Wisconsin Department of Natural Resources
Wastewater Operator Certification

Intro to On-Site Laboratory Testing Study Guide

January 2010 Edition (Revised January 2013)

Subclass J

Wisconsin Department of Natural Resources
Bureau of Science Services
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Preface

This operator's study guide represents the results of an ambitious program. Operators of wastewater facilities, regulators, educators and local officials, jointly prepared the objectives and exam questions for this subclass.

January 2013 Revisions: Minor revisions were made to the January 2010 edition of this study guide. The following key knowledges have been updated: 1.2.7, 2.3.1, 3.3.5, 4.2.2, 5.2.1, 5.2.2, 5.2.4, 5.2.5, 5.2.6, 5.2.7, 5.2.11, 5.2.12, 5.2.20, 5.2.22, 5.2.25, 5.2.29, 5.2.30, 5.3.2, 5.3.6, 5.3.8, 5.3.9, 5.3.13, 5.4.2, 5.4.10, 5.5.1, 5.5.7, 5.6.4

How to use this study guide with references

In preparation for the exams you should:

1. Read all of the key knowledges for each objective.

2. Use the resources listed at the end of the study guide for additional information.

3. Review all key knowledges until you fully understand them and know them by memory.

It is advisable that the operator take classroom or online training in this process before attempting the certification exam.

Choosing A Test Date

Before you choose a test date, consider the training opportunities available in your area. A listing of training opportunities and exam dates is available on the internet at http://dnr.wi.gov, keyword search "operator certification". It can also be found in the annual DNR "Certified Operator" or by contacting your DNR regional operator certification coordinator.

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Chapter 1 - Safety

Section 1.2 - General Safety

1.2.1 Discuss personal protective equipment in a wastewater laboratory.

Always wear safety glasses, goggles, face shields or eye glasses when handling wastewater samples, acids or other chemicals. Eye protection can prevent serious injuries and illness. Protective lab-wear (laboratory coats or aprons) should be worn in the laboratory when ever handling wastewater samples or chemicals. Protective lab-wear should be removed before leaving the laboratory.

1.2.2 Discuss general safety practices in a wastewater laboratory related to eating, drinking and smoking in the laboratory.

Eating, drinking, smoking, chewing gum and applying makeup in the laboratory is not recommended. Pathogens such as hepatitis, typhus, giardia, cryptosporidium, and toxic chemicals could contaminate food, beverages, cigarettes, gum and makeup and be ingested. This can lead to illness and even death. Conversely, food, cigarettes and drinks can also contaminate samples and lead to erroneous results. Tests particularly susceptible to contamination are BOD, TSS and phosphorus. Smoking can also contaminate samples.

1.2.3 Discuss work area safety in a wastewater laboratory.

Keep work areas clean and uncluttered, with chemicals and reagents clearly labeled and stored. Always clean up work areas on completion of lab activities each day.

1.2.4 Discuss fume hoods and ventilation in the laboratory in a wastewater laboratory.

Concentrated acids and solvents should only be used in fume hoods. As a general rule, use a fume hood for any operations that might result in the release of toxic chemical vapors. Total phosphorus digestion should be done in a fume hood or well ventilated area. Muffle furnaces and drying ovens should be vented.

1.2.5 Discuss general pipetting practices in a wastewater laboratory.

NEVER mouth pipet wastewater samples or chemicals. Always use mechanical pipet aids or automatic pipettors.

1.2.6 Discuss how spills of any kind should be dealt with in a wastewater laboratory.

Spills should be cleaned up promptly to avoid injuries. Slips and falls account for a high percentage of industrial accidents and the majority of accidents in wastewater treatment plants. Chemical spills such as acid, should be neutralized with an appropriate "Spill Kit" as part of the clean up process.

1.2.7 Discuss the food storage concerns in a wastewater laboratory.

Food and beverages must not be stored in refrigerators or areas of the laboratory where samples are stored or testing activities take place. Again, pathogens and toxic chemicals could contaminate food and beverages.

1.2.8 List the general safety equipment necessary for the safe operation of a wastewater treatment plant laboratory.

- Eyewash
- Emergency shower
- Spill clean up kit
- Fume hood
- Appropriate chemical storage area

1.2.9 List the personal safety equipment necessary for the safe operation of a wastewater treatment plant laboratory.

- Lab coat or lab apron
- Safety glasses or face shield
- Gloves appropriate for activity

1.2.10 Describe how to safely perform each of the following laboratory tasks:

A. Mixing acid and water.
B. Making glass-to-rubber connections.
C. Using a pipet.

A. Mixing acid and water: Always pour acid into water, and swirl or mix to dissipate the heat of the reaction. This activity should be performed in a fume hood or in a well ventilated area.

B. Making glass-to-rubber connections: Wear heavy gloves in case the tubing slips (and breakage occurs), moisten the connecting ends with water, and use a twisting motion to make the connection.

C. Pipet use: Do not mouth pipet.

1.2.11 Discuss the general concept of a wastewater treatment plan laboratory chemical hygiene plan.

A chemical hygiene plan is a written program developed and implemented by an employer that establishes procedures, equipment, personal protective equipment and work practices that are capable of protecting employees from health hazards presented by hazardous chemicals used in the workplace. Employee information and training is a critical element in the chemical hygiene plan. A chemical hygiene plan should be an integral part of the wastewater treatment plant's safety and health program. All chemical testing laboratories are required to have a chemical hygiene plan (29 CFR 1910.1450).

1.2.12 Discuss the intent of material safety data sheets (MSDS).

Material Safety Data Sheets (MSDS) are designed to provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS's include information such as physical data (melting point, boiling point, flash point etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill/leak procedures. These are of particular use if a spill or other accident occurs. Wastewater treatment plants must have MSDSs for chemicals used at the facility to comply with the Federal Hazard Communication Standard 29 CFR 1910.1200.

1.2.13 Discuss the reasons why muffle furnaces and drying ovens should be vented to the outside or to a fume hood.

This has safety and sample contamination implications. Volatiles driven off during the drying process could consist of hazardous substances which may pose a health risk for those working in the lab. Forced air ovens could aerosolize bacteria and viruses which could expose workers to disease causing organisms. Moisture driven off during sample drying can create a corrosive environment which can cause premature instrument failure. These same substances could also contaminate samples. For example, drying raw wastewater could result in venting ammonia into the air causing samples and blanks to become contaminated.

Section 1.3 - Chemical Safety

1.3.1 Discuss safe storage of laboratory chemicals.

- Chemicals must be stored in a cool dry location out of direct sunlight.

- Storage in a cabinet is recommended.

- Liquid chemicals should be stored below human face level to avoid splattering in case of bottle spillage or breakage.

- Chemicals must be dated upon receipt and the expiration date recorded as well.

- Acids should be carefully stored separately from other chemicals at floor level.

- The operator should always check the label on a chemical container for information regarding hazards with the use or storage of that chemical.

- Keep all Material Safety Data Sheets (MSDS) that come with the chemicals available for review for health, handling and disposal information.

- Chemicals should never be stored in unmarked containers.

- All reagents that are prepared and stored in separate containers should be labeled.

Chapter 2 - Sampling and Sample Handling

Section 2.1 - Definitions

2.1.1 Define Grab Sample

A grab sample represents a single instant in time. It is subject to much more fluctuation than a composite sample. Generally, a grab sample is only used if the sample holding time would significantly change the parameter being tested, or if measurement of a slug loading is desired.

2.1.2 Define flow proportional composite sample

A composite sample represents a longer period in time. It is far more likely to represent the average quality of the water being received or discharged, especially if waste characteristics change with time. Flow proportional composite samples are required for most wastewater plants. The exception is pond effluent, which is thought to have such a long detention time that the variations of loading do not affect it.

Section 2.2 - Sample Collection

2.2.1 Explain the difference between grab and flow proportional composite samples.

Grab samples are used for determining instantaneous values and to identify extreme conditions. Municipal wastewater tests requiring grab samples include: pH, chlorine residual, dissolved oxygen, and, fecal coliform bacteria. Other tests (for industrial wastewater or under toxics regulations) requiring grab samples are: oil and grease, volatile organics, and cyanide.

Composite samples are generally appropriate for the calculation of loading rates. Normally, in municipal wastewater, the tests in this category include BOD, suspended solids, phosphorus, and ammonia.

2.2.2 Discuss the difference between time proportional composite and flow proportional composite samplers.

Time proportional composite sampling is when an automatic sampler is programmed to pull an aliquot sample from the flow stream at predetermined time intervals, such as every 15 minutes. If using time proportional composite sampling, periods of high strength waste flows can be missed during time intervals between samples. When using flow proportional composite sampling, the automatic sampler pulls an aliquot sample from the flow stream directly proportional to the flow rate.

Plant flow meters send an electronic "pulse" to the sampler to indicate a set amount of flow, such as a pulse for every 1,000 gallons. Then the sampler is programmed to pull a set amount of sample at a certain flow pulse interval such as 100 mL every 10 pulses or every 10,000 gallons.

Flow proportional compositing is the most representative way to sample and is required by the Wisconsin DNR.

2.2.3 Describe the guidelines to ensure proper collection of a representative sample.

The WPDES permit states the location where samples must be taken, what type of samples are to be taken, at what frequency, what samples are taken, and which parameters must be tested. For example whether the sample should be flow composite, time composite or grab.

SAMPLER PROBE PLACEMENT CRITERIA
A. In well mixed area
B. In the center of the waste stream
C. Off of the floor of the flume/channel
D. Not against the sides of the channel

It is very important that the flow channel near any metering or measuring device be kept very clean. Debris or grit buildup in a channel can give a false reading, as the channel depth may be affected or the detention time and flow rate changed. Also, grease scum and debris will alter readings when using pressure or ultrasonic (transponder) type devices.

2.2.4 Describe the cleaning and preparation of sample containers and devices.

SAMPLER CLEANING

The sampler should be cleaned twice monthly using the following procedure:

1. Pump HOT tap water through the tubing. Then run the sampler for at least two (2) minutes.

2. Rinse the tubing by pumping a 10% household bleach for at least two additional minutes. Discard the used bleach solution in a safe manner. For phosphorus and metals a 20% hydrochloric acid or a 20% nitric acid rinse may be necessary. This acid rinse may be reused up to four (4) times before it is discarded. Safety precautions are used in the handling and disposal of these acid solutions (wear safety gloves and glasses).

CAUTION: Do not mix the acid and bleach together.

3. Re-rinse the tubing again by pumping HOT tap water through it for at least two (2) minutes.

4. Rinse the tubing by pumping lab reagent water through the system for at least one (1) minute. After one minute, stop the pump and allow the water to stand in the hose for an additional minute. After this minute, continue pumping the water for one (1) final minute. The lab reagent rinse is NOT re-circulated. To ensure the bottles are free of materials that may contribute to the BOD, a weak solution of household bleach (50 mL per 2 L of lab reagent water) can be used as a final step in the cleaning procedure. The final rinse, however, must be sufficient to eliminate any traces of the bleach, which may kill natural seed organisms, leading to low bias in BOD values.

The sampler itself is subjected to a good general washing. Clean the pump, sample container(s), and any internal sampler parts which come into contact with the wastewater. First wash the sampler parts with tap water. Next, vigorously scrub all parts with hot tap water and a non-phosphate detergent. Triple rinse (i.e. rinse and drain, rinse and drain, rinse and drain) these items with tap water after washing. Finally, follow this process up with three (3) rinses using lab reagent water.

2.2.5 Explain, for DMR purposes, how you determine the sample date.

The day on which the majority of the sample was collected is the date of the sample.

For example, if George started the composite sampler at 7am on July 10th and removed the sample from the autosampler at 7am on July 11th, the results of that sample on the DMR would be the July 10th sample since most of the sample was collected on July 10th.

2.2.6 List the information to be recorded and retained for each sample.

For each sample or sample container the following information must be recorded:

A. The sample type (flow proportional composite, time proportional composite, grab, etc).
B. The sample point (where taken).
C. Unique sample identification.
D. The time and date of the sampling (grab) -or- time and date of the first and last sub-samples for a composite sample.
E. Any preservation during sampling - chemical added, refrigerated, etc.
F. The initials of person sampling.

It is also recommended that the operator record sample description (color, odor, turbidity, or other unsual observations). The information recorded on sample containers must be sufficient to trace it back to the sampling information.

2.2.7 Explain how to sub sample or split a wastewater sample

Most composite autosamplers collect wastewater into a single large jug, generally 2-3 gallons in size. When the facility is required to test their wastewater for more than one constituent that requires a different preservative (e.g., metals, phosphorus, suspended solids), it is necessary to sub-sample or "split" the wastewater into one or more containers so the appropriate preservatives may be added. This process must be done in such a way to assure that each sub-sample is identical to the other. Mixing is the most critical step in the sub-sampling process. When the following procedure is followed precisely sub-samples will be homogeneous.

1. Determine the number of bottles needed based on the tests and preservatives required. For example, if metals, phosphorus, ammonia, BOD and suspended solids are needed, you will need at least 3 bottles (see below).
   A. Metals - nitric acid preservative
   B. Phosphorus and ammonia - sulfuric acid preservative
   C. BOD and suspended solids - cooling to less than or equal to 6°C only
2. Remove caps and place bottles in a sink.
3. Thoroughly mix the 2-3 gallon autosampler jug by inverting at least 10 times.
4. Open the lid and quickly fill each bottle about 1/3 full. Don't worry about spilling on the outside of the bottle. It is very important that this be done quickly to make sure solids remain suspended while pouring.
5. Re-cap the jug and mix again by inverting at least 10 times.
6. Open the lid and quickly fill each bottle to about 2/3 full.
7. Repeat steps 5 and 6 to fill the bottles. Remember to leave enough room in the bottles to add preservative.
8. Remove the bottles from the sink, add the appropriate preservative, mix and then wash off the outside of the bottles to remove residual wastewater that splashed on the outside of the bottle during the sub-sampling process.

Other commercial devices such as churn-splitter, cone-splitter, or large scale magnetic stirrers may be used.

The same process may be used to prepare “split” samples. Split samples are samples that are divided into two or more equal sub-samples, each of which is submitted to one or more laboratories for identical analysis. Split samples are used to:

1. Assess the variability from sample processing and preservation.
2. Assess the precision of laboratory analysis by allowing a comparison of analytical results from two parts of the same sample from the same location.
3. Verify compliance with discharge permits when “split” with Wisconsin Department of Natural Resource staff during compliance inspections.

Split samples must be prepared and analyzed for the same parameter(s) by the same method(s) to demonstrate the reproducibility of the split sampling and analytical techniques.

Section 2.3 - Sample Preservation

2.3.1 Identify the maximum holding times and preservation methods for the following samples:

A. Biochemical Oxygen Demand (BOD)
B. Total Suspended Solids (TSS)
C. Ammonia
D. Total Phosphorus
E. Fecal Coliform

A. Sample: BOD
Preservation Method: Cool to 6°C, or less without freezing
Maximum Holding Time: 48 hours after the end of the compositing period

B. Sample: TSS
Preservation Method: Cool to 6°C, or less without freezing
Maximum Holding Time: 7 days

C. Sample: Ammonia
Preservation Method: Add sulfuric acid(H_2SO_4) to pH<2 and cool to 6°C, or less without freezing
Maximum Holding Time: 28 days

D. Sample: Total Phosphorus
Preservation Method: Add sulfuric acid(H_2SO_4) to pH<2 and cool to 6°C, or less without freezing
Maximum Holding Time: 28 days

E. Sample: Fecal Coliform
Preservation Method: Add sodium thiosulfate if sample was chlorinated and cool to 6°C, or less without freezing
Maximum Holding Time: 6 hours

Samples should be refrigerated at a temperature not to exceed 6°C and must not be frozen.

Chapter 3 - Lab Equipment and Instrumentation

Section 3.1 - Definitions

3.1.1 Define Volumetic Flask.

A volumetric flask is a piece of laboratory glassware used in analytical chemistry for the preparation of solutions. It is made of glass or plastic and consists of a flat bottomed bulb with a long neck, usually fitted with a stopper. The stopper is normally made in a chemically resistant plastic such as polypropylene rather than glass. The neck has a single ring graduation mark and a label. The label should show the nominal volume, tolerance, calibration temperature, class, relevant manufacturing standard and the manufacturer's logo.

Volumetric flasks generally come in two different standards. The higher standard flasks (Class A) are made with a more accurately placed graduation mark, and have a unique serial number for traceability. Where this is not required a lower standard (Class B or equivalent) is used for qualitative or educational work.

3.1.2 Define beakers & Erlenmeyer flask.

An Erlenmeyer flask is a widely used type of laboratory flask which features a conical base with a cylindrical neck. They are usually marked on the side (graduated) to indicate the approximate volume of their contents.

The Erlenmeyer flask's counterpart is the beaker. However the main difference is its narrow neck. The neck allows the flask to be stoppered and allows the contents to be swirled or stirred during an experiment (as is required in titration); the narrow neck keeps the contents from spilling. The smaller neck also slows evaporative loss better than a beaker making it a excellent choice for digestion tests such as total phosphorus. Additionally, the flat bottom of the flask makes it unlikely to tip over.

A beaker has straight sides and a curved lip to aid in pouring. The beaker is shaped more like a clear coffee mug without the handle. It also has graduation marks on the side to indicate approximate volume. Water will evaporate quicker in a beaker because it has a larger surface area.

The volume graduation marks on the beaker and the Erlenmeyer flask are approximate only. Neither should be used to measure volume for an analytical test. The marks are only intended to give approximate volumes. For example, the graduation marks on a Erlenmeyer flask are useful for determining when enough sample has evaporated during the total phosphorus digestion.

3.1.3 Define graduated cylinder.

A graduated cylinder is a type of laboratory glassware comprised of a tall cylinder with a range of calibrated markings that is used for visually measuring the volumes of liquids in a quantitative manner. A graduated cylinder can be made of translucent plastic or borosilicate glass such as Pyrex. Graduated cylinders are available as either Class 'A' or 'B' glassware. Graduated cylinders are recommended for measuring the larger volumes (greater than 100

mL) of sample required for the BOD and suspended solids tests.

3.1.4 Define volumetric pipets (mechanical or glass) micro-bore.

Volumetric pipets are calibrated to deliver a fixed volume. The volumetric pipet has a single graduation that allows it to deliver one specific volume to Class A or Class B tolerances. Class A volumetric pipets are most accurate and should be used when ever possible. Class B may be used for less accurate measurements such as adding preservatives. Micro Bore volumetric pipets should not be used for pipetting samples containing particulate matter because they will exclude particles which are representative of the sample.

3.1.5 Define volumetric pipets- macro-bore (wide-bore).

The volumetric pipet has a single graduation that allows it to deliver one specific volume to Class A or Class B tolerances. These pipets have a wide-bore opening which are useful when transferring samples that contain particulates such as raw wastewater. This type of pipet should be used for tests such as the BOD and phosphorus when less than 100 mL of sample is needed.

3.1.6 Define Mohr pipets.

Mohr pipets are measuring pipets in which the graduations stop well BEFORE the tip. Serological pipets are very similar to Mohr pipets except the graduations continue to the tip of the pipet.

Mohr pipets are intended to indicate the delivered volume of liquid by the difference between the initial and final liquid position, with delivery of the maximum calibrated volume leaving the tip of the pipet full of liquid. Mohr pipets come in calibrations with Class A and Class B tolerances.

3.1.7 Define serological pipets (narrow-bore).

Serological pipets are measuring pipets in which the maximum calibrated volume INCLUDES the tip. Serological pipets are very similar to Mohr pipets except, in Mohr pipets, the graduations stop before the tip of the pipet.

A serological pipet is graduated from a zero mark near the top of the pipet to the very tip of the pipet. It can be used to indicate the difference between the initial and final liquid levels similar to the Mohr pipet. However, to deliver the whole calibrated amount, the pipet is blown out with a pipet bulb such that no liquid remains in the tip.

3.1.8 Define serological pipets (wide-bore).

Wide-bore serological pipets are often used in the BOD test to measure sample volumes that are not available in the wide-bore volumetric pipets (e.g., 7, 8, 9, 12, 13 mL, etc.)

Section 3.2 - General Labware

3.2.1 Discuss the appropriate use of:

A. Volumetric Flasks
B. Beakers & Erlenmeyer Flasks
C. Graduated Cylinders
D. Volumetric pipets (mechanical or glass) micro-bore
E. Volumetric pipets- macro-bore (wide-bore)
F. Mohr Pipets
G. Serological pipets (wide-bore)

A. Volumetric Flasks
Volumetric flasks should always be used when making standards, particularly for calibration.

B. Glass beakers or Erlenmeyer flasks
Digestions. NOT for standard preparation or anything requiring volumetric measurements.

C. Graduated Cylinders
BOD & TSS when using 50 mL or more of sample.

D. Micro-bore Volumetric pipets (glass or mechanical)
Standard preparation.

E. Wide bore volumetric pipets
BOD & TSS samples.

F. Mohr pipets
Color reagent (phosphorus) - if using the North Central Laboratories modification.

G. Serological pipets (wide-bore)
BOD influents (low volume samples) also preservation of samples for phosphorus or ammonia.

Figure 3.2.1.1

3.2.2 Discuss the relative merits of various pipet aids:
A. Non Valve Pipet Bulb
B. Wheel Driven Pipet Aid
C. Valve Pipet Bulb
D. Pistol-grip Pipet Aid

Various pipet aids are available to assist analysts in the correct use of pipets. Each type has advantages and disadvantages. Ultimately, the decision of which type to use is analyst dependent. Caution should be used to avoid drawing liquid up into the pipet aid.

A. Non Valve Pipet Bulb

The bulb is placed onto the pipet and then squeezed prior to introduction into the sample. When the bulb is released, sample is drawn up into the pipet. Analyst must quickly remove bulb from the pipet and place finger over opening and then manually lower sample volume to graduated mark.

B. Wheel Driven Pipet Aid

A modification to the basic bulb-type pipet aid. A thumbscrew is used to raise and lower level of the sample into the pipet.

C. Valve Pipet Bulb

The three valves provide a convenience not found in the standard bulb, however, many people require practice using the bulb before using the bulb to deliver a liquid during an

experiment. Valves are typically labeled 'A' (Aspirate, or create a vacuum), 'S' (Suction, to draw sample up into the pipet) and 'E' (Expel, to dispense the sample). The valves can be used to get the sample volume level with the calibrated mark.

D. Pistol-grip Pipet Aid

Designed for one-handed operation. The trigger expels the entire contents at one shot, or you can use the thumb wheel for precision dispensing.

Figure 3.2.2.1

3.2.3 Discuss the types and proper use of pipets, including which should be "blown out" and which should be "drained" out.

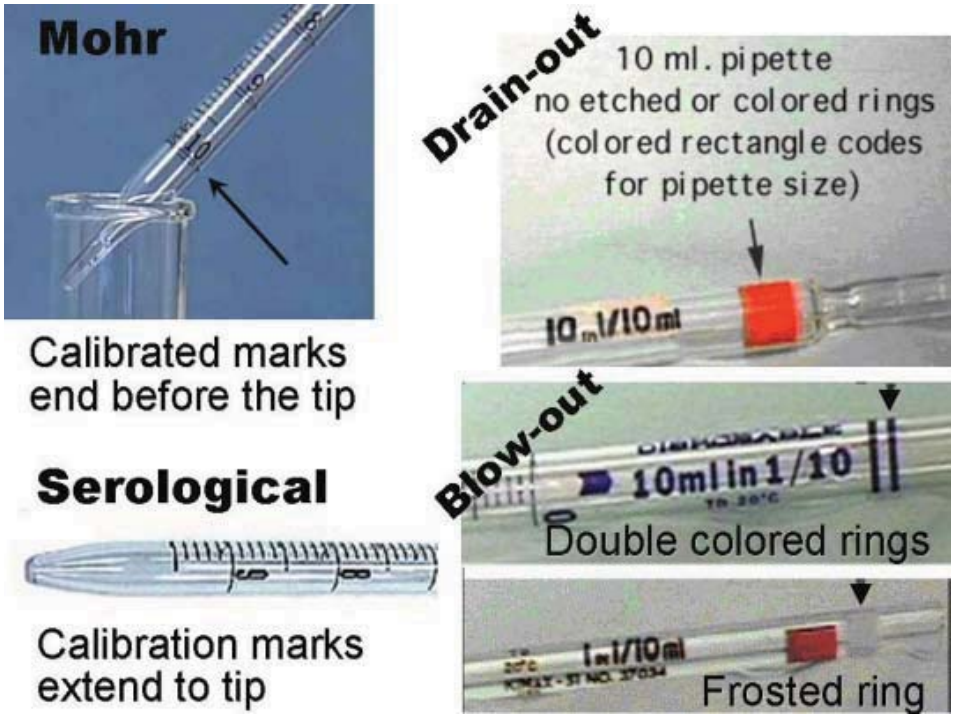
Volumetric pipets are classified as "TD" (To Deliver; the new international designation is "EX") and should never be blown out. These pipets are calibrated to deliver one specific volume and the calibration already takes into consideration the small amount of volume that may remain in the tip. Volumetric pipets may have a colored square at the top of the pipet which is a manufacturer's mark indicating the volume of the pipet.

Serological pipets can be used to measure various volumes from a single pipet due to their volume gradations. There are two kinds of serological pipets,"blow out" and "drain out". Serological pipets from which the volume in the tip should be "blown out" typically are graduated right up to include the entire tip. These pipets are usually identified by the presence of two thin colored or etched (frosted) rings at the top of the pipet.

Serological or Mohr pipets which should be "drained out" typically have their graduated markings stop prior to the tip. Volume should not be drained below the last marking. These pipets are usually identified by the presence of a color-coded rectangle or square (but no

frosted or colored rings) at the top of the pipet.

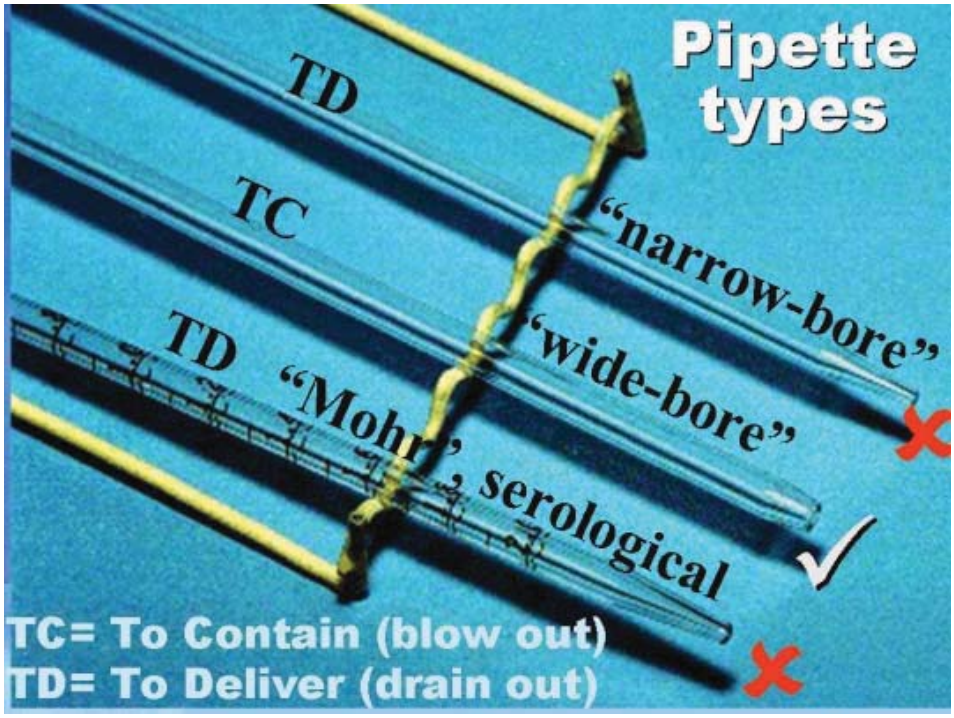
Figure 3.2.3.1



3.2.4 Describe the type of pipets used in BOD and TSS tests.

Only wide bore volumetric pipets should be used for the BOD and TSS tests, other names for these pipets include macro bore, wide mouth tip. Volumetric factory made glassware should not be altered.

Figure 3.2.4.1



3.2.5 Discuss why a single larger volume pipet is more appropriate for measuring volumes than two smaller volume pipets.

1. Use ONE pipet for the job because error is additive.

2. If you pipet 10 mL by using a 5 mL pipet twice, you get double the error that you would using a single 10 mL pipet.

Section 3.3 - Support Equipment

3.3.1 Explain the function of an incubator in a wastewater treatment plant laboratory.

An incubator is an enclosed chamber used in a wastewater treatment plant laboratory to provide a constant temperature in a controlled environment. It is used to maintain required conditions for the development of microbial or chemical analyses.

3.3.2 Explain the function of a desiccator in a wastewater treatment plant laboratory.

A desiccator is used to maintain a humidity-free environment. Oven-dried samples, glassware, and some chemicals, should be stored in a desiccator before weighing to prevent moisture absorption. Absorbed moisture adds weight to the samples, causing incorrect weight measurements. In order for a desiccator to work properly it must contain color indicating desicant and the lid must have a silicone based grease seal.

3.3.3 Describe the color of indicating desiccant.

The color of indicating desiccant changes from blue to pink as it absorbs moisture. Blue silica gel changes from deep blue to pale pink as it absorbs moisture. Once the material turns pink, the desiccant must be re-generated by heating in a drying oven at the

manufacturer-specified temperature to drive off absorbed moisture.

3.3.4 List the conditions that make it difficult for a drying oven to maintain its temperature.

- Repeated opening and closing of the oven door
- An overloaded oven (too many samples for the size of oven)
- Oven defects (a poor door seal, etc.)
- An inadequate or defective thermostat
- Placement under air vent

3.3.5 Discuss the two major types of balances that are used in a wastewater laboratory.

Analytical balance: Analytical balances are most often found in a laboratory where extreme sensitivity (0.1 mg) is needed for the weighing of items. Analytical balances usually measure mass and range in capacity from 0.1 mg to 150 grams. They are typically used for the suspended solids testing in the wastewater laboratory where weight changes of 0.1 mg must be measured.

General purpose or top-loading balances: Another balance used primarily in a laboratory setting. They usually can measure objects weighing around 150-300 g. They offer less readability and accuracy than an analytical balance but allow measurements to be made quickly thus making it a more convenient choice when exacting measurements are not needed. Top-loaders are also more economical than analytical balances and suitable for weighing chemicals when preparing reagents.

3.3.6 Discuss the proper operation and care of a toploader balance.

General Instructions: The operation of a modern electronic top-loading balance is usually very simple and convenient. Most have on-board software and an internal weight to automatically calibrate the balance. This type of balance can generally weigh materials to two or three decimal places depending on the model and price (e.g., 0.01 to 6200 g). If the components of an electronic balance are cold when you start, they will drift while warming up, causing changes in your measurements. Avoid this problem by leaving balance on at all times or by allowing the balance to warm-up before use.

Balance verification – Required at least once per month using one weight in the appropriate range of use.

1. Make sure the balance is placed on a solid and rigid support bench, free from vibrations and shielded from air currents, excess humidity and varying temperature.

2. Check to make sure the balance is level (observe bulls-eye level located near the digital display - bubble should be inside black ring). If not, adjust feet to level.

3. Check if the balance is turned on. If not press the on/off button and wait until the display reads zero (0.000 or 0.00). Check the balance manual to determine the warm-up time required.

4. Select the calibration mode if the balance has this function and/or check the calibration

with ASTM Type 1 weights.

5. Verify the calibration using two external weights to bracket your material that you wish to weigh (select the weight in the mg range and one weight in the gram range).

6. Place a weighing dish or pan on the balance pan and press the TARE button (Do not weigh chemicals directly on the pan. Always use weigh boats or glassine paper). That sets the balance to weigh anything that is placed into the container, but it does not weigh the container itself. When "0.000 or 0.00" shows in the display, it is time to add what you want to weigh to the dish.

7. After the material has been added to the container, record the weight of the material in the container. Then remove the container, clean up any spills, reset the balance to zero, and close the cover.

8. Refer to the Owner's Manual for your balance if you have questions about the use of the balance.

3.3.7 Discuss the proper operation and care of an analytical balance.

The operation of a modern electronic analytical balance is relatively simple and convenient. Virtually all modern analytical balances have on-board software and an internal weight to automatically calibrate the balance. This type of balance is a high quality, precision instrument that can typically measure mass in the range of 0.0001 to 100g. If the components of an electronic balance are cold when you start, they will drift while warming up, causing changes in your measurements. Avoid this problem by leaving balance on at all times or by allowing the balance to warm-up before use.

Balance verification – Required at least once per month using one weight in the gram range and one in the milligram.

Keep the following in mind:

- Electronic balances require adequate warm-up time. If using an electronic balance, follow manufacturer instructions for warm up.

- Balance must be situated on a sturdy foundation. If you observe instability in balance readings, investigate the need for additional equipment such as damping pads, static eliminators or reducing drafts.

- Protect the balance from vibration. Do not bump the balance or table while making measurements. Vibration can significantly affect measurement accuracy, even leaning on a balance table without a damping pad can affect results by several milligrams.

- Balance must be level to function properly. Check the balance bulls-eye (spirit) level to make sure bubble is inside the target.

- Balances need controlled temperature & humidity. Wide swings in humidity & temperature can damage the sensitive parts and electronics. Balance should be placed away from direct sunlight.

- The pan and balance floor must be clean. Use a camel-hair brush (keep near balance). Dust off the balance pan with a clean soft (preferably camel-hair) brush. Use a mild detergent, lab reagent water and lint-free wipe if necessary.

- Samples being weighed must be at room temperature. Warm or hot objects placed in a pan within a closed balance chamber can create air currents that buoy the pan, resulting in erroneous measurements.

- Do not weigh chemicals directly on the pan. Always use weigh boats or glassine paper.

- Sliding doors must be closed when making measurements. Avoid the effects of any draft or air currents on the balance.

- Have the balance checked by a professional. Have balance serviced annually.

3.3.8 Discuss the requirements for and use of certified weights in the laboratory.

Certified weights are used to verify the calibration status of laboratory balances. These weights must be certified to be ASTM Type 1 (formerly Class "S"). Type 1 weights are made from high grade stainless steel. Type 2 (formerly Class "P") are typically made of brass. Type 2 weights should not be used for analytical balance calibration verification, as brass is a soft metal alloy that is subject to oxidative pitting, tarnishing, and scratching. These conditions will result in significant changes to the standard's certified weight. Have weights re-certified at least every 5 years. Remember that these are precision calibration tools. Putting two or more of them in a plastic vials and letting them roll around against each other is NOT appropriate. Never use a metal forceps or your fingers to handle certified weights. Use a plastic forceps to avoid scratching of the weight surface.

3.3.9 Discuss the proper handling, care and storage of certified ASTM Type 1 weights.

Weights can become scratched, wear, corrode from atmospheric conditions and change in weight over time. As a result, industry and certification authorities require weights to be cleaned and re-certified on a regular basis. When ASTM Type 1 weights are purchased they are certified traceable to standards provided the National Institute of Standards and Technology (NIST). This process must be only be done by a certified metrology company.

- When not in use weights should be stored in the original case to keep them as free from contamination as possible.

- Handle Type 1 weights with plastic tipped forceps to prevent contamination of the weight surface from skin acids, and to reduce the effects of heat from the operator's hand.

- Wear soft cotton gloves or soft chamois leather gloves when handling larger weights (for example, weights heavier than 100 grams). Never handle weights with your bare hands

because skin acids and figure prints will damage the weights and change their mass.

- When using weights, it is essential to prevent damage to the surface of weights through metal-to-metal contact, either with other weights or with the pan. Acid-free tissue paper may be used to stand weights on when in use.

- Weights should not be stacked on top of each other or come in contact with other weights.

- If necessary, weights may be cleaned by lightly dusting them with a brush specifically designed for weights such as a soft camel hair brush.

- Always return weights to the original storage box after use.

3.3.10 Discuss the process used to establish acceptance criteria for certified weights.

Weight manufacturers generally recommend a method for determining acceptable tolerances using a statistical approach based on replicate weight measurements on your balance. Until you have generated this data, establish some reasonable tolerances, USE them and TAKE corrective action if they are exceeded.

Use the following INTERIM guidelines until statistical limits have been established:

± 0.3 mg for weights <10mg
± 0.5 mg for weights from 10mg to 100mg
± 1-2% for weights >100 mg

Establish FIRM statistical tolerances as time permits according to the following process:
1. Make 20 replicate measurements of each weight
2. Spread measurements out over several non-consecutive days for best results
3. Determine the mean and standard deviation (sd)
4. The upper acceptable range = certified weight + 3 sd
5. The lower acceptable range = certified weight - 3 sd

3.3.11 Discuss the selection of weights used for verification of calibration of an analytical balance.

The laboratory certification program requires labs to verify the calibration of their analytical balance at least monthly using a minimum of one Type 1 weight in the gram range, and a second Type 1 weight in the milligram range.

Select the weight close to the weight that you typically measure.

For example:
If using a Gooch crucible, use a 20-g or 50-g Type 1 weight.
If using a filter pad and pan, use a 100mg Type 1 weight.

3.3.12 Discuss the process used to verify calibration of an analytical balance.

Calibrate balance daily if it is equipped with an on-board internal calibration feature.
Check balance accuracy monthly in the gram (g) and milligram (mg) ranges.

Use certified Type 1 (formerly Class "S") weights.

The calibration verification procedure is as follows:

1. Perform the internal calibration process if the balance is so equiped.
2. Zero the balance by pressing the "tare" bar (or button).
3. Place the first Type 1 weight on the clean balance pan using plastic forceps.
4. Allow the balance to stabilize. Measure and record the observed weight in the logbook.
5. Repeat steps 3-4 with the other appropriate weights.
6. Compare the observed weights to the laboratory's acceptance ranges for the Type 1 weights. If any weight exceeds the acceptable range, discontinue using the balance and take corrective action.

3.3.13 Discuss appropriate use of thermometers in a drying oven.

Place thermometer bulb in a jar of clean sand or vermiculite (Traceable thermometers like this are available commercially). Doing so will insulate the bulb from rapid temperature shock associated with opening the oven door and provide an accurate temperature of the inside of the oven.

Chapter 4 - General Lab Practices

Section 4.1 - Definitions

4.1.1 Define Reagent Water.

Reagent water is water which has been treated to remove any impurities that may affect the quality of sample analysis. Reagent water is typically classified as either ASTM Type I or Type II.

You may see this referred to as laboratory reagent water, laboratory pure water, distilled water, or deionized water.

- Water used in analyses should not contain contaminants that interfere with test accuracy.

- Water with very low levels of dissolved compounds is used for analyses. There are different classification schemes. Standard Methods for the Examination of Water and Wastewater (Standard Methods) gives three Types (I through III) with I being the best. The classes are differentiated by bacteria, pH, resistivity and conductivity (indirect measures of dissolved solids), etc.

Type I: often required by Standard Methods. It cannot be stored without significant degradation.

Type II: can be used if the presence of bacteria is unimportant. It can be stored for some uses (but not all), but should be stored in materials that will not contaminate it.

Type III: used for cleaning. It can be stored in containers that don't contaminate it.

- Clean water is usually produced using deionizing cartridges. A final step may involve distillation in an all glass still.

- Deionized water doesn't store well, it adsorbs contaminants from the air (carbon dioxide, ammonia, etc.). If you need very clean water, make it fresh every day.

4.1.2 Define Reagent.

A reagent is any substance used in a chemical reaction to detect, measure, examine, or produce other substances.

4.1.3 Define Standard.

A standard is a solution which consists of a known and documented concentration of a target analyte (e.g., 100 ppm phosphorus solution). Standards are used as reference material to determine the concentration of a target analyte in an unknown sample. The instrument response relative to the concentration of standards is compared to that of the unknown samples to determine the concentration. Standards should be prepared in a solution similar to the samples being tested. For example, if you are measuring phosphorus in wastewater, you should prepare the standards in water.

4.1.4 Define Matrix.

The chemical and physical composition of the sample defines the matrix.

The following are examples of matrices that an average wastewater lab may encounter:

1. Raw Wastewater (Influent)
2. Final Wastewater (Effluent)
3. Biosolids or Sludge
4. High Strength (High BOD/ High TSS) Industrial Waste

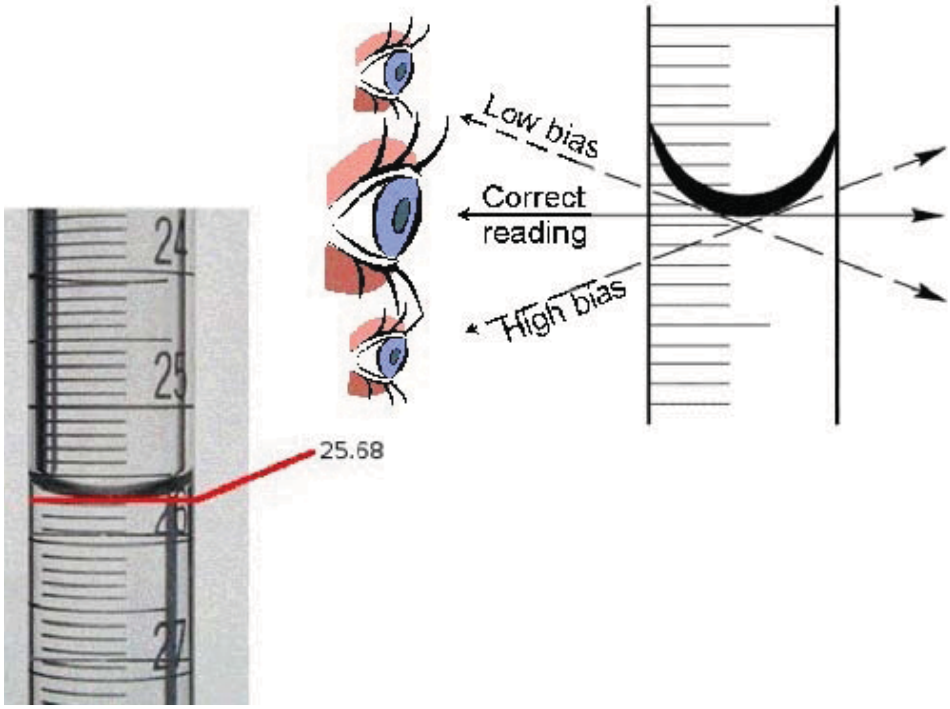
Section 4.2 - Measurement Techniques

4.2.1 Explain the proper way to read a water level meniscus.

When water or liquid is held in a small diameter tube such as a pipet or buret, the water level will form a concave curved surface. The liquid in contact with the container walls will rise slightly, leaving a low point in the center. When taking a measurement, the low point of the curve is considered to be the correct measuring point.

Always read the meniscus at eye level. If the meniscus is not read at eye level, incorrect readings will result.

Figure 4.2.1.1



4.2.2 Discuss the proper use of a volumetric pipet using a bulb type pipet aid.

1. Evacuate the pipet bulb by squeezing.
2. Immerse the tip of the pipet into solution to be delivered.
3. Seat the bulb opening over the top opening of the pipet.
4. Hold the bulb in place while slowly releasing the squeezing pressure.
5. Continue to release the pressure while the solution is drawn into the pipet.
6. Draw the solution up well past the calibration line.
7. Quickly remove the bulb and seal the top of the pipet with the index finger.
8. Keeping the index finger in place, remove the tip from the solution.
9. Rest the tip of the pipet on the side of the container that held the solution.
10. Slowly release finger; allow solution level (meniscus) to drop to calibration line.
11. Place tip of the pipet over the receiving vessel and completely release the finger.
12. Keep the pipet upright and allow to drain completely.

(Note: Many Class A pipets have the drain time imprinted adjacent to the "TD" designation.)

When the draining is complete, touch any remaining droplet at the tip of the pipet to the inside wall of the vessel.

4.2.3 State the meaning of and difference between "TC" and "TD" glassware:

“TC” = To Contain – This is usually found in small print on volumetric flasks. When the liquid is poured out of the flask, it will not be exactly the volume specified, since some of the liquid will adhere to the sides of the flask.
‘TD” = To Deliver – This is commonly found in small print on pipets, particularly volumetric

pipets. Do not blow out the small amount of liquid left in the tip of the pipet; the volume is calibrated with this taken into account. This will ensure deliverance of an accurate volume. Allow the pipet to drain by gravity; do not blow into the pipet to hurry the process.

4.2.4 Explain when to use wide-bore vs. narrow-bore pipets.

The use of either wide or narrow-bore pipets has a significant impact on measurement quality and consideration between the two largely hinges on the sample to be measured. Narrow-bore pipets have a higher level of accuracy by virtue of fluid column control. They provide more accurate measurements for clear liquids. However, when pipetting samples with visible suspended solids, wide-bore pipets are more accurate. This is due to the fact that the narrow-bore can serve to block or strain solids particles from the sample. This is why the use of wide-bore pipets is specified by Standard Methods when determining total suspended solids and BOD.

Section 4.3 - Reagent & Standard Preparation

4.3.1 Identify and explain the use of the following two grades of chemicals used in a wastewater laboratory.

A. Technical Grade.
B. Analytical Reagent Grade (AR).

A. Technical grade chemicals are a lower purity that should not be used in wastewater analysis. They may be used in cleaning equipment where purity is not as critical.

B. Analytical reagent grade (AR) chemicals are typically of a specified purity and the label contains assay information. Only this grade should be used for wastewater analysis.

4.3.2 Proper preparation of calibration standards.

The proper preparation of a calibration curve is required for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

A good calibration curve is dependent on many factors. Mainly it depends on what you do to your sample before you measure it. The first thing you need is a very accurate measuring device for making your calibration standards. When preparing a standard solution, be sure to use a volumetric pipet for accurate volumetric measurement.

If volumetric pipets are inaccurate then your curve is most likely going to show it. Also, you have to make sure your instrument response is linear for the range of measurements you are making. The main thing you need to be careful with is how you handle your standards before you measure them; any errors you make will show in your curve.

Calibration standards are usually made by diluting a stock analyte solution in volumetric flasks. Calibration standards are made so that the typical analyte concentration in the sample falls near the mean concentration of the standards. Enough standards should be made (3 or more) to verify linearity of analyte response. If necessary, samples must be

diluted to fall within the calibration range of the standards.

The stock calibration standard whether purchased or weighed out in the laboratory must be traceable to NIST and or ACS certified. Dilutions must be prepared using reagent water. Problems arise if the water used for a dilution is not of the highest quality—especially if it contains additional analyte or interferences. If a lab does not have a source of high quality water, reagent water can be purchased.

Diluted standards are prepared from concentrated standard solutions. Although concentrated standard solutions are typically stable for many months, most diluted standards should not be considered stable. Diluted standards and samples should be used immediately after preparation and discarded after use.

An accurate dilution is easy to prepare following these basic steps:

1. Use a volumetric pipet to dispense the chosen volume of concentrated standard into a clean volumetric flask.

2. Fill the flask to volume with reagent water. Fill the flask with water until the bottom of the meniscus rests on the top of the volumetric mark that is etched on the flask.

3. Invert the flask several times to thoroughly mix the solution. Once the dilution is prepared, the test can be run on the diluted standard.

Example:
The stock standard solution (Stock A) is 1000 mg/L. Suggested calibration standards for ammonia are: 0.2, 2, 20 mg/L. First we need to decide what volumes of calibration standards are necessary (e.g., 50 mL).

Next we need to calculate what volume of stock would be needed to create 50 mL of 0.20, 2.0 and 20 mg/L standards. Since we want to pipet an accurate amount of sample, we must first make an initial dilution of the Stock A to form Stock B (an intermediate standard). Take 5 mL of Stock A and dilute with reagent water to 100 mL. This is now a 50 mg/L solution. Prepare your low working standards from Stock B. Therefore:

- Standard #1 - 0.20 mg/L = 50 mL x 0.20/50 mg/L (Stock B) = 0.2 ml pipeted into 50 mL
- Standard #2 - 2.0 mg/L = 50 mL x 2.0/50 mg/L (Stock B) = 2 mL pipeted into 50 mL.
- Standard #3 - 20 mg/L = 50 mL x 20.0/50 mg/L (Stock B) = 20 mL pipeted into 50 mL.

Section 4.4 - Reagent Water

4.4.1 Explain how one can determine that purchased water is acceptable for use as reagent water.

Review your BOD blanks. Even if the blanks pass, it could mean that the purchased water contains "bugs" but no other contamination (i.e., a food source). Alternatively, the water might also contain a food source, but no "bugs". Both of these situations could cause

Glucose Glutamic Acid (GGA) to exceed upper control limits, while blanks could meet acceptance criteria. For this reason, you have to review BOTH blank and GGA performance to evaluate water quality. GGAs failing low could be indicative of either toxins present in the water or inadequate seed materials.

For phosphorus analysis, the best means to determine if purchased water is acceptable is to review blank results. If your blanks turn blue upon addition of color reagent, it's a good indication that the water source may be contaminated with phosphorus.

4.4.2 Discuss the proper storage of reagent water.

Store only in materials that protect the water from contamination, such as Teflon and glass for BOD analysis or polyethylene, polycarbonate and polypropylene for other analyses. Avoid storing water for extended lengths of time once it has been opened. If stored water appears cloudy or the general quality has appeared to change, discard.

4.4.3 Discuss the critical maintenance of lab water purification systems including:
A. Deionizers
B. Distillation Systems

A. Deionizers
- Cartridges do not last forever! Cartridges need to be replaced frequently for water purification systems to remain effective.

- Cartridges are subject to breakthrough of organic matter and growth of microorganisms, both of which can interfere with BOD analysis.

- Monitor water quality with conductivity. Increasing conductivity provides an indication that cartridges need to be replaced.

B. Distillation Systems
- Discard the first volume of water obtained through distillation to eliminate contamination from volatile organics or ammonia.

- Regularly remove scale buildup due to hard water or consider installing a softener. Stills lose distillation efficiency when they become "limed up".

4.4.4 Discuss the proper use and maintenance of a lab still.

- Steam out the still and discard first water following manufacturer recommendation.
- Clean still on a regular basis following manufacturer recommendation.

4.4.5 Discuss distillation as a means to produce reagent water.

Distillation heats water until it vaporizes as steam. Minerals, bacteria and other substances are left behind when the steam re-condenses into relatively pure water. Distillers remove bacteria, minerals, trace amounts of metals, many volatile organic chemicals and nitrate. The distillation process is very slow and capacity is usually less than 2-3 gallons per day. The still and receiving container should be either glass or non-metal. Generally minimize the contact of distilled water with any non-glass tubing.

Distillation is subject to carryover of volatile organics, which can interfere with the BOD analysis, and ammonia, which can interfere with the ammonia analysis. Careful temperature monitoring is required to ensure purity and avoid contamination of the purified water. Organics with a boiling point near that of water are very difficult to remove due to carry over into the vapor. In these situations a double distillation system is often required for complete pyrogen removal

Distillation is a high maintenance water purification technique. The still must be regularly cleaned and de-limed.

4.4.6 Discuss deionization as a means to produce reagent water.

Deionization is a complex process that involves a variety of treatment separation steps the collective group of which may be unique for a given laboratory. Deionization alone can produce extremely high-quality water in terms of dissolved ions or minerals, but deonization does not generally remove organics and cartridges can become a breeding ground for bacteria.

Chapter 5 - Lab Analysis

Section 5.1 - Definitions

5.1.1 Define BOD.

BOD = Biochemical Oxygen Demand
BOD is actually a bioassay rather than a chemical test. Bioassay is a shorthand commonly used term for biological assay. Bioassays are typically conducted to measure the effects of a substance on a living organism. Bioassays may be qualitative or quantitative, the latter often involving an estimation of the concentration or potency of a substance by measurement of the biological response that it produces.

BOD is a test used to measure the concentration of biodegradable organic matter present in a sample of water. It can be used to infer the general quality of the water and its degree of pollution by biodegradable organic matter. It is used in water quality management and assessment, ecology and environmental science.

In a nutshell, BOD gives a measure on the impact of a waste(water) on the oxygen content of a receiving system(stream/river/lake). Wastes are broken down by microbial organisms (frequently referred to as “bugs”), and the bugs, in turn, require oxygen. Thus, in order for this test to “work”, you need (1) a food source, (2) a population of bugs, (3) available oxygen fuel, and (4) a system which provides a hospitable environment for the bugs.

A series of dilutions with nutrient-rich, buffered dilution water is performed on each sample. Samples may also be seeded with a population of microorganisms as necessary. An initial measurement of dissolved oxygen is obtained, and then again following a five-day incubation period at 20 ± 1 °C. The amount of oxygen depleted (during the 5 day incubation period) is used to calculate BOD.

5.1.2 Define carbonaceous BOD (cBOD)

Carbonaceous BOD (cBOD) is a specific part of "total" BOD which represents the amount of oxygen demand required to break down carbon sources only. Total BOD measures the oxygen consumption associated with microbial breakdown of a waste, including both carbonaceous and nitrogenous sources.

cBOD is measured by adding a pyridine-based inhibiting agent to the sample prior to incubation during the BOD test. This chemical inhibits the ability of the bacteria, Nitrosomonas sp., to convert ammonia to nitrite; the first phase of nitrification. By inhibiting this activity, the test measures only the oxygen utilized by microorganisms to breakdown carbonaceous waste.

5.1.3 Define dissolved oxygen

Dissolved oxygen (DO) is defined as oxygen (O2) molecules which have actually dissolved in water. Dissolved oxygen cannot be seen, so small bubbles are not actually considered to be "dissolved" oxygen. Oxygen becomes dissolved in water in one of two ways: it either dissolves into water from contact (diffusion) with the atmosphere or is produced by plants as a byproduct of photosynthesis. Dissolved oxygen concentration is related to the following:

1) temperature (as water temperature increases, it is able to hold LESS oxygen)
2) atmospheric pressure (as air pressure increases, water is able to hold MORE oxygen)

5.1.4 Define super-saturation.

Supersaturation means that the water contains more DO than it SHOULD contain according to physical tables. According to tables, the saturation point of oxygen in water at 20° and 760 mm pressure - which is standard temperature and pressure at sea level - is 9.06 mg/L. So, yes, at sea level and 20°C, anything over 9.06 mg/L represents supersaturation.

The method suggests that super-saturation is anything above 9.0 mg/L. However, in reality saturation will vary with temperature and pressure. Consult a DO saturation table.

5.1.5 Define pH

The pH scale is a range of numbers expressing the relative acidity or basicity of a solution. It expresses the “intensity” of an acid or base through a mathematical formula based on the activity of certain ions. The scale ranges from 0 to 14 with 7 being neutral, lower numbers more acidic, and higher numbers more alkaline. Since pH is mathematically based on a factor of 10, a solution of pH 3 is 10 times more acidic than a solution of pH 4 and 100 times more acidic than a solution of pH 5, and so on. pH values are reported as "pH units".

Measurement of pH in samples from the treatment plant is done to determine whether certain processes are functioning optimally, whether some unusually acidic or basic substances are flowing into the plant, and whether the plant is within the effluent pH limits.

5.1.6 Define Total Suspended Solids (TSS)

Total suspended solids (TSS) is a water quality measurement, generally required for

wastewater treatment plant outfalls. This parameter was at one time called non-filterable residue (NFR), a term that refers to the identical measurement: the dry-weight of particles trapped by a filter, typically of a specified pore size.

TSS of a water sample is determined by pouring a carefully measured volume of water through a pre-weighed filter of a specified pore size, then weighing the filter again after drying to remove all water. The gain in weight is a dry weight measure of the particulates present in the water sample expressed in units derived or calculated from the volume of water filtered (typically milligrams per liter or mg/L).

Section 5.2 - Biochemical Oxygen Demand (BOD)

5.2.1 Discuss the key considerations associated with the DO meters and probes.

DO meters can be analog or digital. Most newer instruments have a built-in barometer, thermometer and on-board software to simplify the calibration process. DO meters must be warmed up for at least 30 minutes prior to making measurements.

The polarographic type probe is the most common style. The most important parts of the DO probe are the membrane, the anode and the cathode. Newer electro-optical probes do not have anode/cathodes.

5.2.2 Discuss maintenance of DO probes

Most probe problems are related to membrane failures due to fouling or rupture of the membrane itself. For best results, membranes should be generally replaced every 3-4 weeks, however more frequent replacement may be required due to use and sample conditions. Immediately after replacing the membrane, check to be sure that no tiny air bubbles are entrapped under the membrane surface as these will cause erroneous results. The probe should be allowed to stabilize for at least 12 hours (overnight is best) after membrane replacement before making measurements.

If the cathode (gold tip) appears tarnished, clean it. The gold should have a bright “matte” finish with fine scratches. There is only a very fine layer of gold so DO NOT polish excessively. Use the special sanding disk available from the manufacturer. The anode should have a light silver color. Clean the anode if its color becomes black due to oxidation of the silver. Clean by soaking overnight in household ammonia solution. Rinse thoroughly with tap water, reagent water and electrolyte. Newer electro-optical probes do not have anode/cathodes.

5.2.3 Identify the critical requirements associated with BOD incubators:

Thermostatically controlled at 20 ± 1°C. All light must be excluded to prevent photosynthesis during the 5-day incubation period. The temperature of the incubator must be documented daily when samples are being incubated.

5.2.4 Identify the critical requirements associated with a barometer:

Barometers
A. Electronic Models

The laboratory temperature and barometric pressure must be recorded daily, particularly if the dissolved oxygen (DO) meter is calibrated using the water-saturated air or air-saturated water technique. NIST traceable electronic models are available that will monitor both temperature and barometric pressure.

B. Dial type models

Dial type NIST traceable barometers are satisfactory for monitoring barometric pressure.

All barometers (electronic and dial type) should be checked to verify they are functioning properly. The internet or local airport can provide information that should be used to check laboratory barometers monthly. Barometers are required to be verified at least annually.

5.2.5 Discuss critical equipment to calibrate a DO meter.

Assuming one is not using the Winkler technique for calibration, which requires no additional equipment, the following are required to accurately calibrate a DO meter.

A barometer, or pressure obtained from a local source and adjusted for elevation, is required to obtain an accurate pressure reading that is corrected for altitude.

A thermometer is required to determine the temperatures of the dilution water used to calibrate the DO meter.

A DO saturation table or chart is required to determine the saturation point based on calibration solution temperature and barometric pressure.

5.2.6 Discuss how to prepare and store BOD dilution water.

BOD dilution water nutrient buffer solutions can also be a source of contamination. If you prepare your own solution make sure you store the phosphate buffer in a refrigerator. Discard any solution if it becomes cloudy or has “chunks” floating in the solution. Using single use nutrient buffer pillows will avoid many of the pitfalls.

Nutrient Solutions:
- Magnesium sulfate solution
- Calcium chloride solution
- Ferric chloride solution
- Phosphate buffer

Add one mL of each buffer solution per liter of dilution water being prepared. Alternatively, use one nutrient buffer "pillow" and dilute with the manufacturer's recommended volume of lab reagent water. The pH should be 7.2. Store in a refrigerator at <6°C, but not frozen. Check before each use for contamination (discard any reagent w/ growth). Note that refrigeration is necessary to minimize microbial growth. Phosphorus is the limiting factor in microbial populations, so once the phosphate buffer is added, dilution water becomes a prime growth medium for microorganisms.

Before use bring dilution water temperature to 20 ± 3°C. It is best to bring the temperature

to 21-22°C initially. Saturate dilution water with oxygen by shaking vigorously for several minutes, aerate with organic-free filtered air, or store in cotton-plugged bottles "long enough to become saturated". Shaking is best.

It is preferable to store dilution water in a glass container. Plastic containers can leach BOD.

5.2.7 Discuss DO probe calibration options.

The DO meter can be calibrated by Air-Saturated Water, Water-Saturated Air, or Winkler Titration.

Air-Saturated WATER
- Place the probe in a BOD bottle filled with air-saturated (well-shaken) water.
- Leave probe in the water w/ stirrer operating long enough for the probe temperature to equalize with the water temperature.
- Determine barometric pressure and adjust meter's internal barometer as necessary.
- Check the temperature of source water to be sure the probe thermistor is working correctly.
- Use a detailed DO saturation table to determine the theoretical DO concentration.
- Adjust the meter to read the DO concentration determined from the saturation table.

Water-Saturated AIR
- Place the probe in a BOD bottle containing about 3 cm of water.
- Shake BOD bottle prior to inserting probe to assure saturation. We recommend leaving the stirrer on to speed up equilibration.
- The probe may need to sit in the bottle for 30-35 minutes in order to match the temperature of the air.
- Determine barometric pressure and adjust meter's internal barometer as necessary.
- Check the temperature of the air (in the bottle) to be sure the probe thermistor is working correctly.
- Use the meter's auto-calibration function to calibrate the probe and meter.

Winkler Titration
- Use air-saturated dilution water.
- Use fresh reagents.
- Standardize titrant.
- Perform Winkler titration.
- Check Winkler result against theoretical saturation.
- Set meter equal to the Winkler results.

NOTE: With newer technology probes (LDO, RDO) water-saturated air calibrations work best, as long as a consistent procedure is used.

5.2.8 Discuss when and how atmospheric pressure and temperature affect DO calibration.

The concentration of dissolved oxygen (DO) in water is dependent on both temperature and atmospheric pressure. As the temperature of water increases, its ability to hold oxygen (DO

saturation point) decreases. This explains why fish in an aquarium become stressed and may die if the water temperature is too high. The water can simply no longer hold enough oxygen for the fish to survive. In natural systems, this can result in a "fish kill". On the other hand, as atmospheric pressure increases, the ability of water to hold oxygen (DO saturation) actually increases. Oxygen is a dissolved gas, and gases tend to escape, but increased atmospheric pressure reduces the ability of oxygen to escape the system. This also explains why the oxygen is "thinner" at higher altitudes, where pressure is much lower than at sea level.

In order to make accurate DO measurements for the BOD test, we must calibrate the DO meter. If the Winkler chemistry determination is not being used to calibrate the DO meter, then we must know the temperature of the calibration solution and the atmospheric pressure in order to determine the specific DO saturation point under these conditions. Once we know the saturation point we can calibrate the meter properly. Because temperature and pressure are constantly changing, we need to calibrate the DO meter daily before analysis to ensure accurate results.

5.2.9 Discuss under what conditions super-saturation might occur.

- Super-saturation can occur during winter months (the colder the water, the more oxygen it can hold).
- Super-saturation can also be a problem in localities where algae are actively growing (e.g., lagoons).

5.2.10 Discuss the significance of the BOD test in wastewater testing.

BOD is used to assess the relative strength of a waste. It measures the amount of oxygen required to stabilize a waste if it is discharged to a surface water.

BOD is the most commonly required test on WPDES and NPDES discharge permits. It is widely used in facility design planning and is used to gauge the effectiveness of wastewater treatment plants once in operation. The critical function of the BOD test is to provide a means to assess waste loading on surface waters. By looking at the potential for a waste to utilize oxygen, we can evaluate the potential impacts downstream, including fish.

5.2.11 Explain how you "seed" samples in the BOD test.

You can seed dilution water and then all samples that are diluted to any extent are provided with a population of microorganisms. Alternatively, a small volume of concentrated seed material can be added to individual sample dilutions. Either practice is acceptable. Seeding dilution water ensures all samples are seeded, but seeding individual samples makes calculating seed correction factors a much easier task. Seeding individual samples works best.

If dilution water is seeded, each sample dilution requires a separate seed correction factor to be calculated. When a specified volume of seed is added directly to sample bottles, a correction factor associated with that volume of seed is calculated and that one value is then used to adjust each sample result.

One other drawback to seeding dilution water is that this practice favors those samples which require large dilutions (i.,e., high concentration samples). For those samples with lower BODs, typical of clean effluents, sample volumes of 200 to 300 mL are routinely employed. This means that only a small volume of dilution water -including seed organisms- would be added to individual bottles. This is particularly a problem for labs with very clean effluents, requiring at least one whole bottle (300 mL) of sample. In this case, no dilution water is added, and thus no seed organisms are added.

5.2.12 Discuss the concept of seeding in the BOD test.

The purpose of seeding is to ensure that an adequate population of viable microorganisms is available to breakdown any waste material, utilizing oxygen during the process. Microorganism are a critical component of the BOD test, as it is the oxygen they utilize during the breakdown of organic matter which is measured by the BOD test.

Consequently, whenever there is suspicion that a sample may not contain an adequate population of microorganisms, sample dilutions must be "seeded" with microorganisms obtained as either commercially purchased synthetic seed, or as a portion of process water from a treatment plant.

5.2.13 Discuss what is used for seed in the BOD test.

There are really two options for obtaining a seed source. The first is to utilize a sample from some aspect of the treatment process. Settled primary wastewater is generally the best source. Alternatively, there are several commercially available synthetic seed sources available.

If domestic primary wastewater supernatant is used as a seed source, it should first be allowed to settle at 20°C for at least one hour, but not longer than 36 hours.

Commercial seed (e.g., BOD seed, Polyseed) is supplied as microorganisms which are freeze dried onto an inert material. The general procedure is to open a capsule of synthetic seed into a quantity of BOD dilution water and mix for a period of time. The sequence is important. The seed can only be introduced after nutrients have been added to reagent water and mixed completely.

Some synthetic seeds may require longer or different mixing procedures than the manufacturer recommends. DO NOT mix seed in distilled or deionoized water! Adding a seed capsule to deionized water causes an osmotic imbalance that will kill seed organisms. The purified water -containing no salts- rushes across the bacterial cell walls. The bacteria subsequently swell up and burst (or lyse) which, if it does not kill them, will severely impair their ability to utilize oxygen.

Effluent from a biological treatment system is NOT recommended, as it creates the potential for nitrification to occur. If you do use effluent from a biological treatment system (or recycle effluent) as your seed source, it's very likely you have nitrification and BOD results will be biased high.

Some facilities have managed to obtain acceptable results using final effluent as a seed source. Due to the expected minimal population of microorganisms, final effluent is not an optimal seed source. The bottom line for any seed source, however, is that it be a homogenous sample that when added to a GGA sample will yield acceptable results.

5.2.14 Discuss situations when seeding is required for wastewater samples.

Seeding is required whenever any sample is collected downstream of any disinfection, whenever sample pH requires adjustment, and also when inhibitor is added for cBOD determination.

Sample pH extremes will severely shock or kill microorganisms. Therefore, when sample pH adjustment is required, subsequent sample dilutions must be seeded.

If collected downstream of chlorination, samples must be tested for chlorine residual, and if present, the residual must be quenched prior to sample dilution and analysis. Disinfection agents, such as chlorine, or UV, kill or prevent microorganism populations from reproducing. Therefore, any sample which has an initial chlorine residual, or any sample collected downstream of any disinfection process must be seeded.

Finally, the inhibiting agent used to disrupt the nitrification process for cBOD determinations may have a toxic effect on other microorganisms. Therefore, samples for cBOD determinations (to which inhibitor is added) must be seeded as well.

5.2.15 Explain the importance of a seed correction factor and how it is calculated.

When samples are seeded, an accurate BOD determination depends on the ability to account for the DO depletion due to the seed material in each sample dilution. This is accomplished by determining a seed correction factor. The seed correction factor is generated based on the analysis of "seed control" samples, which are aliquots of dilution water containing specific, known volumes of seed material. The amount of DO depletion in each control is then calculated as the amount of DO depletion per mL of seed material added. The results of seed controls are then averaged to determine the final "seed correction factor" to be used to adjust the BOD calculation of seeded samples.

Seed controls must be treated just like any other sample, since we are really trying to accurately determine the BOD of the seed material. Therefore at least two dilutions of seed material are required, and the method specified DO depletion criteria must be met. Language included in the BOD procedure in earlier editions of Standard Methods suggested that seed control must result in a depletion of 0.6 to 1.0 mg/L. According to Standard Methods the intent of the 0.6 to 1.0 mg/L range is guidance, rather than a requirement. The true test of a seed material is to utilize a sufficient quantity of seed material such that method acceptance criteria for GGA determination are routinely achieved.

Seed control results must be reviewed for consistency in the data. Individual seed control factors (DO depletion per 1 mL of seed) should agree well with each other and the average. If there is a considerable range in these values, it will likely result in high variability in GGA

results.

EXAMPLE:
Seed Control 1
Volume = 10 mL
Initial DO = 8.50 mg/L
Final DO = 5.50 mg/L

Seed Control 2
Volume = 20 mL
Initial DO = 8.50 mg/L
Final DO = 2.70 mg/L

SCF for Seed Control 1:
Depletion = (8.50 - 5.50) - 0 = 3.0 mg/L
SCF 1= 3.0 mg/L ÷ 10 mL
SCF 1= 0.30 mg/L per mL of seed

SCF for Seed Control 2:
Depletion = (8.50 - 2.70) - 0 = 5.80 mg/L
SCF 2= 5.8 mg/L ÷ 20 mL
SCF 2= 0.29 mg/L per mL of seed

Average SCF = (0.30 + 0.29) ÷ 2
Average SCF = 0.295 mg/L per mL of seed

Thus, for each mL of seed added to samples, 0.295 mg/L DO must be subtracted from the individual sample DO depletion prior to calculating BOD.

5.2.16 Explain how seeding affects BOD calculations.

Seed material itself causes a DO depletion. Therefore depletion due to seed material must be subtracted from the total DO depletion of each seeded sample dilution prior to calculating BOD for the original sample.

5.2.17 Discuss the steps involved in performing the BOD test.

The following are the critical steps in preparing and analyzing samples for BOD testing. The BOD test is a method defined (empirical) technique. Consequently, the method must be followed carefully, and must be performed consistently from day to day and analyst to analyst. Potential errors can be encountered in each of the following steps in the BOD test.

1. Prepare dilution water
2. Prepare seed
3. Perform the preliminary testing
4. Determine dilutions required
5. Measure out samples
6. Fill bottles ¾ full with dilution water

7. Add seed to those that need it
8. Top-off samples with dilution water
9. Measure initial DO (DOi)
10. Insert stopper making sure it displaces any air-check for air bubbles
11. As a precaution against drawing air into the bottle during incubation, carefully pour some dilution or reagent water into the area between the stopper and the bottle neck to serve as a water seal preventing oxygen from entering or escaping from the sample
12. To prevent evaporation of water seal, place a cap over bottle
13. Incubate 5 days (± 4-6 hrs)
14. Measure Final DO (DOf)
15. Determine BOD
16. Evaluate the results of samples, GGA, blanks and duplicates and report

5.2.18 Explain why BOD bottles need to be rotated.

The concept of "rotating BOD bottles" does not mean to "turn" the bottles. What we mean is that a specific BOD bottle should not be labeled and consistently used for a given sample. For example, the same BOD bottle should not always be used to prepare BOD blanks, or GGA samples. Some labs inadvertently create this situation by lining up all their BOD bottles in numerical order based on the number that the manufacturer placed on the bottle. If you simply line your bottles up in numerical order, and you always move left to right and begin with a blank, then the lowest number bottle in the sequence will always be your blank.

Why is this a concern? Because the point of randomly assigning BOD bottles to various samples and QC samples is a means of verifying that the bottle cleaning procedure is adequate. If one's cleaning procedure is not adequate for your test, and bottle # 1 is always used for the blank, then you might never detect a problem with the cleaning.

The BOD test is highly susceptible to problems from dirty bottles (contamination from residue left behind that has a BOD demand).

5.2.19 Discuss the incubation time, temperature and other criteria for BOD samples.

Samples must be incubated for 5 days in the dark at a temperature of 20 ± 1°C. The 5-day incubation period was originally set by early BOD pioneers based on the length of time it took water to flow from London, England, down the Thames River to the North Sea. Although this may seem like an arbitrary time, it has been internationally adopted as the “standard” incubation period for the BOD test.

For optimal defensibility of results, you should strive to stay as close to the actual 5 day incubation period as you can, but certainly stay within 5 days ± 4-6 hours. The current edition of Standard Methods indicates ± 6 hours.

5.2.20 Discuss the types of sample pretreatment required for BOD testing.

Because the BOD test is a bioassay, it is critical to maintain optimal conditions for the “bugs” (bacteria) to stay live and viable. Temperature, pH, oxygen levels and the presence of disinfectants can all influence the outcome of the BOD test. Before proceeding with the BOD test, the following items must be checked. In many cases pretreatment may be

required.

pH
pH extremes kill (or at least severely injure) the “bugs”. Consequently, you must test each sample to confirm that the pH is in the appropriate range before proceeding with the BOD test. pH extremes are defined as less than pH 6 or greater than pH 8. pH extremes were undefined prior to the 21st edition of Standard Methods which now states pH 6 – 8; adjust to pH 7.0 to 7.2 (to match international standards).

The acceptable pH range varies between approved editions of Standard Methods. If the undiluted sample requires pH adjustment, adjust the pH with 1N H_2SO_4 or 1N NaOH. Do not dilute sample by >0.5% (1.5 mL in a 300 mL bottle). If more acid or base is needed, use more concentrated solutions (i.e., 5N). Phosphate buffer in the dilution water will often adjust the pH into the acceptable range, particularly in sample volume samples. However, this must be confirmed and recorded.

CHLORINE RESIDUAL/DISINFECTION
Check wastewater samples for residual chlorine unless it can be demonstrated that the sample was collected prior to where chlorine is added. A plant diagram with the sampling point shown in relation to the chlorine contact tank is usually satisfactory. If chlorine is detected, quench the chlorine residual as per Standard Methods and seed the sample(s). If ANY disinfection process is employed (UV, chlorine) and the sample(s) is collected downstream of disinfection, seed the sample(s).

SUPER-SATURATION
Water only has a limited capacity to hold oxygen. This capacity, or saturation point, is driven by temperature and barometric pressure. If the sample DO is greater than saturation point when the bottles are placed in the incubator, oxygen will physically come out of solution (will appear as micro bubbles) and appear to be an oxygen demand. The resulting BOD will be falsely high.

The following summarizes the preliminary oxygen testing requirements for the BOD test.
1. Warm samples to room temperature (20 ± 3°C) and THEN shake them to prevent super-saturation problem. It is best to warm to 21-22°C.
2. Check for super-saturation of oxygen (causes high bias).
3. Know the saturation point at your facility/your conditions.
4. Supersaturation is a concern if DO > 9.0 mg/l at 20°C, but the saturation point could be below 9.0 mg/L.
5. Reduce excess DO by shaking sample(s) or aerate with filtered compressed air.

Make sure there is enough oxygen! Always start with an initial DO close to the saturation point for your facility (typically about 8.2 to 8.5 mg/L in Wisconsin). If the DO is low, shake or aerate with filtered compressed air to increase the DO concentration. Remember, starting with a higher initial DO will allow you to cover a wide BOD range with each dilution.

5.2.21 Discuss reasons why the time between preparing BOD sample dilutions and initial DO measurement must be minimized.

Don't let samples sit too long between adding dilution water and the initial DO measurements. Standard Methods requires measuring the initial DO no longer than 30 minutes after adding the dilution water. The rationale behind this requirement is that some samples exhibit what is termed "instantaneous BOD". These samples contain materials that become oxidized quite rapidly, thereby rapidly reducing the available DO in the diluted sample. In these types of samples, any lag between dilution of the sample and measuring the initial DO will fail to capture this rapid initial utilization of oxygen, which of course results in a low bias for sample results.

You must determine an initial DO on each sample bottle.

5.2.22 Discuss the minimum number of dilutions required for BOD.

With the exception of blanks and GGA samples, a minimum of three (3) sample dilutions is required for any BOD determination.

Note that, while a minimum of three dilutions are required for BOD analysis, three dilutions are not sufficient to identify sample toxicity. When analyzing samples for which they have no previous historical data to guide them, analysts are cautioned to use an appropriate number of dilutions. Standard methods suggests using at least five dilutions for unfamiliar samples.

5.2.23 Discuss how to determine the proper sample volumes for the BOD test.

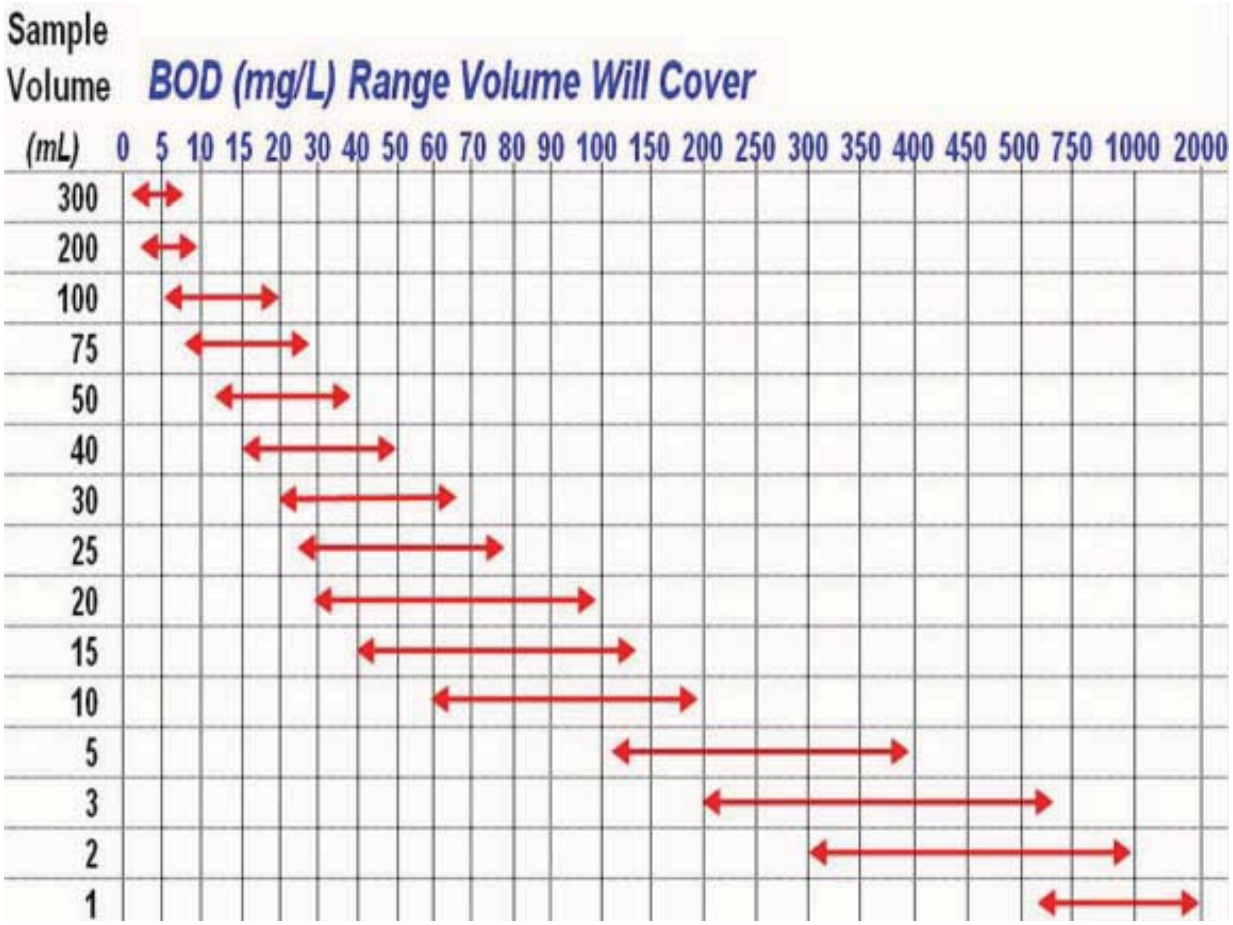
There are tables and charts that have been developed to assist analysts in making the best dilutions for any given sample. To use these charts, however, the analyst needs to have some idea of the sample BOD. All of these tables work off of a simple concept:

- Under typical conditions, at saturation in Wisconsin, initial DO should be about 8.2 - 8.5 mg/L
- The FINAL DO cannot be less than 1.0 mg/L
- Therefore the working range of DOs for any dilution is about 7.5 mg/L

If uncertain, or you have variable BODs, use more dilutions or be flexible in what dilutions you use to ensure that you will have enough dilutions that meet depletion criteria. The correct approach is to always use enough dilutions to ensure that at least two dilutions will meet method-defined DO depletion criteria.

In the absence of prior knowledge, use the following guidelines for dilutions:
-Strong industrial wastes: < 3 mL of sample (<1% dilution)
-Raw and settled wastewater: 3 - 15 mL of sample (1 - 5% dilution)

Figure 5.2.23.1



5.2.24 Discuss the smallest sample volume for BOD that can be used without performing a preliminary dilution.

If less than 3 mL of samples volume is used (<1% dilution), a preliminary dilution is required prior to pipetting the sample into the BOD bottle. This is best done by preparing a simple dilution and then pipetting a portion of the diluted sample into the BOD bottle. For example, if sample volumes of 0.5, 1.0 and 2.0 mL of sample are needed, make a 10 fold preliminary dilution. Prepare the 10 fold dilution by pipetting 10 mL of well mixed sample into a 100 mL volumetric flask (or 100 mL class A graduated cylinder) containing about 50 mL of BOD dilution water. Bring the flask to 100 mL by adding additional BOD dilution water and then mix thoroughly. Then pipet 5, 10 and 20 mL of the diluted sample into the BOD bottles which respresents 0.5, 1 and 2 mL of the original sample.

5.2.25 Discuss how sample volume affects the LOD for the BOD test.

As a bioassay procedure, the BOD test does not lend itself well to the typical EPA procedure (40 CFR Part 136 Appendix B) for determining the limit of detection (LOD). Therefore, the LOD for any given sample is determined based on the method specified minimum required DO depletion (2 mg/L) and the sample volume in the least diluted (i.e., the one with the greatest volume of actual sample) sample.

BOD detection limits are theoretically based. A key assumption on which the concept of detection for BOD is based is that the LEAST amount of depletion allowable is 2 mg/L. The LOD is also based on the highest volume of sample used in a dilution series. What results is the detection capability for a specific dilution series, or sample. This technique should include seed correction, because we want to identify depletion due to the sample itself, and not due to the seed material.

The calculation involved is simply:
LOD mg/L = 2 mg/L X [300 mL ÷ sample in LEAST dilution]

EXAMPLE: sample with dilutions of 100, 50, and 25 mL
LOD = 2 mg/L X [300 mL ÷ 100 mL]
= 2 mg/L X 3
= 6 mg/L LOD for that sample

highest sample volume used	Dilution	Sample LOD
300 mL	1.0	2 mg/L
200 mL	1.5	3 mg/L
100 mL	3.0	6 mg/L
75 mL	4.0	8 mg/L
50 mL	6.0	12 mg/L

In order to report an LOD of 2 mg/L, one sample dilution containing 300 mL of sample must be included.

5.2.26 Discuss DO depletion requirements for BOD testing.

In order to be used to calculate sample results without qualification, dilutions must deplete at least 2 mg/L of DO, and the final (or residual) DO must be at least 1.0 mg/L.

5.2.27 Discuss when and why additional nutrients must be added to BOD samples.

When larger sample volumes are used to prepare BOD dilutions (> 200 mL), it will be necessary to add extra nutrients. Use the following guidance to determine the amount of supplemental nutrients to add:

CURRENT GUIDANCE:
After dilution, BOD bottles containing more than 67% sample (> 200 mL) may be nutrient-limited and subsequently reduce biological activity. The resulting BOD will be biased low. In such samples, add the nutrient/buffer solutions directly to each BOD bottle at a rate of 1 mL/L (0.3 mL/300-mL bottle) or use commercially prepared solutions/pillows designed to dose the appropriate bottle size. When individual nutrient pillows are used, it’s OK to use dilution water that may contain some nutrients already. Generally, it's far easier to use the nutrient pillows designed for individual 300 mL BOD bottle.

5.2.28 Discuss the testing differences between BOD and cBOD.

The only difference between samples analyzed for BOD and those analyzed for cBOD is the addition of a chemical inhibitor to all samples for which cBOD is determined.

If nitrification was occurring in the original sample, the cBOD result is expected to be lower than a BOD result. The difference between the results represents the amount of oxygen which is utilized during the nitrification process. Since the BOD test only measures oxygen utilized, performing both BOD and cBOD is the only way to distinguish the amount of oxygen utilized during decomposition of organic waste from that which is utilized during nitrification.

5.2.29 Given data, calculate BOD for a sample.

1. The formula for the calculation is:

   BOD (mg/L) =[(iDO - fDO) - SCF] × DF

   iDO = Initial DO (mg/L)
   fDO = Final DO (mg/L)
   SCF = Seed Correction Factor (if applicable)
   DF (Dilution Factor) = 300 mL ÷ [sample volume (mL)]

2. Given the following data:

Dilution	IDO	FDO	Sample Volume
A	8.30 mg/L	0.80 mg/L	300 mL
B	8.30 mg/L	1.30 mg/L	250 mL
C	8.30 mg/L	4.25 mg/L	200 mL
D	8.30 mg/L	5.90 mg/L	100 mL
E	8.30 mg/L	7.40 mg/L	50 mL

3. Calulate BOD of individual dilutions:

   BOD #A: Depletion = (8.30 - 0.80) - 0 = 7.5 mg/L
   Excess depletion. DO NOT USE

   BOD #B: Depletion = (8.30 - 1.30) - 0 = 7.0 mg/L
   BOD= 7.0 mg/L × (300 ÷ 250)
   BOD= 7.0 mg/L × (1.2)
   BOD = 8.4 mg/L

   BOD #C: Depletion = (8.30 - 4.25) - 0 = 4.05 mg/L
   BOD= 4.05 mg/L × (300 ÷ 200)
   BOD= 4.05 mg/L × (1.5)
   BOD = 6.075 mg/L

   BOD #D: Depletion = (8.30 - 5.90) - 0 = 2.4 mg/L
   BOD= 2.4 mg/L × (300 ÷ 100)
   BOD= 2.4 mg/L × (3)

BOD = 7.2 mg/L

BOD #E: Depletion = (8.30 - 7.40) - 0 = 0.9 mg/L
Insufficient depletion. DO NOT USE

4. Average all useable results to obtain a BOD for the whole sample.
   Average BOD = (8.4 + 6.075 + 7.2) ÷ 3
   Average BOD = (21.675) ÷ 3
   Average BOD = 7.225
   Report BOD = 7

5.2.30 Given data, calculate BOD for a GGA standard, which requires the application of the seed correction factor.

1. The formula for the calculation is:
   BOD (mg/L) =[(iDO - fDO) - SCF] × DF
   where: iDO = Initial DO (mg/L)
   fDO = Final DO (mg/L)
   SCF = Seed Correction Factor (if applicable)
   = depletion (mg/L) from seed ÷ seed volume (mL)
   DF (Dilution Factor) = 300 mL ÷ [sample volume (mL)]

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2. Given the following data calculate the result for the GGA standard:

DILUTION	IDO	FDO	mL SEED	SAMPLE VOLUME
Seed Control 1	8.45 mg/L	5.45 mg/L	10 mL	N/A
Seed Control 2	8.45 mg/L	3.65 mg/L	15 mL	N/A
GGA	8.30 mg/L	3.75 mg/L	2 mL	6 mL

---

3. Calulate individual seed correction factors (SCF) for Seed Controls:
   SCF for Seed Control 1:
   Depletion = (8.45 - 5.45) - 0 = 3.0 mg/L
   SCF 1= 3.0 mg/L ÷ 10 mL
   SCF 1= 0.30 mg/L per mL of seed

   SCF for Seed Control 2:
   Depletion = (8.45 - 3.65) - 0 = 4.8 mg/L
   SCF 2= 4.8 mg/L ÷ 15 mL
   SCF 2= 0.32 mg/L per mL of seed

   Average SCF = (0.30 + 0.32) ÷ 2
   Average SCF = 0.31 mg/L per mL of seed

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4. Calulate BOD for the GGA solution:

Depletion = (8.30 - 3.75) = 4.55 mg/L
Seed correction = 0.31 mg/L per mL x 2 mL = 0.62 mg/L
Adjusted depletion = (4.55 - 0.62) = 3.93 mg/L
BOD= 3.93 mg/L × (300 mL ÷ 6 mL)
BOD= 3.93 mg/L × (50)
BOD = 196.5 mg/L

5. Evaluate GGA result:
   Result (196.5 mg/L) falls within required 167.5 - 228.5 mg/L

5.2.31 Discuss procedures for reporting results when all dilutions under-deplete (fail to deplete at least 2 mg/L).

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data:

Sample Bottle #	A	B	C
Sample Size (mL)	300	250	200
Initial D.O. (mg/L)	8.48	8.50	8.47
Final D.O. (mg/L)	7.40	7.70	7.95
Depletion (mg/L)	1.08	0.80	0.52
Dilution Factor	1	1.2	1.5
BOD (mg/L)	----	----	----

In the example, all three dilutions fail to meet the minimum requirement of 2.0 mg/L for depletion. Because at least one of the dilutions is a full bottle (300 mL), the requirements to report a result of the target detection limit have been met.

Report result as "< 2 mg/L"

NOTE: If no 300 mL dilution had been made, and the only two dilutions were 250 mL and 200 mL, then the result would have to be reported as "< 2.4 mg/L", based on the dilution with the largest amount of original sample. This situation indicates that a dilution of 300 mL should routinely be incorporated.

5.2.32 Discuss procedures for reporting results when all dilutions over-deplete (final DO less than 1.0 mg/L).

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data:

Sample Bottle #	A	B	C
Sample Size (mL)	300	200	100

Initial D.O. (mg/L)	8.48	8.50	8.47
Final D.O. (mg/L)	< 1.0	< 1.0	< 1.0
Depletion (mg/L)	> 7.48	> 7.50	> 7.47
Dilution Factor	1	1.5	3
BOD (mg/L)	>7.5	>11.3	>22.4

In the example, all three dilutions fail to meet the residual DO requirement of 1.0 mg/L.

Report result as "> 22 mg/L"

NOTE: If this represents an unusual situation, then a comment should be included on the DMR indicating a non-routine event. Additional dilutions may be required over the next several days until the plant settles down. If, however, this situation occurs regularly, then the lab should be routinely using higher dilutions when preparing samples.

5.2.33 Discuss procedures for reporting results when data suggest toxicity.

Each sample dilution must meet the minimum requirement of 2 mg/L DO depletion. There must also be at least 1 mg/L of DO remaining in each sample dilution after 5 days. If there is more than one acceptable dilution, these results must be averaged.

Example: Given the following data:

Sample Bottle #	A	B	C	D
Sample Size (mL)	2	5	10	20
Initial D.O. (mg/L)	8.6	8.5	8.3	8.0
Final D.O. (mg/L)	7.4	6.1	4.3	1.5
Depletion (mg/L)	1.2	2.4	4.0	6.5
Dilution Factor	150	60	30	15
BOD (mg/L)	(> 180)	144	120	98

In the example, dilution/bottle A is not acceptable, because it does not meet the minimum requirement of 2.0 mg/L for depletion. Samples B, C, and D are acceptable.

Because these dilutions show a distinct "sliding" effect, with BOD dropping as dilutions contain more of the original sample. In addition, if the dilution in bottle "A" were calculated out, it would indicate an even higher BOD (> 180). This may be an indication of toxicity. Because there are insufficient dilutions to demonstrate that the threshold inhibition point has been reached (as evidenced by consistent BOD values among a dilution series), we cannot report an accurate result.

The best result, in this case is to report the results from the most dilute sample that meets depletion criteria, and use a ">" sign to indicate that the result is at least this high. Therefore

a result of "> 144 mg/L" should be reported. In addition, a comment on the DMR should be made to indicate that this result is biased low due to indicated toxicity.

5.2.34 Describe the considerations associated with interpretating BOD results.

- Use only dilutions that meet method depletion criteria (depletion must be at lest 2 mg/L; final DO must be at least 1 mg/L).
- If results vary significantly between dilutions, consider using more dilutions to evaluate the potential for nitrification or toxicity.
- If all dilutions fail to deplete at least 2 mg/L, then report “<” plus the LOD associated with the largest sample volume used. Use a larger sample volume if using less than 300 mL (for at least one dilution).
- If toxicity is suspected, report “>” plus the highest BOD result obtained from individual sample dilutions and include a comment explaining results.

5.2.35 Discuss the QC sample types and frequencies required for BOD testing.

- Blanks: Required on each day of analysis
- Seed controls: Required on each day that any samples/QC are seeded
- LCS(GGA): Required at least once per week, or after every 20 samples (whichever is more frequent)
- PT samples: Required for each matrix technology and analyte combination at least once annually. NOTE: A "WP" PT is sufficient for BOD.

5.2.36 Discuss factors that would result in excessive DO depletion in blanks.

- Calibration errors
- Supersaturation
- Contamination (organic matter + micro-organisms)

5.2.37 Discuss blank requirements for the BOD test.

At least one dilution water blank must be prepared on each day that samples are prepared. The maximum allowable depletion in a method blank is 0.2 mg/L.

5.2.38 Explain the potential reasons why GGA results could be unacceptably high or low.

HIGH BIAS of GGA results is caused by:
- Nitrification
- Cold GGA solution
- Contamination: Organic matter
- Contamination: Microorganisms (“bugs”)

NOTE: Contamination from either “bugs” or BOD material alone will cause high bias in GGA but is not likely to cause an exceedance in blanks. There must be contamination from BOTH “bugs” AND waste material for contamination to result in blank exceedances. This explains a common statement from lab analysts that “my GGA is failing high, but my blanks are fine”.

LOW BIAS of GGA results is caused by:
- Not enough seed

- Seed materials too weak or variable
- GGA too old or contaminated

5.2.39 Discuss what is meant by nitrification.

Nitrogenous oxygen demand is the amount of oxygen used during the breakdown or chemical breakdown and conversion of organic nitrogen and inorganic nitrogen forms. This process is called nitrification. We are typically concerned with the inorganic forms, and specifically, ammonia. This means that if the wastewater contains ammonia (and some do, particularly lagoons) AND the nitrifying bacteria are present, then oxygen can be used in the conversion of nitrogen forms. This oxygen used up is measured as BOD, leading to BOD results biased high. The key equations are as follows:

Ammonia + Oxygen -> Nitrite -> Nitrate

5.2.40 Discuss how BOD results relate to TSS results and how it also might relate to nitrification.

Generally speaking there should be a 1:1 relationship between BOD and TSS results for domestic municipal wastewater effluent. This relationship only applies to domestic wastewater effluents. This is because the majority of the BOD comes directly from the material that makes up the TSS. Industrial wastes, such as dairy and food processing wastes, contain a high dissolved BOD component. Milks and sugars from food wastes will pass through the TSS filters causing the BOD to be significantly higher than the TSS values.

If BOD is always significantly higher than TSS (e.g., TSS 10, BOD 25), nitrification is likely occurring. Confirm by performing side-by-side BOD tests with and without nitrification inhibitors. If the inhibited (carbonaceous) BOD results are significantly lower and closer to the TSS results, nitrification is occurring. Repeat side-by-side tests to confirm your findings.

NOTE: Always seed samples when nitrification inhibitor is used.

5.2.41 How might you know if toxicity is occuring in the BOD test.

The first symptom of sample toxicity is evidenced by a decrease in BOD concentration as sample volume increases. What this really means is that we're looking for a trend, and a trend realistically requires more than two data points. If only two dilutions are used, and the dilution with greater sample volume yields a lower BOD result, it COULD merely be a function of sample homogeneity. Having an additional dilution which confirms the initial two dilutions serves as a referee. Therefore, at least three (3) dilutions are necessary to effectively detect sample toxicity.

There must be a distinct trend in the data for the sample to be designated "toxic" and reported as such on the DMR.

Section 5.3 - Total Suspended Solids (TSS)

5.3.1 Discuss the significance of the TSS test in wastewater testing.

Total suspended solids (TSS) are those which are visible and in suspension in the water. They are the solids which can be removed from wastewater by physical or mechanical

means such as sedimentation, flocculation, or filtration. TSS will include the larger floating particles and consist of silt, grit, clay, fecal solids, paper, fibers, particles of food, garbage, and similar materials. Suspended solids are approximately 70% organic and 30% inorganic. TSS determinations may be used to assess wastewater strength, process efficiency, and loadings.

By reducing the TSS in your effluent discharge, you are going to get better disinfection, which will reduce your fecal coliform and/or E. coli counts, allowing you to maintain compliance.

5.3.2 Identify the critical requirements associated with the drying oven.

Drying ovens used for TSS determinations must be able to consistently maintain a temperature of 103-105°C. The purpose of the method temperature is to drive off water but not lose volatile solids.

Drying ovens should be vented properly as a health and safety precaution. Do not position a drying oven directly beneath an HVAC blower vent because drafts can be forced through the top of the oven. Direct contact with blowing cold air prevents the ability of the oven to maintain constant temperature. Ovens can be vented to, but should not be situated inside any hood.

Check and document the oven temperature daily when samples are being dried.

5.3.3 Discuss the required filters for TSS testing.

Standard Methods 2540 D requires glass fiber filters without organic binders such as Whatman 934AH or equivalent. All filter papers are NOT alike! Standard Methods cites the following brands of filters as being equivalent: Millipore AP40, Gelman type A/E, ED Scientific Specialties Grade 161.

Standard Methods 2540D states that, “ Practical filter diameters are 2.2 cm [22 mm] to 12.5 cm [125 mm]”.

5.3.4 Discuss the proper preparation and use of filters for TSS testing.

Filters should be rinsed to remove loose materials and assure the filter weight remains constant.

- PRE-RINSE: Filters must be pre-rinsed with several volumes of reagent water before use. (Omit this step if pre-rinsed/pre-tared filters are used).

- INITIAL WEIGHT: After pre-rinsing, filters must be dried to constant weight at the method specified temperature, dessicated, and then an initial weight obtained. Filters are now ready for use. (Omit this step if pre-rinsed/pre-tared filters are used).

- POST RINSE: During and after sample filtration, direct a fine stream of reagent water along the sides of the filter funnel and over the filter surface. Also rinse any graduated cylinder used for transfer of the sample aliquot with a small volume of reagent water. The

purpose of directing a fine stream of reagent water over the filter surface during filtration is to dislodge any finer particles from clogging the filter pores, and allowing the sample to filter properly.

5.3.5 Discuss the importance of cleaning filter screens.

Ensure filter support screens are not excessively clogged with particulates, which can result in uneven drying. Uneven drying can result in high bias due to “flash” surface drying similar to what can occur if more than 200 mg of residue is captured. In addition, clogged filter screens can lead to trapping of “fines”, or micro-suspended particles that normally would pass through the filter pores but can be held back by clogged pores and larger diameter TSS material.

5.3.6 Discuss the steps involved in performing the TSS test.

1. Select and prepare the proper filter.
2. Determine filter weight and record.
3. Select appropriate volume to filter for each sample.
4. Filter the sample and perform a post wash.
5. Dry, desiccate, and then weigh the filter pad. Repeat as necessary to obtain constant weight, or follow LabCert program allowances in lieu of drying to constant weight.
6. Calculate TSS concentration in mg/L.

NOTE: As an alternative to routinely drying samples to constant weight, samples can be dried overnight (at least 8 hours). If samples are not dried to constant weight and are dried for less than 8 hours, then a quarterly verification of drying to constant weight is required.

5.3.7 Discuss the concept of constant weight.

The attainment of "constant weight" is a process of repeated drying, cooling, and weighing of an object until the weight does not change from the previous weighing, or, until weight loss is less than 0.5 mg or 4 percent, whichever is greater. This process assures that the sample is completely dry and that there is no added weight due to moisture.

5.3.8 Discuss drying time requirements for TSS testing and how it relates to constant weight.

The DNR Lab Certification Program has developed an alternative to performing measurements to constant weight routinely for samples and filters. Samples dried overnight (at least 8 hours) are exempt from determination of constant weight. If samples are dried for less than 8 hours and not to constant weight, on a quarterly basis, labs must compare their procedure on a sample to one that is dried to a constant weight to verify that drying the sample overnight ensures equivalence to a constant weight determination.

5.3.9 Discuss minimum and maximum solids capture weight requirements for TSS testing.

A residue of at least 1 mg, and not more than 200 mg, must be captured. If at least 500 mL of sample volume is filtered and 1 mg of residue is not obtained, the analyst is not required to repeat the analysis using more sample volume.

A minimum capture weight of 1 mg and 500 mL of sample filtered is required to report an LOD of 2 mg/L, the required LOD for Discharge Monitoring Report (DMR) reporting for

effluent samples.

Residue amounts greater than 200 mg on a filter can lead to “flash” surface drying and the formation of a salt crust layer that traps moisture beneath it. This can cause sample results to be biased high. This is generally expected to be a problem related to process control samples with heavier solids loading.

5.3.10 Explain the disadvantages of using Gooch crucibles vs. standard 47 mm filters alone.

Gooch crucibles have significantly higher mass weights, potentially masking differences of only a few milligrams. The larger mass of the crucible also means that a longer drying/desiccating time is typically required. The net effect is that Gooch crucibles typically mean a higher sample LOD, which is generally not acceptable.

Generally Gooch crucibles are available in two sizes, a 13 mL volume (15-16 mm filter diameter) and a 25 mL volume (20-21 mm filter diameter). For reference, a standard TSS filter has a diameter of 47 mm.

NOTE: for other comparisons, Peace Dollar= 38 mm diameter, Jefferson Nickel= 21 mm diameter, and a Roosevelt Dime= 18 mm diameter

Labs using Gooch crucibles typically filter no more than 25 mL, leading to an LOD of 40 mg/L or greater, which is likely unacceptable considering that the TSS permit limit for most facilities is 30 mg/L.

Figure 5.3.10.1

5.3.11 Discuss how sample volume affects the LOD for the TSS test.

Like with BOD, the limit of detection (LOD) for TSS is theoretically based. A key

assumption on which the concept of detection for TSS is based is that the MINIMUM capture weight of residue is 1 milligram (mg). The LOD is also based on the volume of sample used.

The calculation involved is simply:
LOD mg/L = [1 mg residue] ÷ [sample volume (L) filtered]

EXAMPLE: sample volume filtered = 200 mL
1. Convert mL to L:
1 L = 1000 mL, so 200 mL ÷ 1000 mL = 0.2 L

2. Formula for calculation of TSS=
LOD = [1 mg] ÷ [0.2 L]
1 ÷ 0.2 = 5
LOD = 5 mg/L .....for that sample

highest sample volume used	Sample LOD	
500 mL	2 mg/L	[1 ÷ 0.5 = 2]
250 mL	4 mg/L	[1 ÷ 0.25 = 4]
100 mL	10 mg/L	[1 ÷ 0.1 = 10]
50 mL	20 mg/L	[1 ÷ 0.05 = 20]

5.3.12 Discuss the required LOD for TSS and what to do if you can't achieve it.

In order to report a "<" result, the required LOD is 2 mg/L. In order to achive an LOD of 2 mg/L, one must filter 500 mL and fail to achieve a residue of 1 milligram of solids. More volume can be filtered, but as long as you filter at least 500 mL and you do not obtain at least 1 millgram of residue, then a result of "< 2 mg/L" can be reported.

If you are not capturing at least 1 milligram of residue and you are filtering LESS than 500 mL of sample, you will be required to filter a larger sample volume, up to a maximum of 500 mL.

5.3.13 Discuss the QC sample types and frequencies required for TSS testing.

- Blanks: RECOMMENDED (but not required) on each day of analysis
- Balance verification: Required at least once per month using one weight in the gram range and one in the milligram range.
- PT samples: Required for each matrix technology and analyte combination at least once annually

5.3.14 Given data, calculate TSS.

TSS (mg/L) = [Residue weight (mg)] ÷ [sample volume (L)]
TSS (mg/L) = [F+R - FW] ÷ [SV]
where: F+R = Weight (mg) of filter plus residue (after drying)
FW = Filter weight (mg)

SV = Sample volume (L)

Given the following data:
FW = 0.4955 g
F+R = 0.4987 g
SV filtered = 300 mL

Volume (mL) ÷ 1000 = Volume (L)
Weight (g) × 1000 = Weight (mg)

F+R: 0.4987 g × 1000 = 498.7 mg
FW: 0.4955 g × 1000 = 495.5 mg

Residue weight = 498.7 mg - 495.5 mg = 3.2 mg

SV: 300 mL ÷ 1000 = 0.30 L

TSS = [3.2 mg] ÷ [0.3 L]= 10.7 mg/L
TSS = 10.7 mg/L
NOTE: Report: 11; round to nearest whole number.
LOD = [1 mg] ÷ [0.3 L] = 3.3 mg/L for this sample [Report: 3]

Section 5.4 - Ammonia Nitrogen (NH3-N)

5.4.1 Discuss the significance of the ammonia test in wastewater testing.

- Ammonia is a major excretory product of animals and is toxic to organisms at high pH levels.
- Approximately 60% of the nitrogen entering a wastewater plant is ammonia.
- Ammonia toxicity is based on pH and temperature.
- Ammonia affects wastewater plants differently based on type or design.
- High levels of ammonia and the presence of nitrifying organisms will result in increased BOD values.

5.4.2 Discuss approved technologies for ammonia determination in wastewater.

Chapter NR 219, Wisconsin Administration Code, cites the following technologies which are approved for the determination of ammonia:

- Colorimetric, manual, Nesslerization
- Colorimetric, automated, phenate reagent. NOTE: A HACH EPA equivalent colormetric method, using salicylic acid is now approved.
- Ion selective electrode (ISE), either manual or automated
- Titration, manual

Of these techniques, the ion selective electrode generally is most appropriate for use in wastewater treatment plants due to its ease of use, although generally the newer HACH test tube plus method is very easy and reliable.

5.4.3 Discuss why, although an approved technique, the Nessler method is not a good choice for ammonia determinations.

The Nessler technique has concentration ranges limitations; this technique is only appropriate for levels of 0.05 to 1.0 ppm. More important however is the concern over the toxicity of the Nessler reagent itself, both to the environment and the health risks it poses for analysts. Nessler reagent contains significant levels of mercury.

All Nessler samples should be treated as hazardous waste and disposed of accordingly.

5.4.4 Discuss how an ammonia electrode works.

A "gas-sensing" type electrode is used for ammonia analysis. Other types of probes include glass membrane electrodes, solid state electrodes, and liquid exchange electrodes.

5.4.5 Discuss the appropriate storage solution for an ammonia probe.

Essentially, a probe must be stored in an ammonium chloride solution more concentrated than the highest calibration standard.

A probe will be ruined if stored in reagent water for any length of time. This is because the ionic strength of the filling solution (ammonium chloride) is so much greater than that of reagent water. Consequently, the ions (ammonium chloride) move quickly across the membrane to equilibrate with the reagent water. This movement across the membrane causes an internal pH change which is correlated to ammonium concentration.

This also explains why a probe filling solution needs to be refreshed (there's a reason why the manufacturer usually provides a small bottle of electrode filling solution with each probe purchased).

5.4.6 Discuss the critical considerations associated with replacing the membrane on an ammonia probe.

After replacing the membrane on a probe, the new probe must be allowed to equilibrate for at least several hours. For best results, probes should be allowed to equilibrate overnight before use. If the probe is used before it has fully equilibrated, it is likely that the analyst will find that meeting calibration acceptance criteria associated with electrode slope is difficult, if not impossible.

5.4.7 Discuss the importance of allowing a proper amount of time to obtain a stable ammonia measurement on low level samples and standards.

If the response of the low concentration calibration standard is slow, the millivolt response can be biased low relative to that of more concentrated calibration standards which respond much more rapidly. The net result is that the millivolt difference between calibration standards that are a factor of ten different in concentration falls outside of the acceptable range.

As ammonia concentration decreases electrode response time takes longer. The lower the concentration of ammonia in the sample, the fewer the number of ammonia molecules as

gas that pass across the membrane to react with water and trigger a pH change observed as a millivolt increase on the meter. Most meters come equipped with circuitry that identify the point when the reading is stable as the point at which the increase in millivolts slows appreciably.

Unfortunately, many of these computer chips "jump the gun" and lock in on a millivolt response when the millivolts are actually continuing to rise… although quite slowly. For this reason, many manufacturers will suggest techniques such as pressing the "READ" button twice, or allowing an initial minute or two of measurement to be recorded before actually pressing the "READ" button.

This phenomenon explains why blank measurements are often incorrectly believed to be above the limit of detection for ammonia. It also explains why some people have trouble meeting calibration acceptance criteria.

5.4.8 Discuss proper maintenance of ammonia probes.

Routine maintenance should include:
- Regular membrane changes (membranes become pitted, torn, and discolored)
- Filling solution (electrolyte) changes
- Proper storage (in 100 or 1000 ppm ammonia standard)

5.4.9 Discuss the symptoms which indicate the need to replace the ammonia probe membrane.

Membrane failure will cause a shift in electrode potential, drift and poor response. Replace the membrane module if:

- The electrode response becomes very slow
- The results are not reproducible
- The slope becomes too low or shifts
- Visual inspection detects dark spots or discoloration of the membrane

5.4.10 Explain why temperature is so critical when using the ion-selective electrode.

Ammonia electrodes function according to the physical constraints of the Nernst equation, and in that equation temperature is the only variable. Each one degree (°C) change in temperature is associated with a 1-2% error due to changes in the electrode slope. Therefore, calibration standards and samples must be at the same temperature.

5.4.11 Discuss the requirement to distill samples prior to performing ammonia determinations.

All wastewater samples must be distilled. An exemption is provided for domestic municipal wastewater effluents that do not have any significant industrial or waste componant that are analyzed using ion selective electrodes.

5.4.12 Discuss the minimum number of calibration standards required for ammonia determinations.

Calibrations must consist of at least 2 standards. In addition, a blank is required as part of the calibration for colorimetric analyses. Due to the physics governing ion-selective electrodes, a blank must be analyzed, but it is not included as part of the calibration.

5.4.13 Discuss the minimum suggested range between calibration standards when using the ion-selective electrode and why.

Each calibration standard should be exactly 10 times the concentration of the next lowest calibration standard. This is frequently referred to as a "ten-fold" or a "decade" difference.

The reason for this requirement is that electrode response is dictated by the Nernst equation. The Nernst equation states that at 25°C, each ten-fold rise in concentration will result in an increase in electrode response, or slope, of 59.1 millivolts.

Temperature is the only variable involved; as temperature increases, the slope increases.

5.4.14 Discuss the lowest recommended concentration for calibrations and why.

It is not recommended that calibrations be prepared using calibration standards lower than 0.2 mg/L. Below about 0.2 mg/L, electrode response is so slow it can be difficult to obtain a stable reading, resulting in a poor calibration. Although it is a “Good Laboratory Practice” to bracket samples with calibration standards, the lower limit of the calibration range should never extend below the LOD or 0.2 mg/L. The LOQ represents a good lower calibration point.

5.4.15 Discuss the acceptance criteria for ammonia using ion-selective electrode.

The calibration must yield a slope within the range of 54-60 millivolts. A slope within these parameters represents a valid calibration.

Slope is based on the Nernst equation. The only variable is temperature; slope varies directly with temperature.

Theoretical slope is 59.1 mV @ 25°C or 58.1 mV @ 20°C. Some ion meters display the slope as a percentage of the theoretical (based on 25°C).

For example, a 98.5% slope = of 58.27 (which represents 98.5% of 59.1 mV).

5.4.16 Discuss the use and preparation of ammonia-free water.

Ammonia-free water should be used to prepare all reagents and standards. Ammonia-free water can be prepared by either ion-exchange or distillation.

The presence of ammonia gas in the air makes it difficult to store ammonia-free water in the laboratory. To store ammonia-free water properly, store in a sealed glass container. Add about 10 grams of a strong acidic cation-exchange ion-exchange resin. Allow resin to settle; decant ammonia-free water. If blanks are still high, replace the resin or prepare fresh ammonia-free water.

5.4.17 Discuss the purpose of the NaOH buffer solution added to samples and standards and why it should not be added until after the probe is immersed in the sample.

In aqueous solutions, ammonia exists in an equilibrium between two forms, the ionized form (NH_4^+, ammonium ion) and the un-ionized form (NH_3, ammonia gas). The relative amount

of each form present in any sample is controlled solely by pH and temperature. As temperature and pH increase, the predominant form becomes the NH3 (gaseous) form. This is, of course, the form which can cross the gas permeable membrane of the ammonia probe. Since ammonia is a gas, however, it can dissipate quickly from solution. To prevent these rapid losses of ammonia from being measured, the probe is placed into the solution and readings initiated BEFORE the buffer solution is added to a sample or standard.

NOTE: At least one manufacturer sells a buffer solution which remains blue in color as long as the solution pH is above 11.

5.4.18 Discuss the QC sample types and frequencies required for ammonia testing by ion selective electrode.

- Blanks: Required on each day of analysis
- Calibration verification standards: Required after every 20 samples
- LCS: Required every 20 samples per batch of samples processed
- PT samples: Required for each matrix technology and analyte combination at least once annually

NOTE: If using any other technology other than ion selecive electrode additional QC analyses may be required.

Section 5.5 - Total Phosphorus (TP)

5.5.1 Discuss the significance of total phosphorus test in wastewater testing.

Phosphorus is a vital nutrient for plant growth and for metabolic reactions of all other life forms. Phosphorus is often a limiting factor for algal growth. Consequently, the more phosphorus that is discharged to receiving stream systems, the greater the likelihood of algae and aquatic weed growth downstream.

The two forms of phosphate in our natural environment are inorganic and organic phosphates. Natural sources include phosphate-containing rocks and solid or liquid wastes used as fertilizer. The organic form is found in living plants, animals, and animal by-products. Inorganic forms are found attached to soil particles or present in laundry detergents (Alcotabs, commonly used, contain 4% phosphorus as P: 40,000 mg/L). Simple, or "ortho" forms of phosphate are products of natural processes in wastewater. More complex, "poly" phosphates are used for treating boiler waters (to prevent corrosion and scale formation) and detergents.

Phosphorus in wastewater is usually present in the form of organic compounds and phosphates that can easily be removed by chemical precipitation. This process, however, increases the volume and weight of sludge. It has been estimated that the human body releases about a pound of phosphorus per year.

5.5.2 Identify the critical requirements associated with spectrophotometers.

One of the following is required:

1) Spectrophotometer, with infrared phototube for use at 650 or 880 nm, providing a light path of 2.5 cm or longer.
2) Filter photometer, equipped with a red color filter and a light path of 0.5 cm or longer.

Generally handheld colorimeters will not be adequate to make the determinations required for compliance testing.

5.5.3 Discuss critical reagent and standard requirements for total phosphorus testing.

If standards are not purchased, dry chemicals, such as potassium dihydrogen phosphate must be dried at 105°C for at least an hour before preparing standards. This process ensures that bias is not introduced by the absorption of moisture from the air by the dry chemical, thereby increasing its mass.

The ascorbic acid solution, one component used in preparing the color development solution, must be prepared fresh weekly and stored at 4°C in the dark.

Combined color reagent, once prepared, must be used within 4 hours or discarded.

5.5.4 Explain how color reagent is prepared.

Four individual reagents make up the "combined" color reagent. The first three can be purchased commercially. Ascorbic acid must be prepared fresh weekly.

- Potassium antimonyl tartrate reagent: Dissolve 1.3715 g K(SbO)C4 H4 • 1/2 H20 in 400 ml reagent water in a 500 ml volumetric flask.
- Dilute to volume with reagent water. Store in a glass-stoppered bottle.
- Ammonium molybdate reagent: Dissolve 20 g (NH4)6Mo7024 • 4 H20 in 500ml reagent water. Store in a glass-stoppered bottle.
- Sulfuric acid, 5N: Dilute 70 ml conc. H2S04 to 500 ml with reagent water.
- Ascorbic acid, 0.01M: Dissolve 1.76 g ascorbic acid in 100 ml reagent water. Store at 4°C in the dark. Discard after one week.

To prepare Combined Color Reagent (100 mL), add each of the following in the order listed; mix after each addition.

50 mL 5N sulfuric acid,
5 mL potassium antimonyl tartrate,
15 mL ammonium molybdate
30 mL ascorbic acid

5.5.5 Discuss techniques approved for Total Phosphorus analysis in wastewater.

There are three (3) techniques approved by the EPA:
- Single reagent, ascorbic acid [650 or 880nm, BLUE]
- Two reagent, ascorbic acid [650 or 880nm, BLUE]
- Automated, ascorbic acid [650 or 880nm, BLUE]
- HACH procedures, ascorbic acid [890 or 880nm, BLUE]

Bottom line: you must use a procedure that….
(1) measures absorbance at 650, 880 or 890 nm,
(2) involves development of a blue colored solution, and
(3) uses ascorbic acid as a reagent in the color-producing step.

5.5.6 Discuss the different types of total phosphorus digestion apparatus and critical specifications.

- Hotplate: Boil gently 30 to 40 min or until volume is reduced from 50 mL to 10 mL. Do not allow to go to dryness.
- Autoclave: 15-20 psi (98-137 kPa) for 30 minutes.
- Block digester, including COD Reactor (Test N' Tube): 150°C for 30 minutes.

5.5.7 Discuss critical calibration requirements for the total phosphorus test.

- Lab certification code requires at least three calibration standards plus a calibration blank. The more calibration standards used to develop the relationship between phosphorus concentration and absorbance, the more accurate the calibration.
- Calibration standards must bracket sample concentrations.
- Phosphorus calibrations are evaluated using the correlation coefficient which must be greater than or equal to 0.995.

5.5.8 Discuss critical requirements of the color development phase in the phosphorus test.

- 8 mL of combined color reagent must be added to 50 mL of digested sample.
- Combined color reagent must be no more than 4 hours old.
- After a minimum of ten (10) minutes, but no longer than thirty (30) minutes following addition of color reagent, measure the color absorbance of each sample at the appropriate wavelength.
- For Test N' Tube: Read samples between 2 and 8 mins. after addition of PhosVer 3 powder reagent.

5.5.9 Explain how you might know if the ascorbic acid solution needs to be replaced.

- Mixed color reagent is dark yellow.
- Standards or samples don’t turn blue.
- Standards turn yellow, not blue.
- It's over a week old.

5.5.10 Discuss sources and control of contamination in phosphorus analysis.

The phosphorus test is extremely sensitive at even trace levels. Phosphorus is all around us, so it is a very easy test to contaminate. Common sources of contamination include: tap water, oils on fingers, tobacco residue, soap and some detergents, and laboratory buffers or other reagents.

Be sure to clean glassware thoroughly, and maintain laboratory cleanliness. Blanks and standards are critical in monitoring for phosphorus contamination. Keeping records of the absorbance of blanks and standards over time will help detect changes in reagent water quality or reagents.

General considerations to control phosphorus contamination include, but are not limited to:
- Wash glassware well, using a non-phosphate detergent.
- DO NOT use Alconox (contains ~8.7% phosphorus).
- Rinse glassware with dilute (1-10%) hydrochloric acid.
- Never re-use HCl solution to wash glassware (Used acid soon becomes contaminated and can contaminate all of your glassware).
- Even new glassware needs to be washed.
- DO NOT touch inside of glassware with bare hands.
- DO NOT smoke or use air fresheners in the laboratory.
- Cover samples if you use autoclave for digestion.
- Segregate glassware for TP (but still rotate glassware).

5.5.11 Discuss the QC sample types and frequencies required for total phosphorus testing by manual colorimetric technique.

- Blanks: Required on each day of analysis
- Initial calibration verification (ICV): Required immediately after an initial (multi-point) calibration; It should be repaired from a second source standard
- Calibration verification standards: Required at the beginning of each day that an initial calibration is not performed and required after every 20 samples
- LCS: Required every 20 samples per batch of samples processed
- PT samples: Required for each matrix technology and analyte combination at least once annually

NOTE: If using any other technology other than the manual colorimetric technique, additional QC analyses may be required.

Section 5.6 - Total Residual Chlorine (TRC)

5.6.1 Discuss sample handling procedures for total residual chlorine.

- Collect samples in amber, glass bottles treated with bleach to remove chlorine demand.
- Treat bottles by filling with reagent water, adding a few mL of household bleach, allow to soak about 30 minutes, and then rinse thoroughly with tap water followed by reagent water.
- Minimize the time between sampling and analysis (preferably less than 15 minutes) to avoid loss of chlorine.
- Warm samples to room temperature before testing with the ISE method.
- Fill sample completely to minimize contact with the air until samples are tested.

5.6.2 List approved procedures for total residual chlorine analysis.

- Amperometric
- Iodometric
- Back-Titration
- DPD-FAS
- DPD Spectrophotometric
- Ion Selective Electrode

Of these methods, the DPD spectrophotometric method and the ion selective electrode

procedure are the ones typically used.

5.6.3 Discuss the preferred reagent used to prepare standards in the DPD determination of total residual chlorine.

A potassium permanganate (KMnO4) solution is used as a chlorine equivalent. A solution containing 0.891 g/L of potassium permanganate has a chlorine equivalent of 1000 mg/L.

5.6.4 Discuss the Department permit reporting requirements and LOD requirements for total residual chlorine.

Permits are written to include a detection limit requirement of 38 ug/L residual chlorine. However, laboratories are considered to be in compliance as long as their reporting limit for residual chlorine does not exceed 100 ug/L.

5.6.5 Discuss spectrophotometer requirements for the DPD method.

Most brands of spectrophotometers or filter photometers may be used to measure chlorine in wastewater effluent using the DPD method if they meet the following requirements:
- The wavelength can be adjusted to the 515-530 nm range
- It will accommodate a 2 cm or larger cell

5.6.6 Discuss the critical steps for DPD analysis of total residual chlorine.

1. Pipet 10 mL of sample (or standard) into instrument cell.
2. Add DPD color reagent using either of the following options:
- Cut open powder packet, squeeze open the sides and carefully transfer to cell.
- If using the commercially obtained powder dispenser, invert over cell and pull trigger dispenser.
3. Cap cell, mix thoroughly, and start timer.
4. Allow the required time for color development based on recommendations of the manufacturer of the reagent you are using. Some manufacturers recommend 3 minutes, while others suggest only 2 minutes. Experience has shown that a 3 minute color development period works best.
5. Measure absorbance at the appropriate wavelength.
6. When analyzing samples that have natural color or turbidity that may bias the absorbance, record the absorbance both with and without DPD. Subtract the absorbance from the analysis without DPD from the absorbance obtained from analysis with DPD added to get the adjusted (true) sample absorbance. This adjusted absorbance corrects for any natural absorbance of the sample due to color or turbidity.

5.6.7 Discuss the reagent used for standardization in the ion selective electrode determination of total residual chlorine.

A potassium iodate (KIO3) solution is used as a chlorine equivalent. A solution containing 0.1005 g/L of potassium iodate has a chlorine equivalent of 100 mg/L. Chlorine standards can be prepared using household bleach formulations, but standards prepared from bleach are unstable. The iodate standards are much more stable.

5.6.8 Discuss the critical steps for ion selective electrode analysis of total residual chlorine.

1. Add a magnetic stir bar to a 150 mL beaker.

2. Pipet (or pour-graduated cylinder) 100 mL of sample into a clean 150 mL beaker.
3. Add 1 mL of iodide reagent to the beaker.
4. Add 1 mL of acid reagent to the beaker containing the standard.
5. Place beaker on magnetic stirrer and allow solution to mix about 20-30 seconds.
6. Insert electrode into solution and turn off magnetic stirrer, set meter to the mV mode.
7. Allow solution to sit and react for 2 minutes.
8. Allow meter to stabilize. Be patient, it may take 5 or more minutes to stabilize. Record mV readings on bench sheet.
9. Repeat steps 2 - 8 to measure the remaining samples.

5.6.9 Discuss the lowest recommended concentration for chlorine electrode calibrations and why.

It is not recommended that calibrations be prepared using calibration standards lower than 0.1 mg/L. Below about 0.1 ppm, electrode response is so slow it can be difficult to obtain a stable reading, resulting in a poor calibration. Although it is a “Good Laboratory Practice” to bracket samples with calibration standards, the lower limit of the calibration range should never extend below 0.1mg/L, even though an LOD less than 0.038 mg/L can reasonably be achieved.

Section 5.7 - Process Control

5.7.1 Discuss the importance of pH in process control.

pH of the biological mass in treatment facilities must be monitored to determine if the levels are too acidic or caustic for the microbes in the plant or the receiving water. Most wastewater plants in Wisconsin have an effluent discharge limit of 6.0 to 9.0 standard units (s.u.) in their permit, but pH levels of 6.8 to 7.2 are optimum for biological activity of most aerobic organisms. Laboratories may use bench-top pH meters for the analysis, using two or more calibration pH buffers that bracket the pH levels measured. The pH meter should be calibrated daily using fresh buffers. There is no holding time for pH analysis, so all analyses for permit use must be done on-site.

Generally, the lower the pH of wastewater, the greater the disinfection capacity with chlorine compounds, to a point. Below the pH of 4.0, very little disinfection takes place. The higher the pH, the lower the disinfection capability of chlorine. A pH of 5.5 to 7.5 is optimal.

The optimum pH for nitrification is 7.5 to 8.5 su. As ammonia is converted to nitrite and nitrate, alkalinity decreases and pH of the wastewater may drop.

5.7.2 Discuss the importance of the 30-Minute Settleability Test in process control.

The 30-Minute Settleability Test is used to monitor the operations of final clarifier settling action, since it mimics the activity of the activated sludge in that process. It is also used to monitor the general appearance of the biomass. A mixed liquor sample from the end of the aeration basins, usually a one-liter sample, is placed in a one-liter settleometer, graduated cylinder, or beaker. The settling rate is monitored for at least 30 minutes, with a reading taken after a 30 minute settling period. The 30-minute settleability test result is also used to determine the Sludge Volume Index (SVI).

5.7.3 Discuss the importance of Dissolved Oxygen in process control.

Dissolved Oxygen (DO) field analysis is used to monitor the amount of dissolved oxygen in various areas of the facility. A dissolved oxygen meter with a submersible field probe is used. Some facilities also have in-line DO monitors. Aerobic organisms require a minimum DO level of about 2.0 mg/L for proper health. When a facility is accomplishing biological nitrogen and phosphorus removal, the oxygen levels in the anaerobic, anoxic, and oxic selectors must be carefully monitored.

The figure below shows ammonia toxicity with pH and temperature. Multiply the ammonia concentration by the factor for the sample pH and temperature to determine the concentration of un-ionized ammonia.

Figure 5.7.3.1

pH	Temperature													
	42.0 (°F)	46.4	50.0	53.6	57.2	60.8	64.4	68.0	71.6	75.2	78.8	82.4	86.0	89.6
	6 (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0238	.0271	.0310	.0369
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0664	.0743	.0877
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617	.7929
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351	.8585
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271	.9389

5.7.4 Discuss the importance of testing Mixed Liquor Suspended Solids (MLSS) in process control.

Mixed Liquor Suspended Solids (MLSS) are the suspended solids of the mixed liquor in an aeration basin. Mixed liquor is a term referring to the suspended microbiological growth or activated sludge in an aeration tank. Since flows, microorganism levels, and contents of these basins can change daily, the level of microorganisms and other suspended solids needs to be monitored and controlled. The mixed liquor suspended solids test is analyzed in the same way as a total suspended solids test. A larger diameter filter may be used, as the MLSS levels may run between 1100-6000 mg/L. Some facilities use a centrifuge for a quick estimate of the MLSS in the system, but the results need to be frequently compared to the 103-105°C oven procedure for accuracy.

5.7.5 Discuss the importance of Microbiological analysis in process control.

Microbiological analysis is used to assess the biological health of the wastewater system. This is especially important with activated sludge processes. Since biological processes contain a mix of microorganism cultures, the operator should frequently use a microscope as a tool to determine if the proper biological life forms are flourishing. A ratio of types of microorganisms, such as amoeba, grazing ciliates, stalked ciliates, rotifers, nematodes, and other organisms can determine the health of the activated sludge. Microbiological examination can also give the operator a warning of potential problems, such as a predomination of filamentous organisms or other problematic species.

Chapter 6 - QA/QC (Quality Assurance/Quality Control)

Section 6.1 - Definitions

6.1.1 Define Quality Control.

Quality control (QC) is a variety of techniques that the sampler and analyst perform to verify that the sampling and analytical protocols meet the desired goals for data quality. QC functions help to ensure data validity and traceability.

6.1.2 Define Quality Assurance.

Quality assurance (QA) is described as a management function which rests on the documentation and establishment of quality control protocols, and on the evaluation and summarization of their outcomes.

Quality assurance is the system for checking and ensuring that quality control criteria are appropriate based on the desired level of quality (precision and accuracy) of data being generated.

The role of the quality assurance program then, is to continually review all aspects of the quality control program and make adjustments— or initiate corrective action— as needed to achieve the laboratory’s data quality goals.

6.1.3 Define Bias and Precision

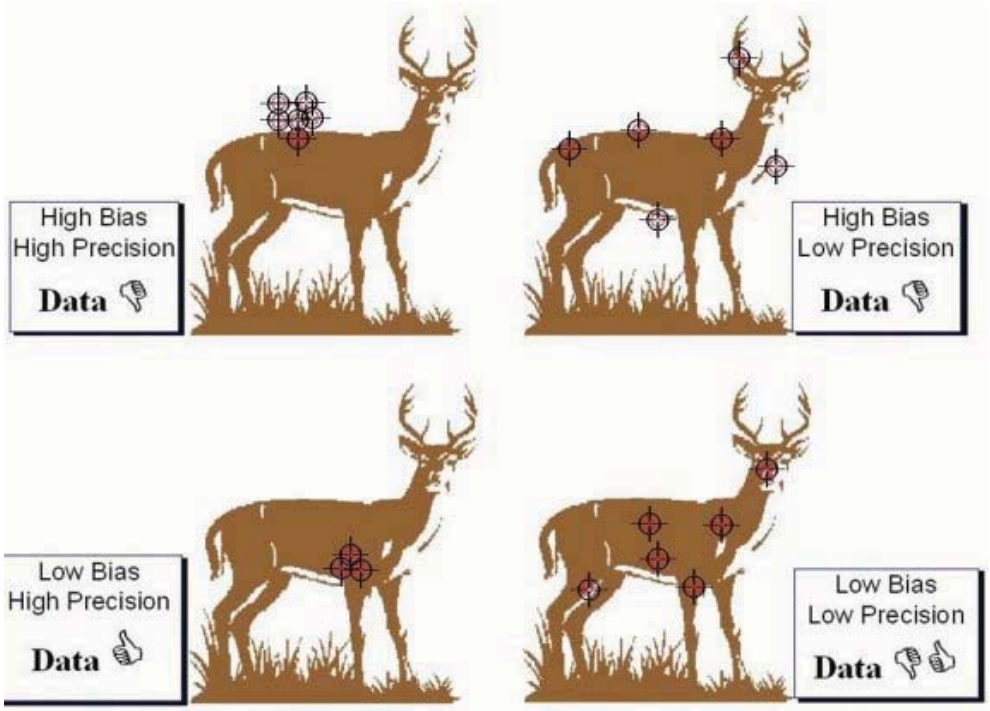
Bias (Accuracy) is the systematic or persistent error in an analysis which results in the expected sample measurement being consistently different than the sample's true value.

A systematic bias is a bias resulting from a flaw integral to the system within which the bias arises (for example, an incorrectly calibrated thermostat may consistently read - that is 'biased' - several degrees hotter or colder than actual temperature). As a consequence, systematic bias commonly leads to systematic errors, as opposed to random errors, which tend to cancel one another out.

Precision is a measure of how closely multiple determinations performed on the same sample will agree with each other. Accuracy is the degree of closeness to the actual value while precision is the degree of reproducibility. Using the typical "target" analogy, if a large number of arrows are fired at the target, the size of the arrow cluster on the target

represents the shooter's precision. When all arrows are grouped tightly together, the cluster is considered precise since they all struck close to the same spot. Note that the cluster may be very precise, by virtue of a tight grouping, but if the cluster is nowhere near the bullseye, then the shooter is precise but not accurate.

Figure 6.1.3.1



6.1.4 Define Percent Recovery

Percent recovery refers to the percentage of the analyte added to a spiked sample that is detected. It is the difference between the mass (weight) detected in the spiked sample and that detected in the un-spiked sample, divided by the mass (weight) of the substance added to the spiked sample. Percent recovery measures accuracy -- the closer to 100 percent recovery, the greater the accuracy. Note that percent recovery can also be calculated based on the concentration (mass per unit volume, e.g. mg/L). Concentration-based calculations, however, are more difficult calculations because dilution of the sample or spike material by the addition of the spike material must be considered.

6.1.5 Define the following terms:
A. Arithmetic Mean.
B. Geometric Mean.

A. Arithmetic Mean.
The arithmetic mean (or simply the mean) of a list of numbers is the sum of all the values divided by the number of values.

If four (4) values are 20, 10, 80, and 10, the arithmetic mean is (20 + 10 + 80 + 10) ÷ 4 = 30.

B. Geometric Mean
The geometric mean of a set of positive data is defined as the 'n'th root of the product of all

the values, where 'n' is the number of values. The geometric mean of a data set is always smaller than or equal to the set's arithmetic mean (the two means are equal if and only if all values of the data set are identical).

If four (4) values are 20, 10, 80, and 10, the geometric mean is the 4th root of (20 × 10 × 80 × 10) or the 4th root of 160,000. The 4th root of 160,000 is 20. [Note that the arithmetic mean (from above) is 30]

The geometric mean is used for microbiological analysis due to the unpredictablity of their exponential growth rate.

6.1.6 Define Standard Deviation

Standard Deviation: The measure of the dispersion of a set of data from it's mean (average). The more spread out the data is, the higher the standard deviation. It is calculated like this:

x = one value in your set of data
avg (x) = the mean (average) of all values (x) in your set of data n = the number of values (x) in your set of data

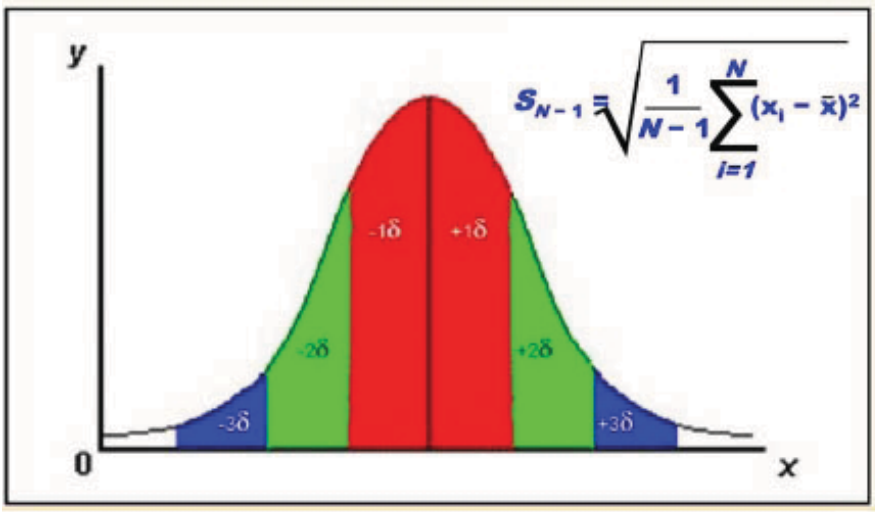
1. For each value (x), subtract the overall avg (x) from x, then multiply that result by itself (otherwise known as determining the square of that value).
2. Sum up all those squared values.
3. Then, divide THAT result by (n-1).
4. Finally take the square root of the result in Step 3. This value is the standard deviation.

To better understand the concept of standard deviation, we have to consider the normal distribution of data, often termed the "bell-shaped" curve.

In a normal distribution, the values falling within one standard deviation of the mean (red areas only), generally represent about 68% of all values. Two standard deviations away from the mean (the red and green areas) account for a little more than 95 percent of the values. Three standard deviations (the red, green and blue areas) account for about 99.7 percent of the values.

The figure below is a graphical representation of standard deviation indicating how much of a set of data should be represented by +/- 1, 2, and 3 standard deviation on either side of the mean.

Figure 6.1.6.1



6.1.7 Define the following terms:
A. Range
B. Relative Percent Difference

A. Range
The range is calculated by subtracting the smallest value from the greatest value and provides an indication of statistical dispersion, or how widely scattered the data is. For duplicates, range is calculated as the larger of the two minus the smaller of the two. It is measured in the same units as the data.

Note that the range cannot be calculated whenever one or both values is a "<" value. Simply record the range as (Not Calculable). If parameters such as ammonia, which can be spiked, are frequently "non-detectable" results, then consider using duplicate spikes to generate precision information.

B. Relative Percent Difference (RPD)
Used with duplicate samples, the relative percent difference measures precision -- the lower the value, the more precise the results. Relative percent difference is calculated by dividing the difference (range) between the two results by their average (mean).

For example:
Sample result = 18 mg/L
Duplicate result = 22 mg/L

Range = (22 - 18) = 4 mg/L
Mean = (22 + 18) ÷ 2 = 20 mg/L
RPD = (4 ÷ 20) × 100 = 20%

Note that the RPD cannot be calculated whenever one or both values is a "<" value. Simply record the RPD as "NC". If parameters such as ammonia, which can be spiked, are frequently "non-detectable" results, then consider using duplicate spikes to generate precision information.

6.1.8 Define the following terms:
A. Duplicate/Replicate Sample.
B. Matrix Spike

A. Duplicate/Replicate Sample:
A duplicate (or replicate) is a sample from which representative portions have been taken and analyzed separately. Duplicate samples are analyzed to evaluate the precision or repeatability of an analysis. Precision answers the question: "If I analyze a sample and get 'X' as a result, if I analyze the sample again, will I still get 'X'?"

B. Matrix Spike:
Spiking the sample refers to the process of adding a known amount of the target analyte to an aliquot of the sample. Samples are spiked before any preliminary treatment step, such as distillation or digestion. Spiked results are then used to determine percent recovery.

6.1.9 Define Laboratory Control Standard (LCS)

“Laboratory Control Standard” or “LCS” means a sample of reagent water spiked with known amount of the analyte of interest. The purpose of an LCS is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

In many EPA methods, the term “lab-fortified blank” is substantially equivalent to a laboratory control sample.

6.1.10 Define Quality Control Standard (QCS).

Quality Control Standard or “QCS” means a solution or sample containing method analyte of known concentration, accompanied by specified analytical acceptance limits, and obtained from a source external to the laboratory and different from the source of calibration standards. These samples are distinguished from proficiency test samples in that the acceptance limits are provided with the sample, rather than after analysis. Quality control standards are used to check either laboratory or instrument performance.

These types of samples are important indicators of quality because instantaneous feedback on performance is provided. This offers the analyst an opportunity to correct any analytical problems in a timely manner. Quality Control Standards are analyzed every four months as a check on analytical performance. Appropriate measures are taken to investigate problems when the result of a QCS analysis is inconsistent with past results.

Unlike PT samples, whose results are not received for some time after the testing is performed, QCS samples can be used at any time there is concern about the control of a specific analysis. Having immediate access to the validated concentrations allows the analyst to take immediate action to identify and correct the problem.

QCS standards are often referred to as "blind standards". QCS sample analysis is not required for tests in which the laboratory incorporates second source standards.

6.1.11 Define the following terms:
A. Warning Limits
B. Control Limits

A. Warning Limits
Warning limits are specified boundaries that indicate a process may be going out of statistical control and that certain precautions are required.

B. Control Limits
Control limits are a specified boundaries that, if exceeded, indicates a process is out of statistical control, and the process must be stopped, and corrective action taken before proceeding.

6.1.12 Define Proficiency Testing (PT) sample.

Proficiency Testing sample or “PT" sample means a sample obtained from an approved provider to evaluate the ability of a laboratory to produce an analytical test result meeting the definition of acceptable performance established by the PT sample provider. The concentration of the analyte in the sample is unknown to the laboratory at the time of analysis. PT samples are used to evaluate whether the laboratory can produce analytical results within specified acceptance limits.

For most tests at least one PT sample must be successfully analyzed each year to renew the lab's certification or registration. Follow-up reference samples are required to be analyzed if the provider acceptance limits are exceeded.

PT samples are also known as performance evaluation samples or reference samples.

6.1.13 Define Outlier

A value in a set of observations that it fails to meet a statistical test for acceptance or is so different from the rest that it is considered to be a member of another set or population.

6.1.14 Define Slope, as it relates to calibrations.

Slope is often used to describe the measurement of the steepness, incline, gradient, or grade of a straight line. In calibrations, this "line" is the line resulting from a linear regression equation.

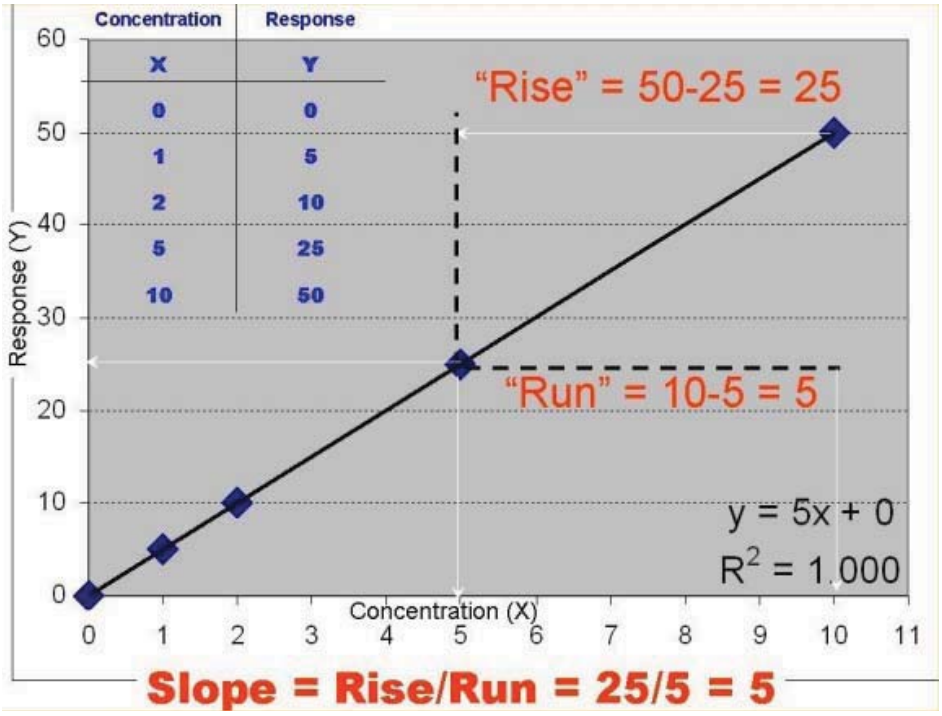
A higher slope value indicates a steeper incline. The slope is defined as the ratio of the "rise" (or increase) of the observed instrument response (Y) divided by the "run" (or

increase) in the measured concentration (X) of calibration standards representing between two points on the regression line.

If Y is a linear function of X, then the coefficient of X is the slope of the line created by plotting the function. Therefore, if the equation of the line is given in the form Y=mX + b then m is the slope.

This form of a line's equation is called the slope-intercept form, because b can be interpreted as the Y-intercept of the line, the Y-coordinate where the line intersects the Y-axis.

Figure 6.1.14.1



6.1.15 Define Correlation Coefficient

The correlation coefficient is usually associated with calculation of a linear regression for calibration. The linear regression equation aims to find a linear relationship between an instrument response and concentration of an analyte. The correlation coefficient, then, offers a measure of how well the linear regression can predict the concentration of an unknown sample from its instrument response.

6.1.16 Define Method Blank.

Method Blank:
A clean sample consisting of all reagent(s), without the target analyte or sample matrix processed simultaneously with and under the same conditions as samples containing an analyte of interest through all steps of the analytical procedure. The response from a sample color blank should be subtracted from the response of the sample.

6.1.17 Define Sample Color Blank

Sample Color Blank:
An untreated sample portion, which is used to account for interferences contained in the sample (such as color or turbidity), particularly for instruments like spectrophotometers.

6.1.18 Define LOD and LOQ.

Limit of detection (LOD) means the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is statistically different from a blank (or, not a false positive value). For department purposes, the LOD approximates the "method detection limit" (MDL, the term used by the EPA and in many methods). The LOD is determined using the procedure in 40 CFR Part 136 Appendix B, Revision 1.11.

Limit of quantitation or (LOQ) means the lowest concentration or amount of an analyte for which quantitative results can be obtained with a specified degree of confidence. The limit of quantitation is typically considered to be a value 10/3 or 3.333 times the limit of detection (LOD).

Section 6.2 - Precision / Accuracy

6.2.1 Discuss the use of range vs. RPD for precision measurements.

Range is preferred at low concentration levels, such as effluent samples.

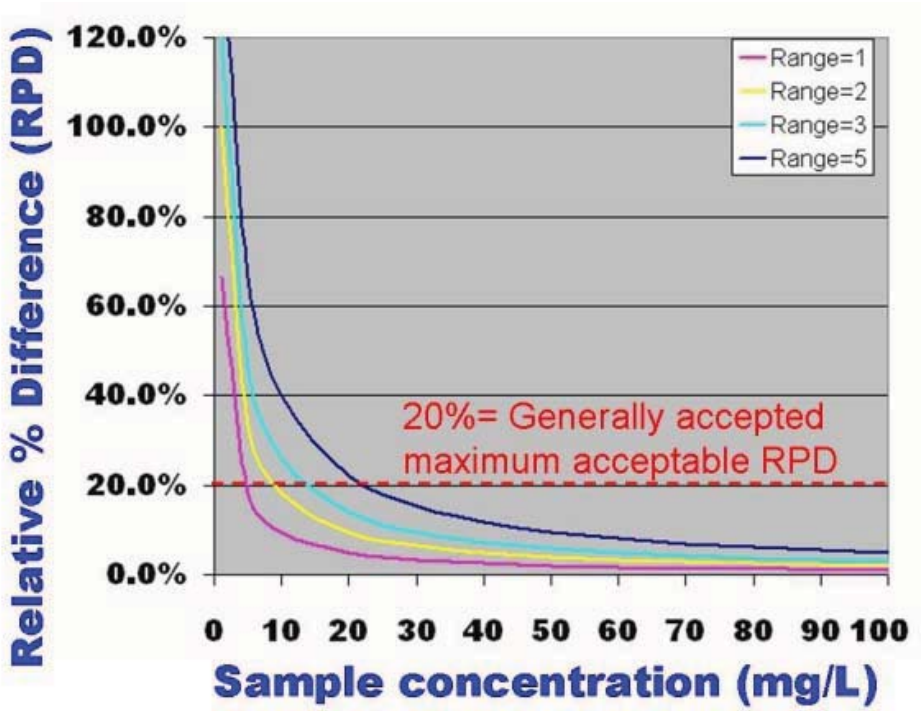
On the other hand, at higher concentrations--typical of influent samples-- larger range values are normalized when using RPD.

The following table illustrates how, for a given range, the RPD increases signifiantly as the value of the two results used to calculate the range decreases. This indicates that as the concentration of replicates approaches the limit of detection, it would be more difficult to ascertain control of the analysis using RPD.

Sample Value 1	Replicate Value 2	Range	RPD
2	4	2	67%
4	6	2	40%
6	8	2	29%
10	12	2	18%
20	22	2	10%
45	50	5	11%
90	100	10	11%
150	170	20	13%
200	225	25	12%
250	300	50	18%

As concentration increases, a point is reached where range fails but RPD passes.
As concentration decreases, a point is reached where range passes but RPD fails.

Figure 6.2.1.1



6.2.2 Discuss the value of Quality Control Charts and how they are developed.

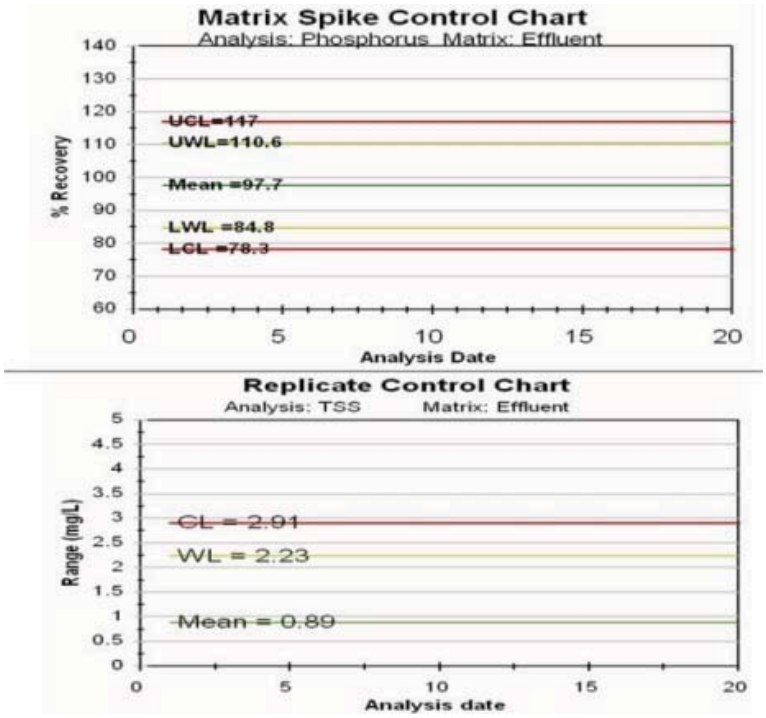
Control charts are not required by NR 149, but are very useful; it is often much easier to identify the source of analytical problems when a graphic representation of the quality control data (i.e., control charts) is reviewed. Once the chart is constructed, quality control values from future analyses are plotted on it. For example when 20 new data points, excluding outliers, have been generated, control limits are again updated.

It is important to remember that the quality control results used to generate limits for a control chart are NOT plotted on that chart. Those limits should be used to construct a new, blank, QC chart - which is filled in over time as future data are generated.

Warning limits are placed at plus two (+2) and minus two (-2) standard deviations of the mean (i.e., at the 95% confidence interval).

Control limits are the mean plus three (+3) and minus three (-3) standard deviations (i.e., the 99.72% confidence level on either side of the central line).

Figure 6.2.2.1



6.2.3 Discuss how control charts can be used to identify potential out-of-control situations before they develop.

Quality Control charts provide a valuable tool for the analyst to “see the big picture”. For example, it’s often difficult to initially assess the cause of a matrix spike that exceeded control limits. By looking at a control chart, however, the analyst may be better able to identify a trend of decreasing recoveries that eventually led to the exceedance. The goal, then, is to use control charts as a form of preventive medicine. Routine review of control charts will enable the analyst to detect –and correct—a potential analytical problem before it leads to an exceedance.

The following conditions indicate an out of control situation. Analysts should be monitoring their control charts for the development of any of these trends. When any QC exceedance is encountered, the operator/analyst must take corrective action, as directed by s. NR 149.14 (3)(h), Wis. Adm. Code.

- Any point beyond the upper or lower control limits.
- Seven successive points on the same side of the mean.
- Another criterion frequently used to identify out-of-control situations is a trend of 5 or more data points moving in the same direction (i.e. 5 points, each of which is successfully higher (or lower) than the previous one).

The 19th edition of Standard Methods suggests the following additional situations warrant corrective action:

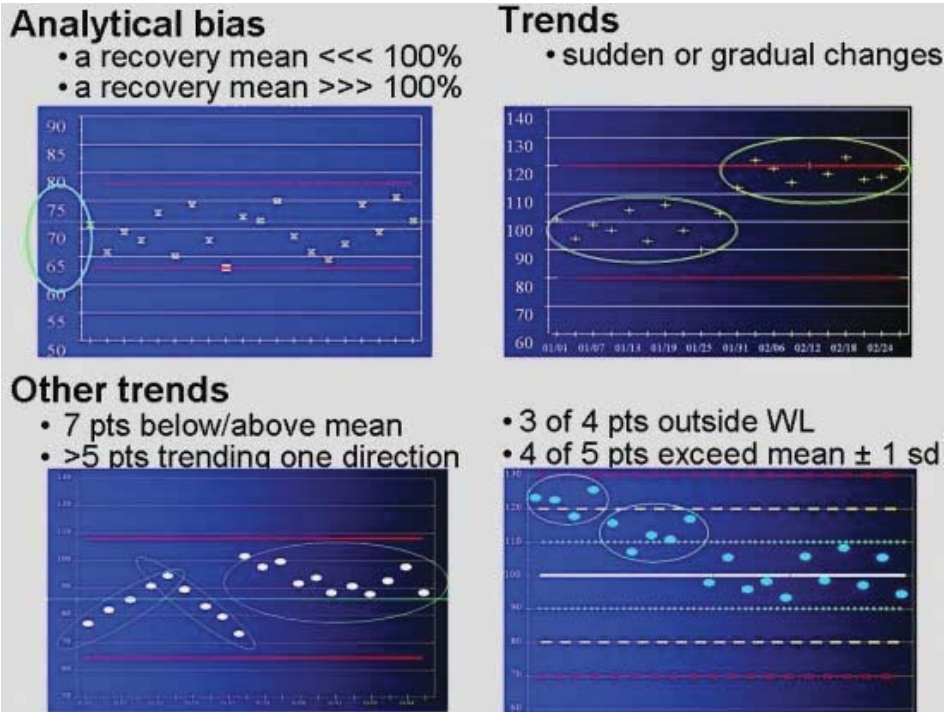
- Repeat analyses exceeding either control limit. If any measurement exceeds a control

limit, repeat the QC analysis once. If the repeat analysis falls within limits proceed, otherwise stop and correct the problem before proceeding.

- 3 of 4 successive data points outside warning limits.
- 4 of 5 successive data points exceeding the mean ± 1 standard deviation.
- 8 successive analyses above (or below) the mean.

If any of these conditions occur, lab priorities should be directed to identifying and resolving the problem. One of the best ways to quickly resolve an out-of-control event, while minimizing the amount of data which must be qualified, is to analyze replicates or matrix spikes for each sample run until the out-of-control situation ceases. The problem and solutions are documented; all results since the last in-control point are reanalyzed or are qualified.

Figure 6.2.3.1



6.2.4 Discuss how outliers are identified.

A number of statistical tests to identify outliers are available, including a procedure used by the USGS, Dixon's test, the t-test, and Grubbs' test. While any of these tests can be employed to identify outlier data, Grubbs' test will be mentioned here due to its relative simplicity.

The Grubbs' test is based on the calculation of a "Z-score" which is then evaluated against a table of criterion values based on the number of data points in the data set.

Z score = [(Mean) - (Suspect result)] ÷ [standard deviation]

Note: if the [Mean - Suspect result] is a negative value, remove the negative sign and use the positive value result in the calculation.

If the resultant Z-score is greater than the corresponding criterion value based on the number of data points, then the suspect value is indeed an outlier, and should be excluded and the control limits re-calculated.

Figure 6.2.4.1

Test the furthest value from the mean

1	110
2	90
3	78
4	91
5	83
6	91
7	81
8	92
9	102
10	80
11	94
12	83
13	79
14	140
15	82
16	92
17	80
18	94
19	98
20	80

Mean= 91
Stdev= 14.3966
N = 20

Highest: (140) – Mean = 49* *CHECK* *
Lowest: Mean – (78) = 13

$$Z = \frac{(140 - 91)}{14.3966}$$

Z= 3.4035726>>> *criterion= 2.709*

∴ 140 is an outlier

N	Critical Z	N	Critical Z	N	Critical Z
15	2.550	27	2.859	39	3.025
16	2.590	28	2.876	40	3.036
17	2.620	29	2.893	50	3.128
18	2.650	30	2.908	60	3.199
19	2.681	31	2.924	70	3.257
20	2.709	32	2.938	80	3.305
21	2.733	33	2.952	90	3.347
22	2.758	34	2.965	100	3.383
23	2.781	35	2.979	110	3.415
24	2.802	36	2.991	120	3.444
25	2.822	37	3.003	130	3.470
26	2.841	38	3.014	140	3.493

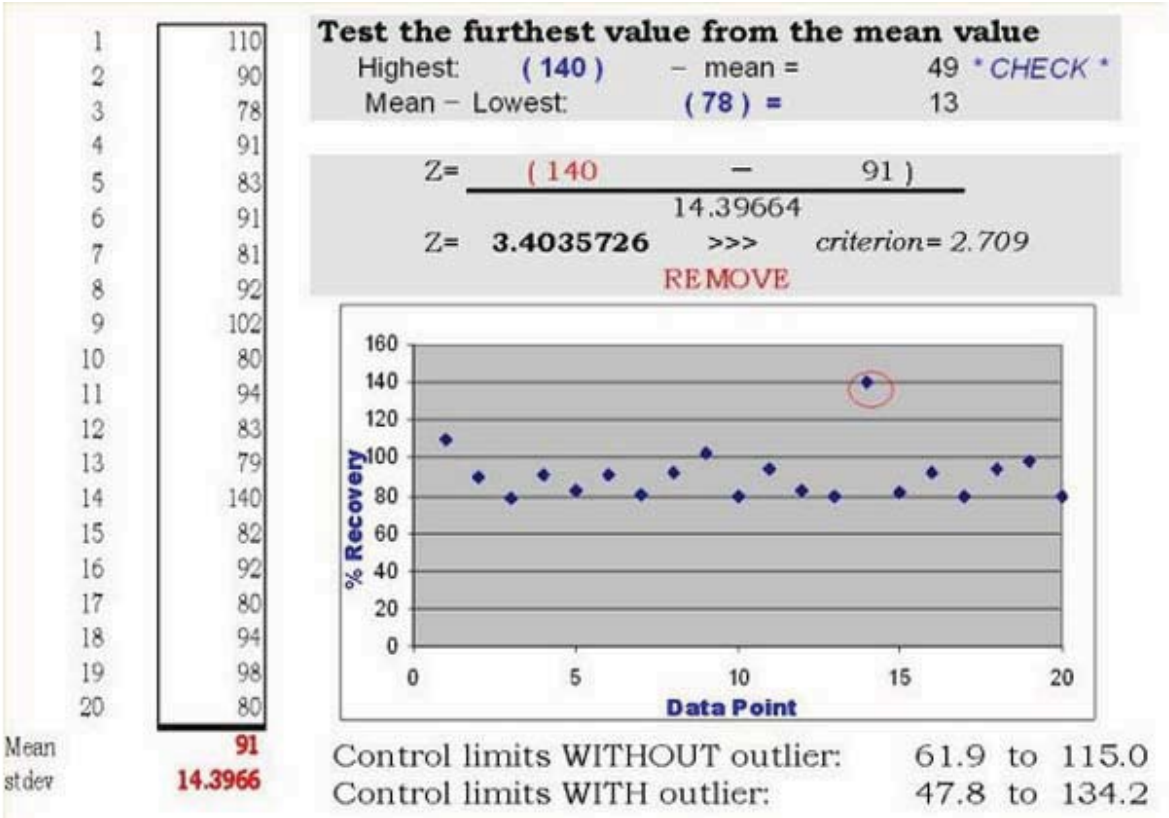
6.2.5 Discuss why outlier rejection is important before calculating control limits.

First, it is critical to understand that the basic premise of any outlier test is simply to assign a statistical likelihood that a given suspect outlier point represents a different population than the other values in a data set. It cannot –and should not—tell you what to do with that point. Because the consequences associated with including outliers (control limits become unreasonably broad allowing inaccurate data) far outweighs the risk of excluding them (control limits become too restrictive and too many data points become suspect), we recommend that outliers be excluded from further use.

A simple example can be seen with a series of five (5) values: 4, 6, 32, 7, 5. If the Grubbs test is performed on this limited set of data, the value, "32" will be identified as an outlier. The mean of the data WITH the outlier is 10.8, yet the mean with the outlier excluded is 5.5. The mean nearly doubles. If one looks at the standard deviation, the standard deviation of the data WITH the outlier is 11.9, yet the standard deviation with the outlier excluded is only 1.3. The inclusion of the outlier increases the standard deviation nearly ten-fold. If control limits were to be calculated based on this small set of data (mean +/- 3 x standard deviation, the control limits WITH the suspected outlier included, the limits would be-24.9 to 46.5. If the outlier is excluded, however, the control limits become a more reasonable 1.6 to 9.4.

Keep in mind that an out of control result is not necessarily an outlier.

Figure 6.2.5.1



6.2.6 Explain why QC samples that exceed quality control (QC) limits should be included on control charts.

Occasionally, after calculating new control limits, the limits are perceived to be too restrictive for ordinary work. An example of this might be matrix spike control limits of 96 to 104%. Clearly, there is little room for error in these situations.

The statistical procedure used in the calculation of control limits is designed to produce limits at the 99% confidence level. In layman's terms, this means that only 1 out of every 100 data points is expected to be judged incorrectly.

The statistical procedure used in the calculation of warning limits is designed to produce limits at the 95% confidence level. In layman's terms, this means that only 5 out of every 100 data points is expected to be judged incorrectly.

It is important to remember that the quality control results used to generate a control chart are NOT plotted on that chart. Those limits should be used to construct a new, blank, QC chart-- which is filled in over time as future data are generated.

6.2.7 Discuss situations that could result in control limits that are too restrictive.

- Exclusion of out-of-control data when calculating control limits. Outliers should be excluded, but all out-of-control points are not outliers.

- Failure to include enough significant figures in QC results. If values are whole numbers (e.g., 10, 89%), you can use one or even two decimal points to include some variability (10.2, 89.3%). This same problem has been observed when a laboratory only reported recoveries to the nearest 5%.

The data below shows that using rounded values (for reporting) to calculate control limits can result in extremely tight control limits.

Figure 6.2.7.1

	Sample	Replicate	Range		Sample	Replicate	Range
1	7	7	0	11	6	6	0
2	8	8	0	12	7	7	0
3	5	5	0	13	8	7	1
4	6	5	1	14	9	9	0
5	8	9	1	15	15	15	0
6	8	8	0	16	19	19	0
7	4	5	1	17	8	8	0
8	7	7	0	18	7	6	1
9	11	11	0	19	10	10	0
10	10	10	0	20	5	6	1

mean= 0.3
st. dev.= 0.47016

Warning Limit: 2.51 X Mean= 0.75
Control Limit: 3.27 X Mean= 1.54

	Sample	Replicate	Range		Sample	Replicate	Range
1	7.4	6.8	0.6	11	6.3	6.1	0.2
2	8.5	7.6	0.9	12	7.3	6.8	0.5
3	5	5.1	0.1	13	8.5	6.6	1.9
4	6.2	4.9	1.3	14	9.2	9.1	0.1
5	7.7	9.4	1.7	15	15.4	14.8	0.6
6	8.2	7.8	0.4	16	18.8	19.4	0.6
7	3.6	5.4	1.8	17	7.9	8.4	0.5
8	6.7	7.3	0.6	18	7.4	5.9	1.5
9	11.3	10.8	0.5	19	9.5	10.2	0.7
10	9.5	10.5	1	20	4.9	6.2	1.3

mean= 0.84
st. dev (SD).= 0.56045

Warning Limit: 2.51 X Mean= 2.11
Control Limit: 3.27 X Mean= 2.75

6.2.8 Discuss what would be reasonable interim accuracy (spikes) and precision (replicates) control limits for the four common wastewater parameters.

Until you have established your own internal control limits the following interim limits should be used to govern the acceptance of initial data. Some method references may specify tighter control limits.

TEST	Accuracy Limit	Precision Limit
Total Suspended Solids	NA	20%
Biochemical Oxygen Demand	NA	20%
Ammonia	80-120%	20%
Total Phosphorus	80-120%	20%

Once you've established your internal control limits, if they are broader than these guidelines, you should investigate the reasons for the differences.

6.2.9 Discuss the term "concentration dependency" and how it relates to QC.

The phrase, "precision is concentration dependent", is a brief way of saying that precision

is difficult to evaluate without knowing the concentration levels involved.

For example, if the range of a pair of replicates is 50 mg/L, an initial impression might be that this represents poor precision. However, if the pair of replicate results are 1,000 and 1,050 mg/L, then a range of 50 mg/L represents pretty good performance. Of course, if the two values are 100 and 50, your initial impression is correct. Recall that the value of precision measures is to answer the question, "If I analyze this sample a second time, will I obtain the same result (or at least very close)"?

Precision data must be further segregated by concentration if a concentration dependency exists. For example, if the influent concentration varies greatly, then some WWTPs may have to further segregate their influent TSS into “low” and “high” concentrations. A good general division is that concentration equal to or greater than ten (10) times the limit of detection (LOD).

For these reasons we recommend using range for effluents and relative percent difference (RPD) for influents.

6.2.10 Discuss what is meant by a "second source" standard and the rationale for using second source standards.

Second source standards are actually a form of QCS sample, analyzed more frequently. The greater frequency helps to identify preparation errors made during dilution of stock standards to prepare working standards. It is recommended that stock standards be purchased from each of two different suppliers or different lots of the same solution may be purchased from a single supplier.

Stock A= primary source used to prepare calibration standards.
Stock B= secondary source used to verify the prepared calibration standards.

Note that second source standards will also help to identify discrepancies in the concentration of original stock standards. Second source standards are frequently used to prepare QC samples, including laboratory control standards (LCS) and matrix spikes.

If there is no independent verification (such as second source standards) and the same solution that is used to prepare calibration standards is also used to prepare spiked QC samples, errors made in the preparation of the stock standard cannot be easily identified.

Second source standards need only be analyzed any time that a new dilution of a stock standard is prepared which will then be used to prepare calibration standards. Many labs, however, routinely include a second source standard, by using second source standards to prepare LCS or matrix spikes. This practice provides a much more frequent, independent verification that standard solutions used to generate calibrations are accurate.

6.2.11 Discuss the critical considerations involved in preparing a matrix spike.

- You must have the same amount of original sample in the matrix spike as in the unspiked sample itself. Therefore, adding a spike to 500 mL of sample represents a different "matrix"

than adding the same spike to 100 mL of sample plus 400 mL of reagent water.

- Spike concentration should be 1-5 X sample concentration.

- Limit spike addition to < 10% of sample volume. If spike addition is < 0.2%, no volume correction is required. The concept here is that the addition of the spike itself, if not based on the addition of a very small (insignificant) volume of a very concentrated solution, can result in a dilution of the background matrix.

Examples:

Correct spike preparation:
- Ammonia: 100 mL of sample is measured to be 0.8 mg/L NH3-N. A spike is prepared by adding 1mL of a 100 mg/L ammonia standard to 100 mL of sample. The spike concentration is 1 mg/L, slightly greater than the sample background concentration.
- Phosphorus: 50 mL of sample is measured to be 0.2 mg/L as P. A spike is prepared by adding 1 mL of a 10 mg/L phosphorus standard to 50 mL of sample. The spike concentration is 0.2 mg/L, or exactly equal to the sample background concentration.

Incorrect spike preparation:
- Ammonia: 100 mL of sample is measured to be 0.8 mg/L NH3-N. A spike is prepared by adding 10 mL of a 100 mg/L ammonia standard to 100 mL of sample. The spike concentration is 10 mg/L, or significantly higher than the sample background concentration. The use of 10 mL of spike solution also creates a significant dilution of the sample matrix.
- Phosphorus: 50 mL of sample is measured to be 0.2 mg/L as P. A spike is prepared by adding 0.5 mL of a 5.0 mg/L phosphorus standard to 50 mL of sample.The spike concentration is 0.05 mg/L, or only 25% of the sample background concentration. It will be quite difficult to obtain an acceptable recovery.

Section 6.3 - Limit of Detection (LOD)

6.3.1 Discuss the procedure for determining an LOD.

The most common, and simplest, procedure for determining the LOD can be summarized as:

1. Determine a spike concentration, which approximates the LOD.
2. Prepare 7 or 8 replicates of reagent water spiked at an appropriate level.
3. Analyze the replicate spikes. NOTE: To ensure that day-to-day precision is considered, these replicates should be analyzed on different, preferably non-consecutive, days.
4. Calculate the LOD: Multiply the standard deviation of replicates by the "t-value" (99% confidence) associated with the number of replicates.
5. Perform the “Wisconsin DNR 5-point” check of the LOD. Repeat the LOD determination as appropriate.

IMPORTANT: This procedure is NOT used to determine the LOD for BOD and TSS!!!!!

NOTE: The EPA procedure for determining detection limits can be found in 40 CFR Part

136 Appendix B, Revision 1.11. The procedure also contains t-table values required for step 4 above.

6.3.2 Discuss the "5-point" check used to evaluate an LOD.

The calculated LOD should be evaluated using several checks to determine if it will meet all of the necessary criteria. The following five actions, which will be collectively referred to as the "Wisconsin DNR 5-Point Check", are simple ways to evaluate a calculated LOD.

1. Does the spike level exceed 10 times the LOD? If so, the spike level is too high.
2. Is the calculated LOD higher than the spike level? If so, the spike level is too low.
3. Does the calculated LOD meet regulatory requirement (i.e., permit limits)?
4. Is the signal/noise (S/N) in the appropriate range of (2.5 to 10)?
5. Are the replicate recoveries reasonable? (e.g., for ammonia and phosphorus, it is not unreasonable to expect 80-120%)

Items 1, 2, and 3 are requirements for all LODs. Items 4 and 5 are not required, but are useful for evaluating the LOD data.

This example calculates the LOD for ammonia-nitrogen using the approved ion selective electrode method. The electrode's manufacturer claims that the probe can detect ammonia as low as 0.05 mg/L. The first step, estimating the LOD, requires familiarity with the analytical procedure. In this case, the manufacturer's claim is a convenient place to start. Multiplying the manufacturer's number, 0.05 mg/L by a factor of 5 gives 0.25 mg/L for a maximum initial spike level (the spike level could be anywhere between 0.05 to 0.25 mg/L). Thus a 0.1 mg/L spike level was selected.

		# replicates	t-value
Rep. 1	0.104	7	3.143
Rep. 2	0.082	8	2.998
Rep. 3	0.096	9	2.896
Rep. 4	0.10	10	2.821
Rep. 5	0.087		
Rep. 6	0.114		
Rep. 7	0.108		

---

mean 0.098714
st dev. 0.011354
t-value 3.143 from table based on # replicates
LOD 0.035684 (t-value) × (std deviation)

The 5-point check

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1. Does the spike level exceed 10 times the LOD?
   No [(0.035 × 10) > 0.1] LOD is OK

2. Is the calculated LOD higher than the spike level?
   No [0.035 < 0.100] LOD is OK

3. Is the LOD < any relevant permit limit?
   N/A

4. Is the signal-to-noise ratio (S/N) between 2.5 and 10?
   Yes [0.098 ÷ 0.011 = 8.69] LOD is OK
   S/N = Mean ÷ std dev. S/N = 8.69

5. Is mean recovery within reasonably expected limits?
   Yes [> 98+% is excellent] LOD is OK
   Mean recovery = mean/spike level × 100 = 98.71%

6.3.3 Discuss selection of an appropriate spike concentration for determining the limit of detection (LOD).

The best spiking level to determine the LOD, as specified in the EPA procedure, is 1 to 5 times the estimated detection level. An estimated detection level can be obtained from the analytical method referenced or from direct analytical experience. Particularly for ammonia, due to slow response of very low concentration samples, a spike level below 0.2 mg/L is ill-advised.

- For ammonia (by the ISE method), a concentration between 0.2 and 0.5 mg/L is appropriate.
- For phosphorus, a spike concentration between 0.1 and 0.2 mg/L should result in a valid LOD.

6.3.4 Discuss the frequency at which LODs must be determined.

The limit of detection will change over time for a variety of reasons, and it is necessary to periodically update the calculated LOD value. Many analytical methods require that the LOD be determined prior to using a new analytical system, and some even require annual updates. The frequency of the determination specified in the analytical method should be followed. If the method does not specify a frequency, the Department recommends that LODs be recalculated whenever a new analyst begins generating data or the performance of the analytical system changes (in addition to the initial LOD). LODs must also be recalculated whenever the analytical procedure is modified (e.g. new analyst and equipment). Laboratories must give their LODs an "expiration date" of one year and check their LODs at least annually.

Section 6.4 - Corrective Action

6.4.1 Explain the concept of corrective action.

Corrective action means any measure taken to correct and prevent the recurrence of the causes of any exceedance of expected analytical operating conditions or quality control acceptance criteria. Corrective action must be documented.

Corrective action must be designed to identify the reason for the failure, and then correct it. There should also be a plan to quickly verify that the action taken has the desired effect.

6.4.2 Discuss the documentation required for corrective action.

- WHEN did you become aware of the problem?
- HOW did you become aware of the problem?
- WHO initiated corrective action to resolve the problem?
- WHAT action did you take to fix the problem?
- WHY do feel the action was appropriate (how do you know the problem has been resolved)?

Chapter 7 - Documentation and Traceability

Section 7.1 - Definitions

7.1.1 Define the following units commonly used in recording laboratory data:

A.mg/L
B.mg/kg
C.% solids

A. mg/L: The weight of a substance in milligrams contained in 1 liter of solution. For solutions in water it is the same as parts per million (ppm). Results for BOD, TSS, ammonia, and phosphorus in wastewater samples are reported in these units.

B. mg/kg: The weight of a substance in milligrams contained in 1 kg of dry solids. This is used for reporting sludge concentrations and is equal to parts per million (ppm).

C. % solids: Percent solids. This is usually used to describe sludge concentrations, and is based on a total solids test. 1% solids is equal to 10,000 mg/L.

7.1.2 Define SOP (Standard Operating Procedure).

Standard operating procedures (SOP) are used to describe a procedure or set of procedures to perform a given task, analysis, or in response to a specifiic event or situation. SOPs often offer guidance where official methods are lacking, or are extremely broad. SOPs are often used to provide practical detail to the sometimes generic language provided in methods or to identify specific situations where choices are available. Every good quality system is based on its standard operating procedures (SOPs).

SOPs may be documents written by laboratory personnel or may consist entirely of copies of published documents, manuals or procedures if the laboratory follows the chosen source exactly. SOPs must indicate their dates of issue or revision.

SOPs may partly consist of copies of published documents, manuals or procedures if:

- Modifications to the published source are described in writing in additional documents.

- Clarifications, changes or choices are completely described in additional documents, when published sources offer multiple options, ambiguous directives or insufficient detail to perform or reproduce an analysis.

Section 7.2 - Standard Operating Procedures (SOP)

7.2.1 Discuss the content of any standard operating procedure (SOP).

Each standard operating procedure for a specific test method must include, address or refer to, at a minimum, the following elements:

- Identification of the test method referenced.
- Method detection capability.
- Potential interferences.
- Equipment and analytical instruments.
- Consumable supplies, reagents and standards.
- Sample preservation, storage and hold time.
- Quality control samples and frequency of their analysis.
- Calibration and standardization.
- Procedure for analysis.
- Data assessment and acceptance criteria for quality control samples.
- Corrective actions and contingencies for handling out of control or unacceptable data.

Other things to consider for an analytical SOP include,

- Definitions (any terms used within the SOP)
- Health & Safety Warnings (are any reagents or equipment dangerous)
- Cautions (other concerns regarding the procedure)
- Personnel Qualifications (what experience or training is required to perform the test)
- Troubleshooting
- Calculations & Data Reduction
- Data and Records Management

Section 7.3 - QA Manual

7.3.1 Discuss the basic contents of a laboratory Quality Manual.

The quality manual should include, or make reference to, at least the following elements:

- Organization and management structure of the laboratory.
- Procedures for handling samples.
- Procedures for retention, control and maintenance of documents used in or associated with analyses.
- Procedures for achieving traceability of standards, reagents and reference materials.
- Lists of major analytical instruments and support equipment.
- Analytical Methods: Lists of all test methods used by the laboratory.
- Procedures for calibration, verification and maintenance of major analytical instruments and support equipment.
- Summary of the types and frequency of analysis of QC control samples for each test.
- Procedures for evaluating quality control samples.

- Procedures for initiating, following up on and documenting corrective action addressing quality assurance and quality control failures, discrepancies or nonconformance.
- Procedures for reviewing analytical data and reporting results.

7.3.2 Discuss the four critical aspects of QC sample analysis that should be considered in a QA Manual.

What is being reviewed? (PARAMETER)
How often does this parameter need to be checked? (FREQUENCY)
What is it being reviewed against (how is it evaluated)? (CRITERIA)
What if it doesn't meet specifications? (CORRECTIVE ACTION)

Examples:

PARAMETER	FREQUENCY	CRITERIA	CORRECTIVE ACTION
TSS oven	Daily	103-105 °C	Adjust temp. up or down
BOD incubator	Daily	19.0 to 21.0 °C	Adjust temp. up or down
Ammonia calibration slope	Daily	-54 to -60 mV	1) Enough time to stabilize? 2) Low standard too low? 3) Change membrane 4) Perform inner body check
Phosphorus known standard	Daily	90-110%	1) Replace standards/reagents 2) If high…contamination? 3) Re-do calibration 4) Reanalyze/ qualify samples

Section 7.4 - Traceability

7.4.1 Explain what data must be recorded when receiving standards and reagents at a laboratory.

Original containers of standards and reagents must be labeled with a receipt and an expiration date.

The laboratory shall document the lot number, manufacturer, date of receipt and the date of expiration of stock standards and reagents separately from their containers to ensure this information will be retained when the containers are discarded.

7.4.2 Explain what data must be recorded when prepairing intermediate solutions and standards.

The laboratory must maintain records that detail the preparation of intermediate and working standards and reagents. These records must link the intermediate and working standards and reagents to their respective originating stocks or neat compounds and must indicate their date of preparation, expiration and the identity of the preparer.

7.4.3 Discuss reasons why laboratory records, including benchsheets, must be generated using non-erasable ink.

Records must be produced in a manner that both ensures their permanence and ensures that they are un-alterable. Records generated in pencil or erasable ink can be easily altered. When original information is altered in this manner, reviewers such as a lab auditor or even the court could suspect that the alterations made represent a fraudulent practice. Consequently, even the use of correction fluid or tape in a laboratory is not recommended.

If you make an error while recording laboratory-related information, draw a single line through the incorrect information and write the correct information above or adjacent to the incorrect information. Always initial and date any such changes you make. It is also a good practice to make a notation regarding the reason the change was made. When data are corrected in this manner it is very clear what error was made, how it was corrected and who made the correction. This is very defensible and it will improve the credibility of the data.

7.4.4 Discuss mechanisms that can be used to ensure that "electronic" records are both permanent and unalterable.

Many labs are routinely using computers to create and store their data. Just as pencil can be erased, so can a value in a spreadsheet be easily deleted with a single keystroke. Security of electronic records begins with the use of password-based computer systems. Access to computer applications used to record and store laboratory records must be controlled through the use of unique user IDs and passwords. These are standard options for virtually all current operating systems. In addition, systems must be designed to automatically log a user off after a certain period of inactivity at the keyboard (or mouse).

All electronic data must be regularly backed up onto media which will survive record retention requirements. This may mean changing media used to back-up data as technology advances.

Whatever system you use, there must be built in assurances that data is free of alteration.

Section 7.5 - Records Retention

7.5.1 Discuss record storage and retention time.

Records such as bench sheets, sampler logs, and DMRs must be unalterable, readily retrievable and stored for a minimum of 3 years.

Chapter 8 - Appendix

Section 8.1 - Abbreviations

8.1.1 Abbreviations:

ACS – American Chemical Society
AR Grade – Analytical Reagent Grade
ASTM – American Society for Testing Materials
BOD – Biochemical Oxygen Demand

°C – Degrees Celsius (temperature)
cBOD – carbonaceous Biochemical Oxygen Demand
CCV – Continuing Calibration Verification
CFR – Code of Federal Regulations
COC – Chain-of-Custody
COD – Chemical Oxygen Demand
DMR – Discharge Monitoring Report
DNR – Department of Natural Resources
DO – Dissolved Oxygen
EP – Extraction Procedure
EPA – Environmental Protection Agency
GGA – Glucose, Glutamic Acid solution (for BOD)
HEM – Hexane Extractable Materials
ICV – Initial Calibration Verification
ID - Identification
IDC – Initial Demonstration of Capability
ISE – Ion Selective Electrode
LCS – Laboratory Control Sample
LOD – Limit of Detection
LOQ – Limit of Quantitation
MDL – Method Detection Limit
mL – milliliter
MLSS – Mixed Liquor Suspended Solids
MS – Matrix Spike
MSD – Matrix Spike Duplicate
MSDS – Material Safety Data Sheets
mV – millivolts
NFR – Non-Filterable Residue (See TSS)
NIST – National Institute of Standards and Technology
NPDES – National Pollutant Discharge Elimination System
NR 149 – Laboratory Certification and Registration Code; Wisconsin Adm. Code
ppb – parts per billion
ppm – parts per million
PT – Proficiency Testing sample (formally known as reference sample)
QA – Quality Assurance
QC – Quality Control
QCS – Reference standard obtained externally that comes with acceptance criteria, formerly known as “blind samples”.
R – correlation coefficient, seen as lower case, “r”
RPD – Relative Percent Difference
RSD – Relative Standard Deviation
SOP – Standard Operating Procedure
SRM – Standard Reference Material
SVI – Sludge Volume Index
TC – To Contain
TD – To Deliver
TDS – Total Dissolved Solids

TRC – Total Residual Chlorine
TSS – Total Suspended Solids
UV – Ultraviolet (a means of disinfection)
WPDES – Wisconsin Pollution Discharge Elimination System
WWTP – Wastewater Treatment Plant

References and Resources

1. **EXAMPLE SMALL WASTEWATER TREATMENT PLANT LABORATORY QUALITY MANUAL**

   2008. 4th Edition. Department of Natural Resources, PO Box 7921, Madison, WI 53707.

   http://www.dnr.state.wi.us/org/es/science/lc/OUTREACH/6QM/A_QualMan_rev0.pdf

2. **WI ADMINISTRATIVE CODE NR 149, LAB CERTIFICATION & REGISTRATION.**

   Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

   http://legis.wisconsin.gov/rsb/code.htm

3. **STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER.**

   1992, 1995 and 1998. 18th, 19th, and 20th editions, American Public Health Association, Washington DC.

   http://www.standardmethods.org/

4. **WI ADMINISTRATIVE CODE NR 219, ANALYTICAL TEST METHODS AND PROCEDURES.**

   Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

   http://legis.wisconsin.gov/rsb/code.htm

5. **Methods for Chemical Analysis of Water and Wastes.**

   1983. EPA-600/-4-79/020, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

6. **A User's Guide to Laboratory Services.**

   1989. Arneson, Ronald. Wisconsin Department of Natural Resources, Office of Technical Services, P.O. Box 7921, Madison , WI 53707.

   http://www.dnr.state.wi.us/org/es/science/lc/PW/Contract.htm#User

7. **Controlling Wastewater Treatment Processes.**

   1984. Cortinovis, Dan. Ridgeline Press, 1136 Orchard Road, Lafayette, CA 94549.

8. **Handbook for Sampling and Sample Preservation of Water and Wastewater.**

   1982. EPA-600/4-82-029. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

9. **Laboratory Safety Manual.**

   1969. Mallinckrodt Chemical Works, Science Products Division, St. Louis, MO 63160. Operation of Municipal Wastewater.

10. Operation of Municipal Wastewater Treatment Plants

Manual of Practice No.11 (MOP 11), 2nd Edition (1990), Volumes I, II, and III. Water Environment Federation (Old WPCF), 601 Wythe St, Alexandria, VA 22314-1994. Phone (800) 666-0206.

www.wef.org

11. Operation of Wastewater Treatment Plants

3rd Edition (1990), Volumes 1 and 2, Kenneth D. Kerri, California State University, 6000 J Street, Sacramento, CA 95819-6025. Phone (916) 278-6142.

http://www.owp.csus.edu/training/

12. Simplified Laboratory Procedures for Wastewater Examination

1985. Water Environment Federation (Old WPCF), 601 Wythe St, Alexandria, VA 22314-1994, Phone (800)666-0206.

www.wef.org

13. Wisconsin Administrative Code, NR 218, Method and Manner of Sampling

Wisconsin Legislative Reference Bureau, One E Main St, Suite 200, Madison, WI 53701-2037 Reference Desk: 608-266-0341

http://legis.wisconsin.gov/rsb/code.htm