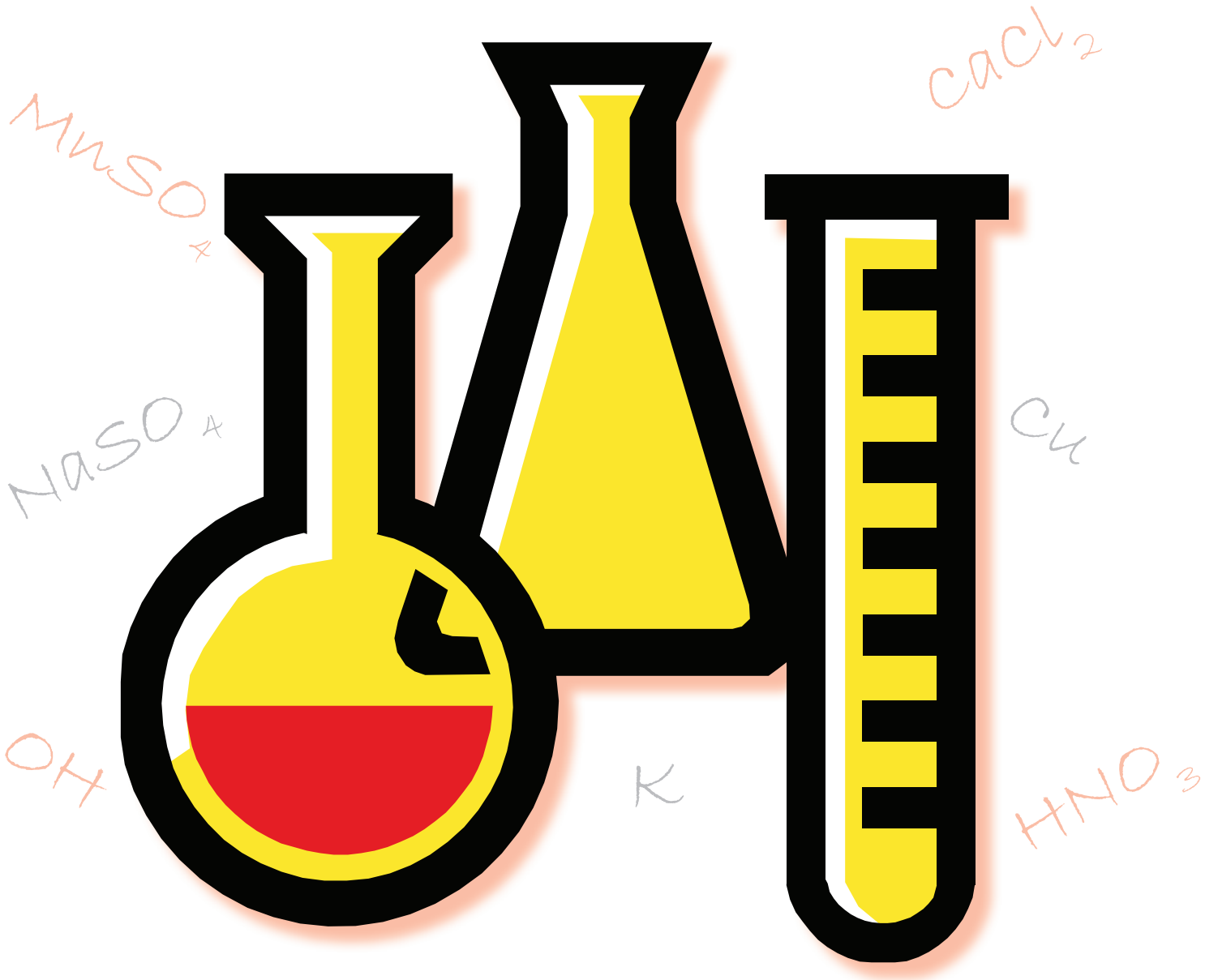
C Level
Wastewater Laboratory Manual

State of Oklahoma
Department of Environmental Quality
Water Quality Division
Operator Certification Section



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GENERAL INFORMATION

INTRODUCTION

This manual is not designed to replace the references "Standard Method for the Examination of Water and Wastewater", 20th Edition, 1998 and "Operation of Wastewater Treatment Plants," Third Edition, California State University, Sacramento, or any other comprehensive procedures manual. This is an introduction to common, inexpensive, and readily available testing materials and simple techniques. Commercially available test kits are emphasized because of their simple techniques, availability, and widespread use. Simplified and inexpensive instrumentation for basic spectroscopic examinations are included in addition to the required routine tests.

Laboratory control tests are the tools by which the efficiency of the processes at the wastewater treatment facility are monitored and the proper process control procedures are chosen. By using accurate laboratory data, the operator can select the most effective process control changes and identify potential problems before they become serious.

This manual is meant to be a study guide for the formal classroom courses required by the rules. The Oklahoma Administrative Code ("OAC") Title 252, Department of Environmental Quality ("DEQ"), Chapter 710, Waterworks and Wastewater Works Operator Certification requires thirty-two (32) hours of approved classroom training presented by various training organizations within the state. In addition to receiving a passing grade for the written examination, an equally important hands-on or practical examination must be taken during the class room instruction. Both, the written and practical examinations must be successfully passed with a grade of at least 70%. These examinations will not be averaged, both, must be passed.

This manual is designed for laboratory analysis for a Class "C" wastewater treatment facility as required by the OAC, Title 252, DEQ Chapter 606, Oklahoma Pollutant Discharge Elimination System ("OPDES") Standards.

The subjects of laboratory safety, basic chemistry, the metric system, glassware, and equipment will not be discussed in this manual. These are indeed important and necessary subjects to know but these particular subjects will be covered in the required thirty-two (32) hours of classroom instruction.

RULES

The OAC Rules 252:710-5-53(a) states that "All duties relating to the laboratory analysis of water quality samples from water treatment plants, groundwater systems, storage and distribution systems (including purchased-water systems) must be performed by or under the general supervision of a laboratory operator certified by the DEQ."

A person who is certified in laboratory operation may perform the laboratory analysis, but the person can not interpret the results of the laboratory data or give orders that will determine the method of working on the works or to change the quality of the wastewater directly or indirectly by order unless that individual is properly certified as a wastewater works operator.

If the wastewater works facility contracts all or a portion of the required laboratory analysis with the associated laboratory, the owner of that works shall notify the DEQ in writing within ten (10) days. The notification shall include:

1. Which of the required analysis will be performed by the associated laboratory;

2. Whether the associated laboratory is currently certified by the DEQ; and/or

3. Whether the laboratory operator has the responsibility or authority to determine the method of working on the works or to change the quality of the wastewater directly or by order.

Certified laboratory operators of the laboratories owned by or associated with wastewater works shall record, at the time of analysis, the results of all laboratory analysis in a bound volume. Each entry shall be dated and signed by the individual who performed the analysis. Each volume shall be kept on file at the laboratory for inspection and review for three (3) years.

LABORATORY PROCEDURES

SAMPLING AND PRESERVATION---------- types and techniques

QUALITY ASSUARANCE ----------quality control and quality assessment

STANDARD AND STOCK SOLUTIONS----------make-up

TEMPERATURE ----------Celsius and Fahrenheit

ALKALINITY ---------- total

CHLORINE RESIDUAL---------- total

PH ----------meter only

DISSOLVED OXYGEN ----------Winkler Azide Modification and Probe

BOD (5 DAY) ---------- unseeded and seeded

SETTLEABILITY ---------- Imhoff Cone and activated Sludge

SOLIDS---------- total, volatile and fixed

SAMPLING AND PRESERVATION

The most common remark a person hears about sampling is that the results of the analysis are no better than the sample. Not only is this statement true, but it is one of the most important aspects of laboratory procedure.

The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and handled in the laboratory, while still accurately representing the material being sampled. This assumes that the proportions or concentrations of the components being tested are the same in the sample as in the body of the material being sampled. It is also assumed that the handling of the material from the act of sampling, to the laboratory, and throughout the analysis has not resulted in any significant change in the composition or concentration of the components being tested.

In many cases, the sampling is to show the compliance with regulatory requirements. Due to the increasing importance of being able to verify the accuracy and representation of data, greater emphasis is placed on the proper sample collection and preservation.

Often in wastewater works, the laboratory determines the specific sampling program based upon the requirements and usage of the results by the wastewater works operator. Such determinations are important to insure that the selection of samples and the methods of analysis provide correct and accurate data necessary to answer the questions that resulted in the sampling being done.

PRECAUTIONS

Note: Check Table #1 for the proper container and method of preservation of samples.

TABLE #1
RECOMMENDATIONS FOR SAMPLING AND PRESERVATION

Measurement	Vol. Req. (mL)	Container	Preservative	Maximum Holding Time
PHYSICAL PROPERTIES				
Color	500	P.G	Cool, 4 C	48 hours
Conductance	500	P.G	Cool, 4 C	28 days
Hardness	100	P.G	Cool, 4 C HNO_3 to pH <2	6 months
Odor	200	G Only	Cool, 4 C	6 hours
pH	25	P.G	Det. on site	Immediately
Residue, Filterable	100	P.G	Cool, 4 C	7 days
Temperature	1000	P.G	Det. on site	Immediately
Turbidity	100	P.G	Immediately	Immediately
Total Solids, Total Volatile Solids	500	P.G	Cool, 4 C	7 days
Settleable solids	1000	P	Cool, 4 C	48 hours
METALS (Fe, Mn)				
Dissolved	200	P.G	Filter on Site HNO_3 to pH <2	6 months
Suspended	200		Filter on site	6 months
Total	100	P.G	HNO_3 to pH <2	6 months
INORGANICS, NON-METALLICS				
Acidity	100	P.G	Cool, 4 C	14 days
Alkalinity	200	P.G	Immediately/within a few hours	
Bromide	100	P.G	None req.	28 days
Chloride	50	P.G	None req.	28 days
Chlorine	500	P.G	Det. on site	Immediately
Cyanide	500	P.G	Cool, 4 C NaOH to pH >12	14 days
Fluoride	300	P	None req.	28 days
Iodide	100	P.G	Cool, 4 C	24 hours
Nitrogen				
Ammonia	500	P.G	Cool, 4 C H_2SO_4 to pH <2	28 days (dechlorinate)
Kjeldahl, Total	500	P.G	Cool, 4 C H_2SO_4 to pH <2	28 days (dechlorinate)
Nitrate plus Nitrite	200	P.G	Cool, 4 C H_2SO_4 to pH <2	28 days (dechlorinate)
Nitrate	100	P.G	Cool, 4 C	48 hours (dechlorinate)
Nitrite	100	P.G	Cool, 4 C	48 hours (dechlornate)
Dissolved Oxygen				
Probe	300	G Only	Det. on site	Immediately
Winkler	300	G Only	Fix on site	8 hours
Phosphorous				
Ortho-phosphate, Dissolved	50	P.G	Filter on site Cool, 4 C	48 hours
Total	50	P.G	Cool, 4 C H_2SO_4 to pH <2	28 days
Total, Dissolved	50	P.G	Filter on site Cool, 4 C H_2SO_4 to pH <2	24 hours
Silica	50	P Only	Cool, 4 C	28 days
Sulfate	100	P.G	Cool, 4 C	28 days
Sulfide	100	P.G	Cool, 4 C 2 mL zinc acetate & NaOH to pH < q	7 days
Sulfite	50	P.G	None req.	Immediately
BOD/CBOD	1000	P.G	Cool, 4 C	48 hours (dechlorinate)
COD	40	G	Cool, 4 C	28 days (dechlorinate)

TABLE #1
RECOMMENDATIONS FOR SAMPLING AND PRESERVATION

H_2SO_4 to pH <2

When no preservative or dechlorinating agent is present in the sample container, rinse the container two or three times with the water that is being sampled.

The choice of the sampling method must be defined in your sampling plan.

For metals, it is appropriate to collect filtered and unfiltered samples to differentiate between total and dissolved compounds.

Determine the acid requirements beforehand (if required) to bring the pH to <2 and add the same amount to all containers.

Filter the samples in the field or at the time of collection before adding the acid to lower the pH to <2.

Make a record of every sample collected and identify every bottle. (Date, time, location, test, sampler initials.)

When sampling a small stream for a grab or catch sample, take it in the middle of the stream at mid-depth. For a larger stream or river, more than one sample is usually required to obtain a representative sample.

When sampling lakes or impoundments: choose the location; depth; number; and frequency of samples depending on the local conditions. Usually several samples mixed together are required to obtain a representative sample because of poor mixing and stratification of the lake waters.

TYPES OF SAMPLES

Grab Sample	A single sample of water collected at a particular time and place, which represents the composition of the water only at that time and place.
Composite Sample	A collection of individual samples obtained at regular intervals, usually every one (1) to two (2) hours during a twenty-four (24) hour time span. Each individual sample is combined with the others in proportion to the rate of flow when the sample was collected. The resulting mixture (composite sample) forms a representative sample and is analyzed to determine the average conditions during the sampling period.

The type and frequency of sampling to satisfy the regulatory requirements are determined by that regulatory agency.

SAMPLING METHODS

Manual Sampling	Manual sampling involves minimal equipment but may be too costly and time-consuming for routine or large-scale sampling programs.
Automatic Sampling	Automatic samplers can eliminate human errors that are frequent in manual sampling, reduce labor costs, and provide a way for more frequent sampling.
Caution	Be sure that the automatic sampler does not contaminate the sample. Periodically, (daily or each use) clean the automatic sampler.

CHAIN-OF-CUSTODY PROCEDURES

It is very important to be sure of the sample integrity from the point of collection to the reporting of the laboratory data. This includes the ability to trace possession and handling of the sample through all the steps from collection to final disposal. This process is called chain-of-custody and is extremely important when legal litigation is involved.

A sample is considered to be under a person's custody if it is in the individual's physical possession, in the individual's sight, secured in a tamper-proof way by that individual, or secured in a restricted area. The following procedures summarize the major aspects of chain-of-custody.

1. Sample Labels – use labels to prevent sample misidentification.

2. Sample Seals – use seals to detect unauthorized tampering.

3. Field Log Book – record all information pertinent to a field survey or sampling in a bound log book.

4. Chain-of-Custody Record – fill out a chain-of-custody record to accompany each sample or group of samples.

5. Sample Analysis Request Sheet – the sample analysis request sheet accompanies the sample to the laboratory. The collector completes the field portion and the laboratory personnel completes the laboratory portion.

6. Sample Delivery to the Laboratory – deliver the sample to the laboratory as soon as practical after collection. If the sample is shipped by commercial carrier, be sure the number and the chain-of-custody record accompanies the sample.

7. Receipt and Logging of Sample – sample custodian inspects the sample, checks the label information and seal against chain-of custody records, assigns a

laboratory number, logs sample in laboratory log book and stores in a secure place.

8. Assignment for Analysis – the laboratory supervisor assigns the sample to an analyst who is responsible for its' care and custody.

QUALITY ASSURANCE

Quality Assurance ("QA") is defined as a set of principles and techniques that if followed and performed exactly according to the written guidelines for sample collection through the analysis will produce data of know and defensible quality. In other words, the accuracy of the results of the analysis can be stated with a high degree of confidence. This is of the utmost importance if the data is questioned by any regulatory authority or in a court of law.

In any laboratory and with any laboratory operator, the quality and accuracy of the data cannot be proven without a well prepared and judiciously carried out program of quality assurance. However, no matter how thorough the quality assurance program is developed and how carefully the program is implemented, the quality assurance program means nothing if accurate and complete records are not maintained. In any inquiry or in any court of law, your quality assurance program is useless without documentation of the proper control and application of all factors which affect the final results of the analysis.

Quality assurance in the laboratory may mean different things to many people, to some it is merely judged on such factors as:

1. Adequately trained personnel;
2. Good physical facilities and equipment;
3. Certified reagents and standards;
4. Frequent servicing and calibration of instruments;
5. A knowledgeable and understanding management;
6. The use of duplicates and known additions;
7. Documentation of lab results and quality control procedures;

All of these things are important, but none by themselves assures reliability of the laboratory data.

Any good quality assurance program must include:

1. Standard Methods – use only methods that have been accepted by Standard Methods or by the regulatory agency;
2. Internal quality control; and
3. External quality control (Quality Assessment).

Everyone realizes that it is a major effort in most small municipalities to retain an operator who is qualified to perform laboratory analysis and have some degree of

confidence in the results reported. Often it is a major accomplishment just to arrive at theses results so why burden your lab operator with another chore called "Quality Control"? This could be answered with the old familiar phrase "If something is worth doing at all, Its worth doing right"!

It is recognized that most lab operators practice quality control to some degree, (whether they know it or not), mostly depending upon the training, professional pride and awareness of the importance of the work they are doing. However, under the pressure of daily workload, many aspects of quality control may be easily neglected or ignored. Therefore, an established and documented routine quality control program applied to every analysis being performed is important to assure the reliability of the final data.

The laboratory operator must always be aware that the recorded laboratory results can be challenged at any time by anyone. This is the reason the operator must be sure that the recorded results are the actual and accurate data obtained. If the original data from the analysis is not recorded or is changed by someone, this is a serious matter indeed. This is the primary reason for the rule OAC 252:710-5-53(b) which states that "Operators of laboratories owned by or associated with waterworks/wastewater works shall record the results of all laboratory analyses in a bound volume for each facility and shall date and sign each entry. Records shall be kept on file at the laboratory for inspection and review for ten (10) years for waterworks and three (3) years for wastewater works." This rule gives protection for the laboratory operator if the data is later changed on the required reporting forms by someone else.

If the data is changed from the original results and then recorded in the bound volume, this gives no protection for the operator. This bound volume must be a volume that no pages can be removed or torn out of without being noticed.

Falsification of data is a very serious offense which can result in revocation of certification, a fine, and/or jail time.

QUALITY CONTROL

Quality control is internal and external control measures. For this discussion, external control will be termed as quality assessment.

The internal quality control methods consist of at least the following seven elements.

1. Certification of operator competency. Before an analyst is permitted to perform reportable work, competency in making the analysis is to be demonstrated.
2. Recovery of known additions. Use the recovery of known additions (Spikes) as a part of your analysis 10% of the time or one for every batch, whichever is greater to verify the absence of matrix effects.
3. Analysis of externally supplied samples. As a minimum, analyze externally supplied standards whenever the analysis of known additions fails to result in 5% acceptable recovery, or at least once a day, whichever is more frequent.

4. Analysis of reagent blanks. Analyze reagents blanks whenever new reagents are used or required by specific methods. Always analyze a minimum of 5% of the sample load as reagent blanks.

5. Calibration with standards. As a minimum, measure at least three different dilutions of the standard when an analysis is started. Afterwards, verify the standard curve daily by analyzing one or more standards within the linear range as specified in the individual method.

6. Analysis of duplicates. When measurable levels of the constituents being determined are present, the analysis of duplicates is valuable in determining precision. Analyze at least 10% of the samples in duplicate.

7. Control charts. Three types of control charts are commonly used in laboratories: a means chart for standards; a laboratory control standards chart; and a calibration check standards chart. For detailed discussion of these charts, consult Standard Methods.

Internal quality control of any laboratory analysis involves consideration and control of the many variables which affect the production of reliable data. The quality of the laboratory services available to the lab operator must be included among these variables:

1. The quality of distilled or de-ionized water;
2. Clean and dry compressed air;
3. Properly regulated electrical power;
4. Clean and proper glassware; and
5. Rotation of the chemical stock, mark date received and use old chemicals first.

Not all of these apply to all laboratories; however, every laboratory must address the importance of a good source of distilled water and clean glassware.

Standard Methods provides a good first step towards a good internal Quality Control program provided there are no "short cuts" employed. The next step is to rule out the introduction of outside interferences that can be controlled within the laboratory itself.

QUALITY ASSESSMENT

Quality assessment is the process of using external control measures to determine the quality and validity of the data produced by the laboratory. This includes the items listed below:

1. Performance evaluation samples. Use samples with known amounts of the constituent being measured supplied by an outside agency or blind additions prepared independently within the laboratory to determine recovery achieved by the analyst. The recovery must fall within the acceptable range of recovery previously established.

2. Performance audits. Make unscheduled performance audits using a check list to document how the sample is treated from receipt of the sample to the final

reporting of the result. The goal is to detect any deviation from the standard operating procedure so that corrective action may be taken if needed.

3. Laboratory intercomparison samples. Commercial and governmental programs supply samples containing different constituents in different matrices. A quality laboratory participates in a well planned inter comparison assessment program.

DATA QUALITY INDICATORS

The primary indicators of data quality are **bias** and **precision**. They are defined as the following:

Bias — Is the measure of systematic error. It has two components, one due to the method, which is measured by a laboratory inter-comparison study, which is the difference between the grand average and the true value.

The other is due to the laboratory's use of the method which is measured by the difference between the laboratory's average recovery and the true value. This bias is measured by recovery of known additions and/or duplicate samples.

Precision — Is a measure of the closeness of results in multiple duplicate samples, repeated analysis of a stable standard or repeated analysis of known additions to samples. The analysis of duplicate samples includes the random errors involved in sampling, as well as, sample preparation and analysis.

Precision is documented by Internal Quality Control

Accuracy — Is defined as the combination of bias and precision.

Accuracy is documented by External Quality Control

The relationship between precision and accuracy can be explained in this way. You are throwing darts at a target. You throw five darts at the bulls-eye and all the darts land very close together, but in a one inch circle in the lower left hand portion of the target. This is very inaccurate because you did not hit what you were aiming at and missed the bulls-eye by a mile.

However, you did throw the darts with precision, because all five darts are very close together. In other words, you repeated the placement of each dart very close to each other.

Then someone else throws the five darts at the bulls-eye and all five darts hit the bulls-eye. This accurate, because all five darts hit what they were thrown at. This is also called precision, because all five darts were very close together.

The external quality control (quality assessment) is a check on whether the laboratory has and practices an acceptable internal quality control program.

STANDARD AND STOCK SOLUTIONS

A standard solution is defined as a solution in which the exact concentration of a chemical or compound is known. A stock solution is defined as a strong solution of a chemical or compound of which the exact concentration is known and is used as a base for further dilutions.

The word "normal" abbreviated N in front of a reagent or compound indicates the strength or concentration. Normal is defined as a solution that contains one gram equivalent weight of a compound/liter of solution. The equivalent weight of an acid is that weight which contains one gram atom of ionizable hydrogen or its chemical equivalent.

Another way to indicate the concentrations of solutions is the a+b system. This means that "a" volumes of a concentrated reagent is diluted with "b" volumes of water. For example: 1+1HCl means 1 volume of hydrochloric acid is diluted with 1 volume of water. If you have a solution with a normality of 0.02 N, this can also be expresses as N/50 or 1/50 N. They all mean the same thing, but are expressed in a different way.

PROCEDURE

When measuring chemicals or compounds for standard solutions, **always** use volumetric pipettes and volumetric flasks to insure accurate measurements.

When preparing a standard solution from a dry chemical, always weigh on an analytical balance for extreme accuracy.

When adding a dry chemical or a liquid solution in preparing a standard solution, add the chemical or liquid to a small amount of water in the volumetric flask and then add water to the volume mark.

As a general rule, stronger solutions usually store for longer periods of time than weaker solutions.

Make up new standards periodically to check against old standards to determine if the old standard has lost strength.

If you have a lesser weight of a compound than it requires to make up 1 liter of solution, you can calculate how much water to add to make up the desired concentration. Use the following formula:

$$\text{Dilute to mL} = \frac{(\text{actual weight, g})(1000\text{ml})}{(\text{desired weight, g})}$$

TEMPERATURE

Temperature is one of the most frequent tests performed in the water laboratory. Temperature has a direct affect on most analysis you will perform in the laboratory or in the field.

The temperature of the water affects the rate of most chemical reactions. It affects pH, the amount of gasses dissolved in water, the amount of solids dissolved in water, biological growth rates, water stability, and many other aspects of water chemistry.

Temperature measurements are usually made with any good mercury filled thermometer. There are two different temperature scales. The one most people are familiar with is the Fahrenheit scale where the freezing point for water is 32° and the boiling point is 212°. The scale used in the laboratory is the Celsius scale where the freezing point for water is 0° and boiling point for water is 100°.

There are two types of glass thermometers; the total immersion type and the partial immersion type. The total immersion thermometer must be completely immersed in the water to obtain an accurate reading. The partial immersion thermometer should be immersed at lest to the solid line (water-level indicator) for an accurate reading.

For the proper quality control of temperature, you must calibrate your thermometers with a National Bureau of Standards and Technology (formerly American Bureau of Standards) thermometer at least once a year.

PROCEDURE

Immerse the thermometer to the proper depth.

Never let the thermometer touch the bottom or sides of the container because the container is usually influenced by the temperature of the surrounding air which will result in false readings.

If you are measuring the temperature of well water or water from a large container or body of water, let the water run into a cup (a polystyrene cup is ideal) and continuously overflow while the measurement is taken. Keep the thermometer in the water and wait for approximately one minute or until the reading stabilizes before recording the reading.

CALCULATIONS

The following calculations show the correct way to convert from one temperature scale to the other.

$$°C=\frac{(°F-32)}{1.8} \qquad °F=1.8(°C) + 32$$

TOTAL ALKALINITY

Alkalinity is a measure of the water's capacity to neutralize acids. The determination of alkalinity levels in wastewater at various points in the treatment process aids in the understanding and interpretation of the treatment processes and the management of digesters and sludge conditioning.

The alkalinity of a water is the capacity of that water to accept protons. Alkalinity is usually imparted by the bicarbonate, carbonate, and hydroxide components of a natural or treated water supply. It is determined by titration with a standard solution of a strong mineral acid to the successive bicarbonate and carbonic acid equivalence points indicated electromagnetically or by means of color. Phenolphthalein indicator enables the measurement of that alkalinity fraction contributed by the hydroxide and half of the carbonate. Indicators responding in the pH range 4-5 are used to measure the alkalinity contributed by hydroxide, carbonate, and bicarbonate. The phenolphthalein alkalinity and total-alkalinity titrations are useful for the calculation of chemical dosages required in the treatment of natural water supplies. The stoichiometric relationships between hydroxide, carbonate, and bicarbonate are valid only in the absence of significant concentrations of weak acid radicals other than hydroxyl, carbonate, or bicarbonate.

COMMERCIAL KITS

There are various commercial kits available for the analysis of total alkalinity and it is very important that the manufacturer's instructions are followed exactly.

Rinse the sample container with the water being tested.

Fill the sample container to the fill line or with the proper volume.

After adding the reagent, **mix well using a magnetic stirrer.**

Titrate using a buret to the end point**. Example of calculation on following page.**

Keep an accurate count of the drops of indicator added.

pH METER AND TITRATION METHOD

APPARATUS:

Buret, 25 ml with stand
Assorted beakers, graduated cylinders, volumetric flasks, and other glassware as needed
pH meter with electrodes
magnetic stirrer.

REAGENTS:

Sulfuric acid, 0.02 N

PROCEDURE

1. Add 100 ml of sample to a beaker (or a volume that will give you less than 50 ml of acid titrant).

2. Put the electrodes of the pH meter into the sample and slowly stir.

3. Titrate to pH 4.5 with 0.02 N sulfuric acid (H_2SO_4). Record the total amount of acid used.

4. Calculate the total alkalinity as shown by the example below.

CALCUALTIONS:

The results of titrations for alkalinity on a water sample were:

Sample size----------100 ml
Total titrant used to pH 4.5----------A = 6.8 ml
Sulfuric acid (H_2SO_4) normality------N = 0.02N

"**Total Alkalinity**" in mg/L as calcium carbonate ($CaCO_3$)

$$= \frac{A \times N \times 50{,}000}{\text{ml of sample}}$$

$$= \frac{(6.8\text{ ml}) \times (0.02) \times 50{,}000}{100\text{ ml}}$$

$$= 68 \text{ mg/l}$$

CHLORINE RESIDUAL

Chlorine residual is the amount of chlorine left in the water after the demand has been satisfied. There are three types of residuals to be measured.

Free residual chlorine refers to the chlorine that is contained in the compounds chlorine (Cl_2); hypochlorous acid (HOCl), and the hypochlorite ion (OCl-).

Combined residual chlorine refers to the chlorine in the compounds resulting from the reaction between ammonia and chlorine. The most important of these compounds are monochloramine (NH_2Cl), dichloramine ($NHC1_2$) and trichloramine ($NC1_3$).

Total chlorine residual is the sum of the free chlorine and the combined chlorine residuals.

In wastewater, the differentiation between free and combined chlorine is usually not made because wastewater chlorination is seldom carried far enough to produce free chlorine.

The three (3) most common methods of total chlorine analyses in wastewater are the amperometric titration method, the Iodometric method, and the DPD Titrimetric method. The Orthotolidine method is no longer accepted by the DEQ or by the EPA.

AMPEROMETRIC TITRATION METHOD

This method is the most accurate and the least subject to interferences. However, it requires more operator skill than the other methods and it requires relatively expensive equipment. This method will not be outlined in this manual but the method is described in Standard Methods.

COMMERCIAL KITS

This method is quick, simple, inexpensive, and suitable for field use, as well as, laboratory use. There are various manufacturers of DPD kits that measure both the free chlorine and the total chlorine levels. There are slight variations in the instructions and usage of the kits available.

PRECAUTIONS

Only certain commercial kits are accepted by the Oklahoma DEQ and by EPA to measure total chlorine in wastewater.

Make certain that if you use a commercial kit for chlorine analysis, choose a kit that is accepted by the regulatory agencies.

Analysis must be made immediately after sampling. (within 15 minutes)

Rinse the sample bottle with the water being tested.

Add the proper chemical as instructed, **mix well.**

Fill one viewing container with the treated sample, stopper and be sure there are no air bubbles present then do the same with the untreated sample.

When measuring the residual, always try to use the same light source and intensity when you make your reading. Do not use direct sunlight, but try to use the same source of artificial light.

You should always strive to perform the test at the same temperature each time (preferably at room temperature).

DPD TITRAMETRIC METHOD

This method is usually more accurate than the color comparison method and is easier to perform than the amperometric method.

APPARATUS:

graduated cylinder (100 ml)
pipettes (1.0 & 10 ml)
Erlenmeyer flask (250 ml)
buret (10 ml)
magnetic stirrer and stirring bar
analytical balance

REAGENTS:

H_2SO_4 solution add 10 ml of concentrated sulfuric acid to 30 ml distilled water then cool.

Standard ferrous ammonium sulfate solution (FAS titrant), 0.00282 N:dissolve 1.106 g of $Fe(NH_4)_2(SO_4)_2*6\ H_2O$ in distilled water containing 1.0 ml of the acid solution described above and dilute to 1.0 liter using freshly boiled and cooled distilled water.

Phosphate buffer solution pH 4.

DPD indicator solution.

Potassium iodide (KI) crystals.

PROCEDURE

TOTAL RESIDUAL

1. Place 5 ml of buffer solution and 5 ml of DPD indicatior solution in a 250 ml Erlenmeyer flask and mix.

2. Add 1g KI Crystals.

3. Add 100 ml of sample and mix.

4. Let stand for 2 minutes and titrate with FAS titrant to clear color (red disappears).

CALCUALTIONS:

For a 100 L sample, 1.0 ml of standard FAS titrant = 1.0 mg/L of total chlorine residual

IODOMETRIC METHOD

APPARATUS:

Graduated cylinder, 250 ml
Pipets, 5 and 10 ml
Erlenmeyer flask, 250 ml
Buret, 5 ml
Magnetic stirrer & stirring bar

REAGENTS:

Standard Phenylarsine Oxide (PAO) solution 0.00564 N.

Potassium Iodide crystals (KI).

Acetate Buffer solution.

Standard Iodine titrant 0.0282 N.

Starch Indicator.

For reagent makeup, refer to Standard Methods.

PROCEDURE:

1. Pipette 5.00 ml of 0.00564 N PAO solution in an Erlenmeyer flask.

2. Add excess KI (about 1 gm).

3. Add 4.0 ml of acetate buffer solution (or enough to lower the pH within range of 3.5 to 4.2).

4. Add 200 ml of sample and mix well.

5. Add 1.0 ml of starch solution and titrate at once with 0.0282 N iodine to a blue color.

CALCULATIONS:

$$\text{Total Residual C1} = \frac{(A - 5B) \times 200}{C}$$

When A = ml of 0.00564 N PAO
B = ml of 0.0282 N Iodine solution
C = ml of sample used

PRECAUTIONS:

pH control is essential. The pH of the sample, buffer and the DPD indicator must be between 6.2 and 6.5.

If oxidized manganese is present in the sample, add 5 ml of acetate buffer solution and 0.5 ml of sodium arsenite to the titration flask.

Higher temperatures lead to false positive results and increase color fading.

Copper (Cu) interference up to about 10 mg/L is overcome by the addition of EDTA.

Chromate above 2 mg/L will interfere with the end point determination - add barium chloride to mask this interference.

The analysis of chlorine residual must be made as soon as possible after sampling. You **cannot store** chlorine samples to be analyzed at a later time, because the chlorine will dissipate from the sample.

pH—METER METHOD

The measurement of pH is another important and misunderstood measurement in water chemistry. Almost every phase of water treatment chemistry from prechlorination through coagulation to final treatment is pH dependent. pH is used in many measurements, such as alkalinity, carbon dioxide, dissolved oxygen, stability, and many more measurements of acid-base equilibrium. The hydrogen ion activity or pH is the intensity of the acid-base relationship at any given temperature. pH is defined as the logarithm (base 10) of the reciprocal of the hydrogen ion activity or $- \log (H^+)$. Because the pH is based on the logarithm expression, pH cannot be averaged.

The pH scale is from 0 to 14. The neutral point is pH 7, above pH 7 is basic and below pH 7 is acidic.

As the pH decreases from neutral (pH 7) toward a pH of 0, the H^+ activity increases at a logarithmic rate and the hydroxyl (OH^-) activity decreases as the solution becomes more acidic. When the pH increases from pH 7 towards pH 14, the effect is opposite from that above and the solution becomes more basic.

It cannot be stressed enough how important pH measurement is in controlling chemical reactions, the rates of chemical processes, and the accuracy of laboratory analysis.

APPARATUS:

pH meter and probe
small beaker

REAGENTS:

pH standards of pH 4, 7, and 10.

PROCEDURE:

Plug in the meter and let it warm up for five to ten minutes. Standardize the meter using the pH 7.0 and either the pH 4.0 or the pH 10.0 standards. The actual sample pH should fall between pH 7.0 and the other standard used. Usually with ground water the two standards will be pH 7.0 and pH 10.0. The standards must be purchased from a supply house instead of making up the standards in the lab. In either case, pour enough of each standard into a small beaker to immerse about the lower one inch of the pH probe. Using each standard, follow the manufacturer's instructions for standardizing the meter.

Place the sample in a beaker or flask and swirl to mix, or use a magnetic stirrer.

The pH is read directly from the meter, no conversions are necessary.

After shutdown, follow the manufacturer's instructions for the proper care of the probe.

PRECAUTIONS:

Even though the meter will automatically compensate for the temperature changes, temperature differences will cause a change in the pH. Therefore, always bring the standards and the sample to the same temperature before measurement (preferably room temperature).

Rinse the probe with distilled water after each and every reading.

At the end of the day or when you are finished using the meter, do not pour the used standards back into the bottle. **THROW AWAY THE USED STANDARD.** This prevents you from contaminating the unused portion of the standard in the bottle.

Never use indicators such as methyl orange or other organic indicators to calibrate the pH meter. The end points are not accurate enough because of the individual interpretation of the end point and the many interferences that may change the end point.

DISSOLVED OXYGEN (DO)

Dissolved oxygen (DO) levels in natural waters and wastewaters depends on the physical, chemical, and biological activities in the body of water. This test procedure determines the amount of oxygen dissolved in the sample of wastewater. This is one of the most important tests in determining the water pollution control activities and waste treatment process control. The amount of oxygen dissolved in any water is dependent upon the temperature, the number of oxygen consuming organisms in the sample, and the previous treatment or source of the sample.

The methods described here are the Sodium Azide Modification of the Winkler Method and the Membrane Electrode Method.

Under normal conditions, the following ranges of DO levels, in mg/L, from different sources can be expected.

Plant Influent	normally 0, >1 very good
Primary Clarifier Effluent	normally 0, re-circulated from filters >2 good
Secondary Clarifier Effluent	50% to 95% saturation, or 3 to 8
Oxidation Ponds	>2 to supersaturation
Activated Sludge Mixed Liquor	>2

APPARATUS:

Buret, graduated to 0.1 ml with stand
BOD bottles, 300 ml glass stoppered
Erlenmeyer flask, 500 ml or greater
Pipette, 10 ml measuring

REAGENTS:

Manganous Sulfate Solution	Dissolve 480 g manganous sulfate crystals ($MnSO_4 \cdot 4H_2O$) in 400 to 600 ml of distilled water. Filter through filter paper, add distilled water to the filtered liquid to make 1 liter.
Alkaline Iodide Solution	Dissolve 500 g of sodium hydroxide (NaOH) in 500 to 600 ml of distilled water; dissolve 150 g of potassium iodide (KI) in 200 to 300 ml of distilled water in a separate container.
Sodium Azide Solution	Dissolve 10 g of Sodium Azide (NaN_2) in 40 ml of distilled water.

Caution: Sodium Azide Solution is poisonous, do not breathe the fumes.

Note: Fresh sodium azide will destroy nitrate that interferes with this test. The sodium azide solution will decompose and is no good after three months. It is preferred that the solution be made fresh for each day of analysis.

Alkali-Iodide-Azide Solution — Add the sodium azide solution to the cooled alkaline iodide solution very slowly, and then add distilled water to make a 1 liter volume. Both solutions should cool to room temperature before mixing.

Caution: Mix these chemicals in pyrex glass bottles using a magnetic stirrer. Add the chemicals to the distilled water slowly and with care. Avoid breathing the fumes or making body contact with the chemicals. When you add these chemicals to the water heat is produced and the chemicals are **very caustic and will cause sever burns**.

Keep a water faucet running when working with these chemicals in order to wash hands frequently to avoid burns.

Sulfuric Acid — Use concentrated reagent grade sulfuric acid (H_2SO_4).

Phenylarsine Oxide Solution (PAO) 0.025 N

This solution is available standardized from commercial sources.

Sodium Thiosulfate Solution 0.025 N

Dissolve 6.206 g of sodium thiosulfate crystals ($Na_2S_2O_3 \cdot 5H_2O$) in freshly boiled and cooled distilled water and dilute to 1 liter. For preservation, add one pellet of sodium hydroxide (NaOH).

Note: You may use either the phenylarsine or the sodium thiosulfate solutions as the titrant. The sodium thiosulfate solution is only good for two weeks. Preferably, make fresh solution for each analysis.

Starch Indicator Solution — Dissolve 2 g of laboratory grade soluble starch and 0.2 g of salicylic acid (as a preservative) in 100 ml of hot distilled water.

ANY OR ALL OF THESE REAGENTS ARE AVAILABLE FROM COMMERCIAL LABORATORY SUPPLY COMPANIES.

PROCEDURE:

1. Place the sample in a 300 ml BOD bottle being very careful to avoid any aeration of the sample or any trapped air bubbles. Fill the bottle to overflowing and add the stopper.

2. Remove the stopper. Using a pipette with the tip below the liquid surface, add 1.0 ml of manganous sulfate solution.

3. Add 1.0 ml of alkaline-iodide-sodium azide solution below the surface of the liquid as described above.

4. Replace the stopper, and avoid trapping any air bubbles. Mix well by inverting the bottle several times. Let the floc settle about half way. Repeat the mixing and let the floc settle about half way a second time.

5. Add 1.0 ml of concentrated sulfuric acid by pipette and let the acid run down the inside neck of the bottle above the surface of the liquid.

6. Replace the stopper and mix well until the precipitant is dissolved by the acid. The sample is now ready to titrate.

7. Place 201 ml of the sample in a 250 ml Erlenmeyer flask and titrate with 0.025 N PAO or with 0.025 N sodium thiosulfate to a pale yellow color.

8. If the sample has no brown color or a very light straw color, add a few drops of the starch indicator. If no blue color develops the DO is 0 mg/L. If a blue color develops, proceed with step #9.

9. Titrate again with POA or sodium thiosulfate until the blue color disappears. Record the ml used in titration.

If blue color returns, this indicates the presence of nitrates. If this occurs check your sodium azide solution to see if it has gone bad.

CALCULATIONS:

Calculate the amount of DO in the sample.

With a 201 ml sample and 0.025 N titrant:

1.0 mg/L DO = 1.0 ml of PAO or sodium thiosulfate

Using the temperature/oxygen saturation table below you can calculate the % saturation.

$$\text{DO Saturation \%} = \frac{\text{DO in sample, mg/L} \times 100}{\text{DO mg/L at 100\% saturation}}$$ (from Table #2)

PRECAUTIONS:

1. **These reagents must be added in the exact quantities, in the exact order, and by the exact procedure as outlined above.**

2. Before taking DO measurements, be sure all reagents and the sample are at room temperature.

3. Be sure no air bubbles are trapped in the BOD bottle.

TABLE #2
EFFECT OF TEMPERATURE ON OXYGEN SATURATION

°C	°F	mg/L DO at SATURATION
0	32.0	14.6
1	33.8	14.2
2	35.6	13.8
3	37.4	13.5
4	39.2	13.1
5	41.0	12.8
6	43.8	12.5
7	44.6	12.2
8	46.4	11.9
9	48.2	11.6
10	50.0	11.3
11	51.8	11.1
12	53.6	10.8
13	55.4	10.6
14	57.2	10.4
15	60.0	10.2
16	61.8	10.0
17	63.6	9.7
18	65.4	9.5
19	67.2	9.4
20	**68.0**	**9.2**
21	69.8	9.0
22	71.6	8.8
23	73.4	8.7
24	75.2	8.5
25	77.0	8.4

The table above should impress upon the laboratory operator the importance of maintaining the same temperature throughout the analysis.

MEMBRANE ELECTRODE METHOD:

In many circumstances, measurements of the dissolved oxygen (DO) concentration with a probe and an electronic readout meter is a satisfactory substitute for Sodium Azide Modification of the Winkler Method. Membrane covered electrode systems have a sensing element (probe) protected by an oxygen permeable membrane (polyethylene or fluorocarbon material) that serves as a diffusion barrier against impurities.

Various types of membrane electrode instruments are commercially available. In all of these instruments the diffusion current is linearly proportional to the molecular oxygen concentration in the test sample. This method for DO determination is recommended for those samples containing materials that interfere with the Azide Modification Method, such as sulfite, thiosulfite, free chlorine, color, turbidity, and biological flocs.

APPARATUS:

Oxygen- sensitive membrane electrode with appropriate meter.

PROCEDURE:

Follow the manufacturer's instruction exactly.

Calibration: Take a sample that does not contain any interfering substances for the probe method or the Modified Winkler method. Split the sample. Measure the DO in one portion of the sample using the Modified Winkler method and compare the results with the reading of the probe method for the other portion of the sample. Adjust the probe reading to agree with the results of the Modified Winkler method.

If you are calibrating the probe in an aeration tank of the activated sludge process, do not try to measure the DO in the aerator and then adjust the probe. The biological floc will interfere with Modified Winkler method. Take an aerator effluent sample and measure the DO by the probe method and the Modified Winkler method. Then, adjust the probe to match the Modified Winkler results.

PRECAUTIONS:

1. Periodically check the calibration of the probe.

2. Prevent the membrane from drying out.

3. High concentrations of dissolved inorganic salts can influence the readings of the probe.

4. Reactive compounds like reactive gases can interfere with the probe's results.

5. Make sure you do not take readings directly over a diffuser. This will give false high readings.

6. Follow all precautions recommended by the manufacturer.

7. Take extreme care when changing the membrane to avoid contamination of the sensing element and be sure you do not trap minute air bubbles under the membrane.

8. Check frequently to verify if temperature compensation is correct.

9. If the probe is used to measure the initial and final DO for the BOD test, be certain that after each reading you thoroughly rinse the probe with water.

BIOCHEMICAL OXYGEN DEMAND (BOD_5)

GENERAL

The method consists of filling an air tight bottle of a specified size to overflowing with the sample and incubating it at the specified temperature for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between the initial and final DO.

SAMPLE HANDLING

Collected samples for BOD analysis may degrade significantly between the time of sampling and analysis. It is of utmost importance that the proper sampling technique is used to insure that the sample is indeed a true representation of the wastewater being analyzed. Equally important is the proper storage of the sample if analysis is not started immediately. The greatest errors produced in the BOD test are usually caused by improper sampling, poor preservation, or the lack of adequate mixing during compositing and testing.

GRAB SAMPLES

If analysis is to begin within two (2) hours of collection, cold storage is not necessary. If analysis is not begun within two (2) hours, keep the sample at or below 4°C from the time of collection. Always begin analysis within forty-eight (48) hours of collection.

COMPOSITE SAMPLES

Keep samples at or below 4°C during the compositing time frame. Limit the compositing time frame to a maximum of twenty-four (24) hours. Use the same criteria for storage as for the grab samples and start the measurement of holding time from the end of the compositing period.

The amount of sample required at each sampling time is calculated by dividing the total volume of sample needed to run all the tests by the number of sampling times. This gives you the multiplication factor.

EXAMPLE: 2,000 ml needed with 6 sampling times.

$$\frac{2{,}000}{6} = 333.33 \text{ or the factor of } 333$$

Sample Freq.	MGD Flow	Factor	Portion Size (ml)
10 AM	0.5	333	166.5
11 AM	0.7	333	233.1
12 NOON	0.9	333	299.7
1 PM	1.1	333	366.3
2 PM	1.5	333	499.5
3 PM	1.3	333	432.9

PRECAUTIONS

Check samples for chlorine, dechlorinate with sodium thiosulfite if necessary.

Dechlorinated samples should be aerated if sample volume for any dilution is over 90 ml.

Check and record sample pH (after composite is complete). The sample pH should be between 6.5 and 7.5. If necessary, adjust pH with sodium hydroxide (NaOH) or sulfuric acid (H_2SO_4) and record as adjusted pH.

Check and record sample temperature. Temperature should be as close as possible to 20°C.

CLEANING OF THE DILUTION WATER JUG

The dilution water bottle must be thoroughly cleaned every time it is refilled. Clean the dilution water jug using at least 1 gallon of 10% aqueous sulfuric acid. The acid bath must be in contact with all of the inside of the surface of the jug for at least ten (10) minutes. This also includes draining the acid back into the jug through the drain tube mechanism. Rinse the jug about ¼ full with the drain tube inside of the jug with deionized water. Shake the jug and empty, fill the jug with deionized water and empty. Repeat this rinsing process four (4) or five (5) times. Take the air hose out of the jug and rinse thoroughly.

Use only a good grade deionized water – do not use tap water. Do not use soap unless you think soap is needed. If you use soap, rinse the jug at least six (6) times with hot water before the acid wash.

CLEANING BOD BOTTLES

Note: BOD bottles are of 300 ml capacity with ground glass stoppers.

Usually, the bottles are washed after each use with soap and water. After running them through the dishwasher, rinse at least three (3) times with DI water. After washing and

rinsing, set them upside down to air dry. The ground glass stoppers and plastic water seals must be washed, rinsed, and dried the same way.

ACID WASHING BOD BOTTLES

At least every three (3) months the BOD bottles should be acid washed to maintain quality control. Fill one bottle with 10% sulfuric acid. Pour the acid from one bottle to the next while swirling and rotating each bottle. Continue this until all the bottles have been acid washed and tap water rinsed. Put the ground glass stoppers in several beakers and acid wash and rinse them the same way. Then put the bottles and stoppers in the washer. Run a full cycle with soap and a full cycle without soap. Finally, rinse with the DI water and air dry as described above for storage.

REAGENTS:

Phosphate Buffer Solution

In about 500 ml of DI water dissolve the following:

8.50 g KH_2PO_4 (Potassium Phosphate Monobase)
21.75 g K_2HPO_4 (Potassium Phosphate Dibasic)
33.40 g $Na_2HPO_4 \cdot 7H_2O$ (Sodium Phosphate Dibasic Heptahydrate)
1.70 g NH_4CL (Ammonium Chloride)

Dilute to 1 liter – pH should be 7.2 without adjustment, if not adjust.

Magnesium Sulfate Solution

Dissolve in DI water and dilute to 1 liter:

22.5 g $MgSO_4 \cdot 7H_2O$ (Magnesium Sulfate Heptahydrate)

Calcium Chloride Solution

Dissolve in DI water and dilute to 1 liter:

27.5 g $CaCl_2$ (Calcium Chloride Anhydrous)

Ferric Chloride Solution

Dissolve in DI water and dilute to 1 liter:

0.25 g $FeCl_3$ (Ferric Chloride Hexahydrate)

Preparation of Glucose-Glutamic Acid Solution

Dry reagent grade glucose and reagent grade glutamic acid for one (1) hour at 104°C. Dissolve in DI water and dilute to 1 liter.

150 mg glucose
150 mg glutamic acid

Note: This reagent must be prepared daily before using.

Preparation of Dilution Water

Fill the clean dilution water jug to the desired volume with deionized water.

For every liter of water add 1.0 ml each of:

Phosphate buffer solution
Magnesium sulfate solution
Calcium Chloride solution
Ferric chloride solution

You may obtain commercially prepared nutrients. This dilution water should be made up at least one (1) day before being used.

Sample Dilutions/Volumes

A number of different dilutions must be set up to obtain and average BOD. At least two (2) dilutions must be set up to obtain an average BOD. The minimum number of dilutions suggested is three (3). The number of dilutions is usually determined by the experience of the operator with that particular sample.

QUALITY CONTROL

Dilution Water Blanks

As a test of the quality of the dilution water, **blanks** are to be set up with every set of BOD determinations. Prepare three (3) bottles with dilution water only and label these bottles. One of the bottles is to check initial DO using the Winkler Azide Method and the two remaining bottles are to be incubated for five (5) days using the Winkler Azide Method. The DO depletion should not be over 0.2 mg/L and preferably not over 0.1 mg/L after five (5) days of incubation. If the depletion is over 0.2 mg/L, the results of the BOD test using that dilution water **must be rejected. This rejection could be due to bad dilution water, dirty BOD bottles or contamination of the dilution water during test set up.** It is to the operators advantage to check these possibilities and correct the problem before setting up the next test.

Glucose-Glutamic Acid Check

There is no measurement for establishing bias for the BOD procedure because bias cannot be determined for a biological procedure using live organisms. The glucose-glutamic acid check is intended to be a reference point to check technique, seed quality, and dilution water. A glucose-glutamic acid check should be run once a week or at least on 10% of the samples run.

Determine the BOD of a 2% dilution (6 ml in a 300 ml bottle). Using the same techniques and depletion criteria as for any BOD. Simply use the 6 ml of glucose-glutamic acid as if it were the sample. Handle the standard like any other sample. The checks should have a BOD of 198 mg/L + or – 30.5 mg/L. If the control limits are outside of this acceptable range re-evaluate the control limits and investigate the source of the problem. Label these bottles.

If the BOD for the glucose-glutamic acid check is outside the control limit range, **reject the tests made with that glucose-glutamic acid and dilution water.**

APPARATUS:

300 ml BOD bottles with ground glass stoppers
Incubator, 20° C =/- 1° C
Pipets, 10 ml with large opening
Buret and stand
Erylenmeyer flask, 500 ml

DO probe (if used instead of the modified Winkler method for DO)

PROCEDURE: (Use your own labeling so you can differentiate)

1. Fill two BOD bottles to overflowing with dilution water only (blanks). Stopper and be sure there are no air bubbles trapped inside of the bottles. Label these bottels. Set up dilutions of the sample using 2 bottles/dilution (preferably three different dilutions) to cover the estimated range of BOD concentrations. Fill the 300 ml BOD bottles to overflowing and stopper tightly. Be sure there is no air bubbles trapped inside of the bottles. For example, the dilutions are 3 ml, 6 ml and 9 ml of sample in 300 ml bottles. This gives you dilutions of 1/100, 2/100 and 3/100.
2. Label the 3 ml dilution bottles, the 6 ml dilution bottles and the 9ml dilution bottles and the glucose-glutamic acid check (if you run them) and bottle.
3. On one bottle for each dilution, the dilution water blank, and the glucose-glutamic acid check, immediately determine the initial DO.
4. Incubate the other bottles in the dark at 20° C for FIVE (5) days.
5. At the end of five days (+/- 3 hours) determine the residual DO in the dilution water blanks, glucose-glutamic acid check, and each dilution bottle using the azide modification of the Winkler method or the DO probe method.

PRECAUTIONS:

Be sure that you bring the samples, dilution water, and blanks to room temperature (20°C) before the DO measurements.

Be sure that the water used for all solutions and preparation of the samples is of the highest quality. Distilled water bought in grocery stores is seldom good enough. The distilled or deionized water must contain less than 0.1 mg/L of copper and be free of chloramines, caustics, acids, or organic material.

Be sure there are no air bubbles left in the stoppered bottles.

To be reliable and valid, the DO depletion must be at least 2 mg/L and the residual DO must be at least 1 mg/L. If these criteria are not met, that test cannot be used to calculate the BOD.

Make enough different dilutions so you will have at least two (2) valid tests to average for the BOD value.

If the DO depletion for the dilution water blank is over 0.2 mg/L after five (5) days of incubation, you cannot use any of the tests using that dilution water.

BOD CALCULATIONS:

Example:	BOD bottle volume	= 300 ml
	Sample volume	= 9 ml
	Initial DO of diluted sample	= 8 mg/L
	Residual DO after incubation	= 4 mg/L

BOD mg/L =

$$(\text{Initial DO of sample} - \text{DO of incubated sample}) \times \frac{\text{BOD btl vol mg/L}}{\text{sample vol. mg/L}}$$

$$= (8 \text{ mg/L} - 4 \text{ mg/L}) \times \frac{300 \text{ ml}}{9 \text{ ml}}$$

$$= 4 \times 33.3$$

$$= 133 \text{ mg/L}$$

CHLORINATED (SEEDED) SAMPLES

All chlorinated samples must be dechlorinated and then seeded because by chlorination kills most of the microorganisms in the sample.

By dechlorinating you have removed the disinfectant.

By adding seed you are reintroducing the microorganisms to the sample.

A settled raw sample usually makes an adequate seed. Allow the raw sample to settle at room temperature for at least one (1) hour but no longer than thirty-six (36) hours. Commercially made seeds are available.

Whatever seed you use, you must run BOD's on the seed to determine the seed correction to be used. This is called the Seed Control. Try to make dilutions of the seed so that the largest quantity of seed used results in at least a 50% DO depletion. The seed control must meet the same depletion criteria of the BOD test; a depletion of 2.0 mg/L and at least 1.0 mg/L of DO residual.

From the value of the seed control, you then determine the seed DO uptake of the seeded samples. The DO uptake (seed correction) should be between 0.65 and 1.0 mg/L. This is calculated based on the ml of seed in the sample divided by the ml of the seed in the seed control. This is called the seed factor.

REAGENTS:

Dechlorinating Solution

Dissolve in DI water and dilute to 1 liter

1.575 g Na_2SO_3 (Sodium Sulfide)

Note: This solution is unstable and must be prepared daily.

The other reagents used are the same reagents as stated for the unchlorinated samples.

PROCEDURE:

1. Set up two (2) BOD bottles for each different dilution of the seed material being used. In this example, let's use 12 ml of sample, 15 ml, and 18 ml.
2. Determine the initial DO of one bottle of each seed dilution.
3. Place the remaining bottles in the incubator at 20° C +/- 1° for five (5) days.
4. After five (5) days of incubation, determine the residual DO for each sample.
5. Calculate the seed correction as explained below:

In this example, 4 ml of seed in each sample bottle was chosen. This choice must be made by the operator through experience or by guess. This must be done in order to calculate the F factor.

TABLE #3
EXAMPLE OF CALCULATION FOR SEED CORRECTION

SEED CONTROL				**Depletion x Factor = Seed Correction**			
Bottle #	**ml of seed in control**	**(B1) Int. D.O.**	**(B2) Final D.O.**	**Depletion mg/L**	**F Factor**	**Seed Single**	**Correction average**
#1	12	7.3	4.9	2.4	0.33	0.79	
#2	15	7.2	4.1	3.1	0.27	0.84	0.84
#3	18	7.2	3.2	4.0	0.22	0.88	

F = $\frac{\text{ml seed in sample (see \#5 procedure above)}}{\text{ml of seed in control (Table \#3)}}$

$\frac{\text{4 ml seed in sample}}{\text{12 ml seed in control}} = 0.\underline{33}$ this is the Factor

The 12 ml dilution in the seed control has a depletion of 2.4 mg/L

Seed Control depl. (2.4 mg/L x Factor (0.33) = 0.79 mg/L (seed corr.).

Do the same calculation for the 15 ml dilution and the 18 ml dilution.

Average the three seed corrections as in the above example to obtain the average correction factor to be used on all the seeded sample determinations. The average of 0.84 is called the seed correction.

You now have determined the seed correction and are ready to use this in the actual BOD calculations. The first decision you must make is what range of sample dilutions will result in the DO depletions that will satisfy the required criteria. These requirements are a minimum DO depletion of 2.0 mg/L with a minimum residual DO level of 1.0 mg/L.

If you do not know the average level of the BOD in the sample then you must estimate what the level might be for your particular sample. For domestic wastewater the estimated average BOD levels are:

Influent	150 to 400 mg/L
Primary Effluent	60 to 160 mg/L
Secondary Effluent	5 to 30 mg/L
Digester Supernatant	1,000 to 4,000+ mg/L

It is strongly suggested that the laboratory operator set up at least three (3) different sample dilutions. After you have estimated the average BOD by experience or by guess, use the middle dilution as the sample volume chosen for duplication and averaging. This is important because the final BOD value must be an average of all dilutions which meet

the depletion requirements. If you have only one determination that meets the requirements, you cannot obtain an average?

Set up two bottles for each sample dilution. Label the BOD bottles for the sample dilutions of 30, 60, 60, and 80 ml.

In this example we used two sets of the dilution using 60 ml of sample to ensure that we will obtain at least two BOD tests that meet the criteria for the average.

PROCEDURE:

Continuation from step #5 of the seed correction determination.

6. Determine the initial DO of one bottle 30, 60, 60, and 80.
7. Incubate the remaining bottles at 20° C +/- 1° for five (5) days.
8. After five (5) days of incubation, determine the residual DO for the sample bottles.
9. Calculate the BOD values as shown in the example below.

TABLE #4
SEEDED BOD CALCULATION

SAMPLE – final eff. **Depl. –seed corr./P =BOD mg/L**

mls sample	ml seed	(D1) Int. D.O.	(D2) Final D.O.	Depl.	Seed Corr.	Depl. Seed Corr.	P	BOD mg/L Single	BOD mg/L Avg.
30	4	7.5	6.1	1.4	0.84	0.56	0.10	-	
60	4	7.6	4.7	2.9	0.84	2.06	0.20	10.3	
									10.8
60	4	7.6	4.5	3.1	0.84	2.26	0.20	11.3	
80	4	7.5	0.9	6.6	0.84	5.76	0.28		

By subtracting the seed correction from the seeded sample, you are correcting for the added D.O. uptake caused by the seed material.

Note: Bottles 30 ml and 80 ml do meet the requirements and **cannot be used** in the BOD determination.

30 ml is invalid and cannot be used because the DO depletion is less than the required 2 mg/L. 80 ml is invalid because the final DO is less that the required minimum residual DO of 1.0 mg/L.

This is the reason that a minimum of three (3) different dilutions should be run in order to get at least two (2) valid tests so you are able to obtain and average DO depletion.

Label so you can differentiate

SEED CORRECTION

3 bottles, 3 seed volumes

SAMPLE DILUTIONS + 4 ml of SEED

3 dilutions, 2 bottles per dilution
1 repeat dilution with 4 ml of seed

BLANKS AND CHECKS

2 bottles dilution with water only (dilution water blank)
2 bottles with 2 ml of glucose-glutamic acid in dilution

CALCULATION OF SEEDED BOD VALUES

BOD is calculated as the net depletion of a sample divided by the decimal volumetric fraction of the sample used. To be considered valid, the final DO must have a depletion of at least a 2.0 mg/L and a residual of at least 1.0 mg/L. The following formula is used in the calculation:

$$\frac{(D_1 - D_2) - (B_1 - B_2)xF}{P} = BOD\,\frac{mg}{L}$$

D_1 = Initial DO, mg/L (DO of diluted sample immediately after preparation)
D_2 = Final DO, mg/L (DO of diluted sample after five (5) days incubation)
D_1 - D_2 = mg/L Depletion
P = ml of sample/ ml of BOD bottle volume
B_1 = DO mg/L of seed control before incubation
B_2 = DO mg/L of seed control after five (5) days
F = ml of seed in sample/ml of seed in seed control
B_1 - B_2xF = mg/L Seed Correction

GLUCOSE-GLUTAMIC ACID CHECK

Determine the BOD of a 2% dilution (6 ml in a 300 ml bottle), using the same techniques and depletion criteria as for any seeded BOD. Simply use the 6 ml of glucose-glutamic acid as if it were the sample. Handle the standard like any other seeded sample. The checks should have a BOD of 198 mg/L +/- 30.5 mg/L. If the control limits are outside of this acceptable range re-evaluate the control limits and investigate the source of the problem. Label these bottles.
If the BOD for the glucose-glutamic acid check is outside the control limit range, **reject the tests made with that seed and dilution water.**

TALBE # 5
EXAMPLE OF THE GLUCOSE-GLUTAMIC ACID CHECK

SAMPLE – Final Eff. **Depletion – Seed Corr./P = BOD mg/L**

ml seed	mls gluc glut	(D_1) Int. D.O.	(D_2) Final D.O.	Depl.	Seed Corr.	Depl. Seed Corr.	P	BOD	198 mg/L + or – 30.5 Yes	198 mg/L + or – 30.5 No
4	6	7.6	3.0	4.6	0.84	3.76	0.20	188	X	
4	6	7.6	2.9	4.7	0.84	3.86	0.20	193	X	

SETTLEABLE SOLIDS (IMHOFF CONE)

Settleable solids in raw influent wastewater is important in determining the volume of solids the primary clarifier will remove from the waste stream.

APPARATUS:

Imhoff cone, 1 liter, glass or plastic with stand
Glass rod

PROCEDURE:

1. Fill 1 liter Imhoff cone to the 1 liter mark with a well mixed sample.
2. Let the sample settle for forty-five (45) minutes.
3. Gently loosen any solids that adhere to the sides of the cone with a glass rod.
4. Let the sample settle for fifteen (15) minutes more.
5. Record the volume of the settleable solids in the cone as mL/liter.

SETTLEABILITY
(ACTIVATED SLUDGE MIXED LIQUOR OR RETURNED SLUDGE)

The settled sludge volume of biological suspensions is useful for the routine activated sludge process control. However, be aware that differences in temperature, sampling, agitation methods, the diameter of the settling column, and the time between sampling and starting the test can significantly affect the results. The same procedure and apparatus should be used every time in order to minimize the differences that may result.

Settleability is important in determining the relative ability of the solids to separate from liquid in the final clarifier.

The suspended solids test should be run on the same sample of mixed liquor as the settleability. This will allow the operator to calculate the Sludge Volume Index (SVI) and the Sludge Density Index (SDI).

APPARATUS:

graduated cylinder, 1000 ml graduated in 10 ml

PROCEDURE:

1. Fill a 1 liter graduated cylinder with a well mixed sample.
2. The test should begin immediately after the sample is taken.
3. The mixed liquor sample should be taken at the effluent of the aeration tank.
4. The returned sludge sample should be taken somewhere between the effluent of the final clarifier and where it is mixed with the influent of the primary clarifier.
5. Care should be taken to minimize any vibrations or other disturbing motions that could change the settling rate.
6. After thirty (30) minutes, record the volume of settled sludge. This reading is used for plant control purposes.

CALCULATION:

$$\% \text{ Settled Sludge} = \frac{\text{ml/L of settled sludge in thirty (30) minutes} \times 100}{1{,}000}$$

SOLIDS

TOTAL SOLIDS

Total solids of a sample include all suspended solids and all dissolved solids.

APPARATUS:

Evaporating dishes, 100 ml, made of porcelain, platinum, or high-silica glass
Muffle furnace, for operation at 550°C
Drying oven, for operation at 103°C to 105°C
Desiccator, provided with either a color indicator or an instrumental indicator for moisture concentration
Analytical balance, capable of weighing to 0.1 mg

PROCEDURE:

1. Prepare evaporating dish. If volatile solids are to be determined, place clean evaporating dish in a muffle furnace at 550°C for one (1) hour. If only total solids are to be determined, heat a clean dish in drying oven at 103°C to 105°C for one (1) hour.
2. Store and cool evaporating dish in the desiccator until use.
3. Take the evaporating dish from the desiccator and weigh, repeat heating and colling, weigh again, repeat until you achieve a difference in weight of 0.5 mg or less.
4. Place a sample in the evaporating dish. Choose a sample volume that will yield a residue between 10 and 200 mg.
5. Place the sample in the evaporating dish into the drying oven at 103°C to 105°C for one (1) hour.
6. Place sample in desiccator to cool and balance weight and temperature.
7. Weigh the evaporating dish with the sample residue.
8. Keep repeating the cycle of drying, cooling, desiccating, and weighing until you achieve a difference of weight between cycles of less than 0.5 mg.

CALCULATION:

$$\text{mg of total solids/L} = \frac{(A-B) \times 1{,}000{,}000}{\text{sample volume, mL}}$$

A = weight of dried residue + dish in mg
B = weight of dish in mg

VOLATILE SOLIDS & FIXED SOLIDS

Volatile solids are those solids that are lost by volatilization when heated to 550°C.

PROCEDURE:

1. Ignite dried residue from the total solids measurement at 550°C in a muffle furnace for one (1) hour.
2. Cool the sample and place in desiccator to balance temperature and weight.
3. Repeat the igniting (thirty (30) minutes), cooling, desiccating, and weighing until a difference in weight of less than 0.5 mg is achieved.

FIXED SOLIDS

Fixed solids are the total solids that remain after the volatile solids are lost.

CALCULATION:

$$\text{Total solids, mg/L} = \frac{(A-B) \times 1{,}000{,}000}{\text{Sample volume, mL}}$$

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

$$\%\ \text{Volatile solids} = \frac{(A-D) \times 100}{A-B}$$

$$\%\ \text{Fixed solids} = \frac{(D-B) \times 100}{A-B}$$

A = weight of dried residue + dish in mg/L.
B = weight of dish in mg.
C = weight of wet sample + dish in mg.
D = weight of residue + dish after ignition in mg.

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