydroxide (NH_4OH)
Concentrated = 29% or *15N*
Volume of Concentrated, 15*N* NH_4OH to prepare 1 L of
5*N* Solution = 333 mL
3*N* Solution = 200 mL
0.1N Solution = 13 mL

To calculate the volume of the more concentrated acid or alkaline solution needed to prepare a less concentrated acid or alkaline solution, use the following equation:

$N_1 \times V_1 = N_2 \times V_2$

where

N_1 = Normality of the more concentrated acid or alkaline solution

V_1 = Volume, mL of more concentrated acid or alkaline to use

N_2 = Normality of the less concentrated acid or alkaline solution being prepared

V_2 = Volume, mL of less concentrated acid or alkaline solution being prepared

so

$V_1 = (N_2 \times V_2)/N_1$

C) QUALITY ASSURANCE/RECORD KEEPING

1. When making an acid or alkaline solution, record it in the Reagent Preparation Log located [state location].
Include the solution ID, strength, date prepared, date expired, lot number of the concentrate, and analyst.

2. Label the glassware containing the solution with the solution ID, ratio, date prepared, date of expiration and analyst.

D) SAFETY

Fumes of acids and bases are severe eye and respiratory system irritants. Likewise, liquid and solid acids/bases can severely burn the skin and eyes. When acids are heated to increase digestion, they pose an even more significant threat because the fumes increase, and hot acid reacts more quickly with the skin.

Store acids and bases separately in a well-ventilated area. Work with strong acids/bases only in a properly functioning chemical fume hood. Always add acids/bases to water to avoid splattering. If skin contact is made, thoroughly wash the area with water and seek medical attention. If eye contact is made, immediately flush both eyes with water for at least 15 minutes and seek medical attention. Dispose of any clothing that comes in contact with acids or alkalines.

Whenever working with concentrated acids/bases or with large volumes of acidic/alkaline solutions, you must wear the lab-supplied PVC apron, face shield, and nitrile gloves while working under the fume hood.

When working with less concentrated, smaller volumes of acids/bases, analysts must wear a laboratory coat, eye protection, and nitrile gloves.

In the event of a large spill (approximately the size of a saucer), acid and alkaline spill kits are

located [state location]. How to guides should be posted on the spill kit doors for safely cleaning up spills.

Always label any container containing an acid or alkaline solution with a Hazard Identification Label. The label must include the solution ID and its hazard ratings.

E) ACID CLEANING PROCEDURE

1. Prepare the sulfuric acid or hydrochloric acid bath.
    - 10% sulfuric acid solution (used for cleaning BOD glassware)
      Slowly pour three 500 mL bottles of concentrated sulfuric acid into a 5 gallon Rubbermaid container containing 13.5 liters of laboratory reagent water.

    - 1:1 hydrochloric acid solution (used for cleaning phosphorus glassware)
      Pour one 4 liter container of concentrated, 12N hydrochloric acid into a 2.5 gallon Rubbermaid container containing 4 liters of laboratory reagent water.

2. Select dishwasher-cleaned glassware to be acid-stripped. Submerge the glassware in the acid bath, ensuring that there are no air bubbles in the glassware.

3. Let the glassware soak in the acid bath for at least 24 hours.

4. Remove the glassware from the acid bath and rinse 3+ times with laboratory reagent water. Use pH paper to ensure that all glassware is properly rinsed (The pH will be neutral if the glassware has been properly rinsed).

5. Let the glassware dry completely. If the glassware is not to be used immediately, label each piece with the date and which solution it has been acid-cleaned with.

F) REFERENCES

Standard Methods 21st edition. Preparation of Common Types of Desk Reagents – located on inside of front cover
Standard Methods 21st edition, Method 1090 B, Pages 1-43.

SAMPLING PROCEDURES for WASTEWATER and BIOSOLIDS

1 General Information

1.1 Purpose

The purpose of this procedure is to document both general and specific procedures, methods and considerations to be used and observed when collecting wastewater samples for field screening or laboratory analysis. **Please note:** these are general guidelines, always refer to regulated permits or permitting agencies for specifics that would better define your specific sampling requirements.

1.2 Scope/Application

This document describes both general and specific methods to be used by field personnel when collecting and handling wastewater samples in the field. On the occasion that sampling personnel determine that any of the procedures described in this section are inappropriate, inadequate or impractical and that another procedure must be used to obtain a wastewater sample, the variant procedure will be documented in the field log book, along with a description of the circumstances requiring its use.

1.3 Documentation/Verification

This procedure was prepared by persons deemed technically competent based on their knowledge, skills and abilities. The procedure has been tested in practice and reviewed by a subject matter expert.

1.4 General Precautions

1.4.1 Safety

Proper safety precautions must be observed when collecting wastewater samples. The precautions should only be used to complement the judgment of an experienced professional. Address chemicals that pose specific toxicity or safety concerns and follow any other relevant requirements, as appropriate.

1.4.2 Procedural Precautions

The following precautions should be considered when collecting wastewater samples.

- Special care must be taken not to contaminate samples. This includes storing samples in a secure location to preclude conditions which could alter the properties of the sample. Samples shall be custody sealed during long-term storage or shipment.
- Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.
- If samples are transported by the sampler, they will remain under his/her custody or be secured until they are relinquished.
- Shipped samples shall conform to all U.S. Department of Transportation (DOT) and/or International Air Transportation Association (IATA) hazardous materials shipping requirements.
- Documentation of field sampling is done in a bound logbook.

• Chain-of-custody documents shall be filled out and remain with the samples until custody is relinquished.
• All shipping documents, such as air bills, bills of lading, etc., shall be retained by the sampling facility and stored in a secure place.

2 Special Sampling Considerations

2.1 Special Precautions for Wastewater Sampling

• A clean pair of new, non-powdered, disposable gloves will be worn each time a different location is sampled, and the gloves should be donned immediately prior to sampling. The gloves should not come in contact with the media being sampled and should be changed any time during sample collection when their cleanliness is compromised.
• Sample containers for samples suspected of containing high concentrations of contaminants shall be stored separately.
• Sample collection activities shall proceed progressively from the least suspected contaminated area to the most suspected contaminated area. Samples of waste or highly contaminated media must not be placed in the same ice chest as environmental (i.e., containing low contaminant levels) or background/control samples.
• If possible, one member of the field sampling team should take all the notes and photographs, fill out tags, etc., while the other members collect the samples.
• Field investigators must use new, verified certified-clean disposable or non-disposable equipment cleaned according to approved procedures for collection of samples for trace metals or organic compound analyses.

2.2 Sample Handling and Preservation Requirements

1. All sample collection and preservation procedures will comply with the requirements outlined in *40 CFR Part 136.3 (e),* Table II
2. Wastewater samples will typically be collected either by directly filling the sample container or by using an automatic sampler or other device.
3. During sample collection, if transferring the sample from a collection device, make sure that the device does not come in contact with the sample containers.
4. Place the sample into appropriate, labeled containers. Samples collected for VOC analysis must not have any headspace (see Section 7.4, Volatile Organic Compounds). All other sample containers must be filled with an allowance for head space.
5. All samples requiring preservation must be preserved as soon as practically possible, ideally immediately at the time of sample collection. If preserved VOC vials are used, these will be preserved with concentrated hydrochloric acid prior to departure for the field investigation. For all other chemical preservatives, sampling personnel will use the appropriate chemical preservative generally stored in an individual single-use vial. The adequacy of sample preservation will be checked after the addition of the preservative for all samples, except for the samples collected for VOC analysis. If it is determined that a sample is not adequately preserved, additional preservative should be added to achieve adequate

preservation.

6. All samples preserved using a pH adjustment (except VOCs) must be checked, using pH strips, to ensure that they were adequately preserved. This is done by pouring a small volume of sample over the strip. Do not place the strip in the sample. Samples requiring reduced temperature storage should be placed on ice immediately.

2.3 Quality Control

Equipment blanks should be collected if equipment is field cleaned and re-used on-site or if necessary to document that low-level contaminants were not introduced by the sampling equipment.

2.4 Records

Information generated or obtained by sampling personnel will be organized and accounted for in accordance with the facility's records management procedures. Field notes, recorded in a bound field logbook, will be generated, as well as chain-of-custody documentation according to accepted procedures.

3 General Considerations

3.1 Wastewater Sampling Design

Wastewater sampling studies focus primarily on collecting wastewater samples of the Influent or Effluent at domestic and non-domestic facilities. Sampling activities are usually conducted for National Pollutant Discharge Elimination System (NPDES) compliance, compliance assistance, civil and criminal investigations, and water quality studies. Collection of wastewater samples is necessary in order to obtain reliable data that can support compliance or enforcement activities. The main considerations in developing a wastewater sampling strategy are:

- Type of study (Compliance Sampling Inspection, Diagnostic Evaluation, etc.).
- Regulated or target pollutants in the wastewater stream to be sampled.
- Selection of the projected sampling locations to satisfy the study objectives.
- Quality control criteria of the parameters to be sampled (oil and grease samples need to be collected as grab samples, trip blanks are taken into the field for the collection of samples for volatile organic compound analyses, etc.).

Complexity of the sampling program will vary with a number of factors. Some primary factors are:

- The number of sampling stations to be monitored. This will be dependent on NPDES permit requirements and the type of study.
- Special handling requirements of the target pollutants (sampling equipment for trace organic compounds require special cleaning procedures, etc.).
- Laboratory conducting the analyses (use of a contract laboratory may require shipping from the field, etc.).
- Accessibility to sampling stations.
- Process and operation criteria of the source generator (i.e., batch operation versus continuous discharge).

• Coordination of participating organizations in the study (i.e., state assistance with the sample collection).
• The length of time for sampling activities will dictate logistical considerations (i.e., shipment of samples, additional supplies, etc.).

3.2 Sampling Techniques and Equipment

The wastewater sampling techniques and equipment described in Sections 4 through 9 of this document are designed to minimize effects on the chemical and physical integrity of the sample. If the procedures in these sections are followed, a representative sample of the wastewater should be obtained. The variety of conditions at different sampling locations requires that considerable judgment be exercised regarding the methodologies and procedures for the collection of representative samples of wastewater. Each sampling location warrants attention commensurate with its complexity. There are, however, basic rules and precautions generally applicable to sample collection. Some important considerations for obtaining a representative wastewater sample include:

• The sample should be collected where the wastewater is well mixed. Therefore, the sample should be collected near the center of the flow channel, at approximately 40 to 60 percent of the water depth, where the turbulence is at a maximum and the possibility of solids settling is minimized. Skimming the water surface or dragging the channel bottom should be avoided. However, allowances should be made for fluctuations in water depth due to flow variations.
• In sampling from wide conduits, cross-sectional sampling should be considered. Dye may be used as an aid in determining the most representative sampling locations.
• If manual compositing is employed, the individual sample portions must be thoroughly mixed before pouring the individual aliquots into the composite container. For manual composite sampling, the individual sample aliquots should be preserved at the time of sample collection.

3.3 Site Selection for Wastewater Sampling

Where applicable, wastewater samples should be collected at the location specified in the NPDES permit (if the source has a permit). In some instances, the sampling location specified in the permit, or the location chosen by the permittee, may not be adequate for the collection of a representative wastewater sample. In such instances, the investigator is not limited by permit specifications and may collect a sample at a more representative location. When a conflict exists between the permittee and the regulatory agency regarding the most representative sampling location, both sites should be sampled, and the reason for the conflict should be noted in the field notes and the inspection or study report. Recommendations and reasons for a change in sampling locations should be given to the appropriate permitting authority.

3.3.1 Influent

Influent wastewaters are preferably sampled at locations of highly turbulent flow in order to ensure good mixing; however, in many instances the most desirable location is not accessible. Preferable Influent wastewater sampling locations include:

1) the up-flow siphon following a comminutor (in absence of grit chamber)

2) the up-flow distribution box following pumping from main plant wet well
3) aerated grit chamber
4) flume throat
5) pump wet well when the pump is operating
6) downstream of preliminary screening. When possible, Influent samples should be collected upstream from side stream returns.

3.3.2 Effluent

Effluent samples should be collected at the site specified in the permit, or if no site is specified in the permit, at the most representative site downstream from all entering wastewater streams prior to discharge into the receiving waters. If a conflict exists between the permittee and inspector regarding the source being sampled or the location of the most representative site, follow the procedures previously described in Section 3.3, Site Selection for Wastewater Sampling.

3.3.3 Pond and Lagoon Sampling

Generally, NPDES Permits specify grab Effluent wastewater samples should be collected from ponds and lagoons (refer to your permit to make sure you are collecting the correct type of sample). Even if the ponds or lagoons have long retention times, composite sampling is necessary because of the tendency of ponds and lagoons to have flow paths that short circuit which changes the design detention time.

4 Sample Types

4.1 General

For NPDES sampling, two types of sampling techniques are used: grab and composite. For these procedures, the NPDES permit specifies the appropriate sample type.

4.2 Grab Samples

Grab samples consist of either a single discrete sample or individual samples collected over a period of time not to exceed 15 minutes. The grab sample should be representative of the wastewater conditions at the time of sample collection. The sample volume depends on the type and number of analyses to be performed.

4.3 Composite Samples

Composite samples are collected over time, either by continuous sampling or by mixing discrete samples. A composite sample represents the average wastewater characteristics during the compositing period. Various methods for compositing are available and are based on either time or flow proportioning. The choice of a flow proportional or time composite sampling scheme depends on the permit requirements, variability of the wastewater flow or concentration of pollutants, equipment availability and sampling location. The investigator must know each of these criteria before a sampling program can be initiated. Generally, a time composite is acceptable. However, in enforcement cases where strict adherence to permit requirements are necessary, a flow proportional sample is preferable, if possible.

A time composite sample consists of equal volume discrete sample aliquots collected at

constant time intervals into one container. A time composite sample can be collected either manually or with an automatic sampler.

A flow proportional composite sample can be collected using one of two methods. One method consists of collecting a constant sample volume at varying time intervals proportional to the wastewater flow. For the other method, the sample is collected by varying the volume of each individual aliquot proportional to the flow, while maintaining a constant time interval between the aliquots. Flow proportional samples can be collected directly with an automatic sampler that is connected to a compatible flow measuring device. An automatic sampler can also be used to collect discrete samples. At the end of the compositing period, the discrete samples are composited by volume versus flow chart readings. Field personnel can use the facility's primary flow device and flow measurement system when their accuracy can be verified. Prior to collecting flow proportional samples, the facility's flow measuring system should be examined for proper installation and accuracy. If the facility's primary flow measuring device does not meet standard conditions or is in an unsafe or inaccessible location, then the investigator may choose to collect time composite samples or install a portable primary flow device. If the flow measurement system is acceptable, samples should be collected using the appropriate flow proportioning methods.

5 Automatic Samplers

5.1 General

Automatic samplers may be used to collect composite or grab samples when several aliquots are to be collected at frequent intervals or when a continuous sample is required. For composite sampling applications, the automatic samplers may be used to collect time composite or flow proportional samples. In the flow proportional mode, the samplers are activated and paced by a compatible flow meter. Flow proportional samples can also be collected using an automatic sampler equipped with multiple containers and manually compositing the individual sample portions proportional to the flow. Automatic samplers must meet the following requirements:

- Sampling equipment must be properly cleaned to avoid cross-contamination which could result from prior use.
- No plastic or metal parts of the sampler shall come in contact with the water or wastewater stream when parameters to be analyzed could be impacted by these materials.
- The automatic sampler must be capable of providing adequate refrigeration during the sampling period. This can be accomplished in the field by using ice.
- The automatic sampler must be able to collect a large enough sample for all parameter analyses.
- The individual sample aliquot must be at least 100 mL if the sampler uses a peristaltic pump.
- The automatic sampler should be capable of providing a lift of at least 20 feet and the sample volume should be adjustable since the volume is a function of the pumping head.
- The pumping velocity must be at least 2 ft./sec. to transport solids and not allow solids to settle.

• The intake line leading to the pump must be purged before each sample is collected.
• The minimum inside diameter of the intake line should be 1/4 inch.
• An adequate power source should be available to operate the sampler for the time required to complete the project. Facility electrical outlets may be used if available.
• Facility automatic samplers should only be used if 1) field conditions do not allow for the installation of investigator's sampling equipment and 2) the facility sampling equipment meets all the requirements detailed above. Specific operating instructions, capabilities, capacities, and other pertinent information for automatic samplers are included in the respective operating manuals.

5.1.1 Conventional Sampling (Inorganic Parameters)

Conventional sampling includes all inorganic parameters (i.e., BOD_5, TSS, COD, nutrients) that can be collected using an automatic sampler. New tubing (Silastic7, or equal, in the pump and either Teflon7 or Tygon7, or equal, in the sample train) will be used for each sampler installation. Installation procedures include cutting the proper length of tubing, positioning it in the wastewater stream, and sampler programming. Protective gloves should be worn to reduce exposure and to maintain the integrity of the sample. For a time composite sample, the sampler should be programmed to collect sufficiently sized aliquots (at least 100 mL if using a peristaltic pump) at a frequency that provides a representative sample and enough sample volume to conduct all required analyses. For a flow proportional sample, the sampler should be programmed to collect a minimum of 100 mL for each sample aliquot with the interval predetermined based on the flow of the monitored stream. At the end of the compositing period, the sample collected should be properly mixed and transferred into the respective containers, followed by immediate preservation, if required. For routine inspections, the permittee should be offered a split sample.

5.1.2 Metals

When an automatic sampler is used for collecting samples for metals analyses, the entire sample collection system should be rinsed with organic-free water and an equipment blank should be collected. Approximately one-half gallon of rinse water should be pumped through the sample tubing into the composite container and discarded. Nitric acid must be added to the metal's blank container for proper preservation. The sampler may then be positioned in the appropriate location and the sampler program initiated. If the automatic sampler tubing is attached to a metal conduit pipe, the intake tubing should be carefully installed upstream and away from the conduit to prevent metals contamination. This can be accomplished by clamping the tubing upstream of the conduit using laboratory clamps and wrapping the submerged portion of conduit pipe with a protective barrier (i.e., duct tape).

5.1.3 Extractable Organic Compounds, Pesticides and PCBs

When an automatic sampler is used for collecting samples for the analyses of extractable organic compounds, pesticides and/or PCBs, the installation

procedures include cutting the proper length of new Teflon7 tubing, rinsing of the entire sampler collection system with organic-free water and collection of appropriate equipment blanks for organic compounds analysis. For the organic free water rinse, approximately one-half gallon is initially pumped into the composite sample container and discarded. An additional one and one-half (1 ½) gallons (approximate) are then pumped into the composite sample container for distribution into the appropriate blank container. Finally, the collection tubing should be positioned in the wastewater stream and the sampler programmed and initiated.

5.2 Automatic Sampler Security

Field investigators should take whatever steps are necessary to prevent tampering with sampling equipment. A lock or custody seal may be placed on the sampler to detect tampering. However, this does not prevent tampering with the sample collection tubing. If necessary, seals may be placed on the sampling pole and tubing line to further reduce tampering possibilities.

5.3 Automatic Sampler Maintenance, Calibration and Quality Control

To ensure proper operation of automatic samplers, and thus the collection of representative samples, the following maintenance and calibration procedures should be used, and any deviations should be documented in the field logbook.

Prior to being used, the sampler operation should be checked by the field investigator to ensure proper operation. This includes operation (forward, reverse, and automatic) of at least one purge-pump-purge cycle; checking desiccant and replacing if necessary; checking the 12-volt batteries to be used with the sampler; and repairing any item if necessary. During each field trip, prior to initiating the automatic sampler, the rinse and purge pump-purge cycle shall be checked at least once. The pumping volume should be checked at least twice using a graduated cylinder or other calibrated container prior to initiating the sampler. For flow proportional sampling, the flow meter that activates the sampler should be checked to ensure that it operates properly.

Upon returning from a field sampling episode, the structural integrity of the sampler should be examined and repaired, if necessary. The desiccant will be checked and replaced if appropriate. The operation (forward, reverse, automatic, etc.) will be checked and required repairs will be made and documented. The sampler will then be cleaned thoroughly. The automatic sampler should be checked against the manufacturer's specifications and documented whenever one or more of the sampler functions appear to be operating improperly.

6 Manual Sampling

Manual sampling is normally used for collecting grab samples and/or for immediate on site field analyses. However, it can also be used in lieu of automatic equipment over extended periods of time for composite sampling, especially when it is necessary to evaluate unusual waste stream conditions.

The best method to manually collect a sample is to use the actual sample container which will be used to transport the sample to the laboratory. This eliminates the possibility of

contaminating the sample with intermediate collection containers. If the water or wastewater stream cannot be physically reached by the sampling personnel or it is not safe to reach for the sample, an intermediate collection container may be used, from which the sample can be redistributed to other containers. If this is done, however, the container used to collect the sample must be properly cleaned and must be made of a material that meets the requirements of the parameter(s) being investigated. Samples for oil and grease, bacteria, and most volatile compounds (both organic and inorganic; see Section 7.4 for specific requirements) must always be collected directly into the sample container.
In some cases, it may be best to use a pump, either power or hand operated, to withdraw a sample from the water or wastewater stream. If a pump is used, it is imperative that all components of the pump that come in contact with the sample are properly cleaned to ensure the integrity of the sample. In general, samples are manually collected by first selecting a location in the waste stream that is well mixed then dipping the container in the water or wastewater stream, so the mouth of the container faces upstream. The container should not be overfilled if preservatives are present in the container.

7 Special Sample Collection Procedures

7.1 Organic Compounds and Metals

Trace organic compounds and metals detection limits are usually in the parts per billion or parts per trillion ranges, so extreme care must be exercised to ensure sample integrity. All containers, composite bottles, tubing, etc., used for sample collection for trace organic compounds and metals analyses should be prepared specifically for the parameter(s) being sampled.
When possible, the sample should be collected directly into the appropriate sample container. If the material to be sampled cannot be physically reached, an intermediate collection device may be used. This should be a Teflon7, glass or stainless steel (for nonmetals only) vessel on a pole or rope, or Teflon7 tubing via a peristaltic type pump and a Teflon7 vacuum container attachment which converts a sample container into a vacuum container. The device which is used should be cleaned specifically for the parameter(s) being sampled.
Sample collection for trace-level mercury analysis will be conducted in accordance with the procedure in US EPA Method 1669.

7.2 Bacteriological

Samples for bacteriological analyses must always be collected directly into the prepared glass or plastic sample container. The sample container should be kept unopened until it is to be filled. When the cap is removed, care should be taken not to contaminate the cap or the inside of the bottle. The bottle should be held near the base and filled to within about one inch of the top without rinsing and recapped immediately. During sample collection, the sample container should be plunged with the neck partially below the surface and slightly upward. The mouth should be directed against the current.
When the sample container must be lowered into the waste stream, either because of safety or impracticality (manhole, slippery effluent area, etc.), care must be taken to avoid contamination.

7.3 Immiscible Liquids/Oil and Grease

Oil and grease may be present in wastewater as a surface film, an emulsion, a solution or as a combination of these forms. Since it is very difficult to collect a representative sample for oil and grease analysis, the sampler must carefully evaluate the location of the sampling location. The most desirable sampling location is the area of greatest mixing. Quiescent areas should be avoided. The sample container should be plunged into the wastewater using a swooping motion with the mouth facing upstream. Care should be taken to ensure that the bottle does not over fill during sample collection.

Because losses of oil and grease will occur on sampling equipment, an automatic sampler should not be used to collect samples for oil and grease analysis. Individual portions collected at prescribed time intervals must be analyzed separately to obtain the average concentrations over an extended period.

7.4 Volatile Organic Compounds

Samples to be analyzed for volatile organic compounds (VOCs) should be collected in 40 mL septum vials with screw caps with a Teflon7-lined silicone disk (septum) in the cap to prevent contamination of the sample by the cap. Samples for VOC analysis must be collected using either stainless steel or Teflon® equipment.

When sampling for VOCs, triplicate samples should always be collected from each location. The investigator should determine if the water to be sampled contains chlorine. If the water contains no chlorine, three pre-preserved 40 mL vials should be filled with the sample. The samples may be held for up to 14 days before analysis. When preservation is not feasible, samples can be held up to 7 days before analysis. In the great majority of cases, the preserved vials are used to take advantage of the extended holding time. In some situations, however, it may be necessary to use the unpreserved vials. For example, if the wastewater sample contains a high concentration of dissolved calcium carbonate, there may be an effervescent reaction between the hydrochloric acid and the water, producing large numbers of fine bubbles. This will render the sample unacceptable. In this case, unpreserved vials should be used, and arrangements must be confirmed with the laboratory to ensure that they can accept the unpreserved vials and meet the shorter sample holding times.

If the water contains chlorine, collect the sample in an 8 ounce sampling container with two (2) drops of a 25% ascorbic acid solution. The 8 ounce sampling container should be prepared with acid should be prepared prior to sample collection). Cap and mix thoroughly but gently by swirling to eliminate residual chlorine. Transfer the sample to three pre-preserved 40 mL vials. The ascorbic acid and preservative must be added in this order and in two separate steps.

The 40 mL vials should be completely filled to prevent volatilization, and extreme caution should be exercised when filling each vial to prevent any turbulence which could also produce volatilization. The sample should be carefully poured down the side of the vial to minimize turbulence. As a rule, it is best to gently pour the last few drops into the vial so that surface tension holds the water in a "convex meniscus." The cap is then applied, and some overflow is lost, but air space in the bottle is eliminated. After capping, turn the bottle over and tap it to check for bubbles. If a bubble or bubbles are present, the vial should be topped off using a minimal amount of sample to re-establish the meniscus. Care should be taken not to flush any preservative out of the vial during topping off. If,

after topping off and capping the vial, bubbles are still present, a new vial should be obtained, and the sample re-collected.

8 Special Process Control Samples and Tests

During diagnostic evaluations, process control tests may be conducted to evaluate and troubleshoot the performance of the biological treatment processes of a municipal or industrial wastewater treatment facility. The EPA *Activated Sludge Process Control Manual* is the standard reference used by EPA inspectors for activated sludge process control testing. The manual includes a complete description of the step-by-step procedures for each test and the interpretation of the results. The six basic activated sludge process control tests are:

- Sludge settleability (settleometer)
- Centrifuge spins
- Aeration basin Dissolved Oxygen (DO) profiles
- Oxygen uptake rate (OUR) measurements
- Mixed liquor microscopic examinations
- Sludge blanket depth (SBD) measurements

Additional references are available that provide a more comprehensive evaluation of the methods used to conduct a diagnostic evaluation.

9 Biosolids Sampling

An important part of the Biosolids Program is based upon valid analytical data derived from relatively small samples. **The collection of a sample and its proper preservation during shipment is fundamental to obtaining reliable** analytical results.

The concentration of nutrients, pathogens, and pollutants in Biosolids are variable. In addition, pathogenic organisms are both time and temperature sensitive. Establishing a written protocol is important to collect samples that are both representative and consistent.

Analysis of pollutants (so-called 503 metals) and pathogens provide the basis for establishing Class B Biosolids. Nutrient concentrations are used to determine agronomic rates when Biosolids are land applied. In addition to sampling Biosolids, soil sampling at land application sites provides important crop nutrient data. The nitrogen status of the soil is combined with nitrogen of the Biosolids and the predicted crop uptake to develop the agronomic rates at which the Biosolids are applied to the land. Accurate assessment of soil nitrogen is dependent on good sampling techniques.

This sampling and analysis plan (SAP) will describe the processes involved with sampling Biosolids at a wastewater treatment plant. The intent is to be complete and concise so that sample collection, preservation, and shipment to a lab for appropriate analysis may be performed by personnel with little or no assistance outside this document.

9.1 Biosolids Sampling Protocol

A number of tools and personal protection equipment will be required to complete the task aseptically to avoid contamination of one's self or the sample. Collected samples will be placed in the appropriately sized hi-density polyethylene (HDPE) containers and chilled to 4^0 C using ice, blue ice, or dry ice.

9.2 Tools Required for Sample Collection

1. Nitrile gloves (be sure to have plenty on hand)
2. Sample containers from laboratory (properly labeled and dated HDPE containers; contact the laboratory at least two weeks prior to sampling event to request sample containers)
3. Ice chest with ice, "blue ice", or dry ice.
4. Shipping containers (may be the ice chest or other Styrofoam type container)
5. Sharpie® pens, ink pens, labels for sample containers
6. Chain-of-Custody forms
7. Stainless steel spatula or disposable plastic spatula for sample collection
8. Stainless steel bowl or container to composite grab samples
9. Packing tape
10. Packing material to fill air space within shipping container (air-filled bags, paper, etc.)
11. Shipping labels
12. Directions to nearest shipping location and drop-off times

9.3 Sampling for Nutrients, Metals, Total & Volatile Solids

1. Ensure that you have the appropriately labeled and dated sample containers before sample collection.
2. Put on your nitrile gloves.
3. The best place to collect Biosolids samples is typically where the material exits the belt-press or other dewatering equipment. (This sentence should be written to reflect the treatment plant's situation. Lagoons will need a sample map and description of some means for collection).
4. Using a gloved hand or clean spatula, collect and place 10-15 small, separate, grab-samples of Biosolids into a stainless bowl or container.
5. Mix the grab samples together thoroughly in the stainless bowl. The total sampled amount should be more than the amount needed to send to the lab.
6. Once the grab samples have been thoroughly mixed in the stainless bowl or container, take a portion of the total and place it in the lab supplied container. Fill the container so that the lab has enough Biosolids for the analysis.
7. Place the sample on ice and into the shipping container to begin preservation

9.4 Sampling for Fecal Coliform - 7 Samples Method

1. Ensure that you have the appropriately labeled and dated sample containers before sample collection.
2. Put on nitrile gloves.
3. The best place to collect Biosolids samples is typically where the material exits the belt-press or other dewatering equipment. (This sentence should be written to reflect the treatment plant's situation. Lagoons will need a sample map and description of some means for collection).
4. Using a gloved hand or a clean spatula, collect a single sample and place it in the lab supplied container.

5. Immediately place the sample on ice and into the shipping container to begin preservation.
6. Seven individual samples need to be collected over a two week period for fecal coliform analysis. This process needs to be repeated 7 separate times over 14 days. It does not have to be exactly every other day, but individual samples shall be taken on different days.
7. Fecal coliform samples shall arrive at the accredited lab conducting the analysis within 6 hours.

10 References

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Water Environment Federation and ASCE. 1998. Design of Municipal Wastewater Treatment Plants. Manual of Practice No. 8, Fourth Edition.

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TEMPERATURE and THERMOMETER CALIBRATION

SCOPE/APPLICATION:
Temperature readings are used in the calculation of various lab procedures and in general laboratory operations. Temperature measurements are generally made with glass or metal Celsius thermometers. As a minimum, the thermometer should be scale marked, or graduated, for every 0.5°C with markings etched on the capillary glass. The thermometer should have a thermal capacity to permit rapid equilibration. To assure accuracy, all thermometers should be checked annually against a precision thermometer with NIST certification or one that is traceable to NIST conforming specifications.

The type of thermometer used most frequently in the lab is the partial-immersion thermometer (PIT). It has a line around it at the immersion distance from the bottom. It indicates that the bulb and the liquid column to the line should be exposed to the temperature being measured and the remaining, emergent stem is to be at ambient temperature. The thermometer is most accurate when this line is correctly placed (when the line is even with the liquid's surface that the thermometer is being immersed in). Maintain partial-immersion thermometers in water or glycerol for all air incubators and refrigerators. In the drying oven, however, it is not possible to maintain the thermometer in a liquid immersion due to the excessive heat and evaporation of the liquid.

A second form of thermometer used in the laboratory is the infrared thermometer. This is a handheld, gun-like device that measures the thermal radiation emitted by an object's surface. The measured radiant energy is expressed in units of temperature. The only circumstance when it is permissible to use this form of temperature measuring is when it is not possible to use a probe-type thermometer. Some examples when infrared thermometers are used in the wastewater laboratory are 1) Measuring the temperature of the muffle furnace since its temperature is out of the measuring range of the probe-type thermometers employed in the lab, 2) Measuring the temperature of the HACH TNTplus vials since it is impossible to insert the probe-type thermometers into the vial openings, 3) Temperature of samples received in the laboratory and 4) Measuring the temperature of samples that need warmed to room temperature prior to analysis. In addition, other occasions may occur when the infrared thermometer is more useful for temperature measurement or if a sample is particularly "dirty" and temperature is NOT a permit-required analyte, the analyst may elect to measure temperature with the infrared thermometer in lieu of the PIT.

A third form of thermometer used in the laboratory is the sensor located on analytical electrodes, called thermistors. These are sensors on the electrode circuit that automatically measure and express temperature readings while measuring for analytical values. When thermistors are utilized, the analytical values are automatically corrected for temperature compensation. Thermistors are available for temperature measuring on the CBOD electrode and the pH electrode.

MATERIALS NEEDED:
5 Partial Immersion Thermometers
2 Electrode Thermistors
1 Drying Oven Thermistor

1 Infrared Thermometer
1 Clear, Glass Bottle
1 Double Thermometer Spacer

REAGENTS NEEDED:
Laboratory Reagent Water

PROCEDURE:
EQUIPMENT TEMPERATURES
1) Partial-immersion thermometers are to be permanently placed in the following pieces of laboratory equipment:

E. coli Incubator
Sample Refrigerator
Lab (Reagent) Refrigerator
CBOD Incubator

2) With the exception of the drying oven (temp too excessive for liquid immersion), all thermometers should be placed in a clear, glass bottle containing a rubber thermometer spacer. The bottle should be almost full of laboratory reagent water and the thermometer immersion line should be placed at the water's meniscus.

3) When calibrating the Drying Oven temperature display against the NIST-Certified thermometer, accuracy must be ascertained by eliminating the influence of ambient temperature change when the Drying Oven door is opened to view the NIST thermometer reading. Therefore, the thermometer must be placed in a clear, glass bottle containing a rubber thermometer spacer and the bottle must be filled with sand to the NIST thermometer immersion line in lieu of laboratory reagent water. Sand will hold the drying oven temperature long enough to record an accurate reading. Calibrate the Drying Oven against the NIST Certified thermometer every six months.

4) Record all equipment temperatures at the beginning of each shift on the Daily Operating Temperatures bench sheet. Ranges for accuracy are given at the head of each column. If any equipment temperature exceeds the allotted range, make a notation on the bench sheet and adjust the equipment accordingly. Be certain to monitor the out-of-range equipment for the remainder of the shift to ascertain that there is not equipment failure.

CALIBRATING THERMOMETERS
Every thermometer employed in the laboratory, regardless of type, must be calibrated annually against the NIST traceable thermometer. These thermometers and their calibration points include:

1. pH Probe (20.0°C)
2. CBOD Electrode (20.0°C)
3. Infrared Thermometer Gun (20.0°C, 6.0°C)
4. Room/Sample Thermometer (20.0°C)

   NOTE: Laboratory should be maintained at 20.0°C at all times.
5. *E. coli* Incubator (35.0°C)

6. Sample Refrigerator (6.0°C)
7. Lab/Reagent Refrigerator (6.0°C)
8. BOD Incubator (20.0°C)
9. Drying Oven (104.0°C)

1) Partial-immersion thermometers:

- Fill a clear, glass bottle almost full with laboratory reagent water (or sand). Place a double thermometer spacer in the liquid filled bottle and allow the water to stabilize to the equipment or room temperature.
- Place the thermometer in one space and the NIST traceable thermometer in the other space, following proper immersion procedure.
- Once the readings have stabilized, record each thermometer's reading in the Equipment Manual, noting a difference in readings if applicable.

2) Thermistors:

- Measure the temperature of a laboratory reagent water sample using the electrode.
- Measure the same laboratory reagent water sample using the NIST traceable thermometer.
- Record each thermometer's reading in the Equipment Manual, noting a difference in readings if applicable.

3) Infrared Thermometer:

- Measure the temperature of a laboratory reagent water sample using the infrared thermometer.
- Measure the same laboratory reagent water sample using the NIST traceable thermometer.
- Record each thermometer's reading in the Equipment Manual, noting a difference in readings if applicable.

Once the thermometer has been calibrated to its specific set-point(s) and the difference is noted in the Equipment Manual, it must be labeled. For partial-immersion thermometers, construct a "flag" that contains the data listed in this section and adhere the flag to the thermometer. NOTE: The thermometer itself must be labeled – NOT the piece of equipment in which the thermometer is located. For infrared thermometers or electrode thermistors, construct a label that contains the data listed in this section and adhere the label to the infrared thermometer gun or the meter that is connected to the electrode.

- Hazmat info for the thermometer
- Thermometer "name"
- Thermometer serial number
- Serial number of NIST spec conforming thermometer in which it was calibrated against
- Date of calibration
- Notation of correction (example being -2.0°C if the thermometer read 2.0°C high)

QUALITY ASSURANCE/QUALITY CONTROL:

1) Calibrate each thermometer annually except the *E. coli* incubator which is calibrated semiannually. Partial immersion thermometers are to be calibrated in January of each year. Electrode thermistors and infrared thermometers are to be calibrated in July of each year. The Drying Oven thermistor is to be calibrated in January and July of each year.

2) Calibrate the NIST specification conforming thermometer against an NIST traceable thermometer every five years. Calibrate the thermometer to the following set-points: 6.0°C, 20.0°C, 35.0°C, 104.0°C. Calibration should be done using an outside, professional calibration laboratory and should be presented with a certificate of calibration accuracy. The following laboratory is suggested for NIST calibration:

Laboratory Name: Indiana Standards Laboratory
Address: 2919 Shelby Street Indianapolis, IN 46203
Phone: (317) 787-6578

Parameters and Thermometer Calibration Frequency

CBOD	**TSS**	**pH**	**TS/VS**
1) Calibrate the probe thermistor against the NIST annually or when probe is installed, whichever is most recent. 2) Record incubator PIT temperature daily on Operating Temps Bench Sheet (OTB). 3) Calibrate incubator PIT annually against NIST.	2) Record drying oven temperature daily on the OTB. Temperature may be read via the digital display on the drying oven door. 3) Calibrate the drying oven thermistor twice annually against NIST.	1) Calibrate the probe thermistor against the NIST annually or when probe is installed, whichever is most recent.	1) Record the muffle furnace temperature on the OTB each day it's employed. 2) Use the infrared thermometer to temp the muffle furnace.
E.coli	**Ammonia/Phosphorus**	**Samples**	**Infrared Thermometer**
1) Record incubator PIT temperature daily on the OTB. Thermometer may be read without opening the incubator door. 2) Calibrate incubator PIT semiannually against NIST.	1) Record the lab fridge PIT temperature daily on the OTB. This is where the TNT vials are stored. 2) Use the infrared thermometer to temp all TNT vials. 3) Calibrate lab fridge PIT annually against NIST.	1) Use the PIT Sample Thermometer to temp samples. Wipe the PIT off with an alcohol swab after each probe. 2) Sample temps should be between 18.0°C and 22.0°C. 3) Record sample fridge PIT temperature daily on Operating Temps Bench sheet (OTB). 4) Calibrate sample fridge and sample PITs annually against NIST.	1) Calibrate the thermometer annually against the NIST for the following set points: 6.0°C 20.0°C

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