Franklin Township Municipal Sanitary Authority

Laboratory Standard Operating Procedures

Effective Date: January 18, 2016

Revisions: July 28, 2006 (original)
June 21, 2007 (rev. 1)
January 4, 2010 (rev. 2)
April 14, 2010 (rev. 3)
April 27, 2011 (rev. 4)
October 15, 2012 (rev. 5)
December 21, 2012 (rev. 6)
October 23, 2013 (rev. 7)
October 1, 2015 (rev. 8)
January 18, 2016 (rev. 9)

Approving Name: Kevin Kaplan Title: Manager

Approving Signature:____________________________ Date:__________________________

The analyst has read, understands and is performing the methods as written in these SOPs.

Analyst Name (Print)___________________________ Initials:___________

Analyst Signature______________________________ Date:_________________________

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% Total Solids 42

Distribution List:

Kevin Kaplan - Manager
Gene Greco - Plant Superintendent
Laboratory

Identification of Methods:

Ammonia

Distillation of, - *Standard Methods* (SM), 18th ed. 4500-NH3 B.

Nesslerization Method - *Standard Methods* (SM), 18th ed. 4500-NH3 C.

Chlorine, Residual (4500-Cl G.)- DPD Colorimetric

pH ,Electrometric Method (*Standard Methods 18th ed.* 4500-H^+ B.)

TSS - Total Suspended Solids Dried at 103 - 105 °C (*Standard Methods 18th ed.* 2540 D.)

CBOD - Carbonaceous Biochemical Oxygen Demand, BOD5 (*Standard Methods 18th ed. 5210 B.)*

Dissolved Oxygen - Membrane Electrode Method (*Standard Methods 18th ed. 4500-O G.)*

Fecal Coliform

Membrane Filtration Technique (*Standard Methods 18th ed. 9222 D. Membrane Filter Technique)*

Multiple Tube Fermentation - Fecal Coliforms (Biosolids) in A-1 Broth (*Standard Methods 18th ed. 9221 E.)*

Ammonia, Distillation of (4500-NH3 B)

Scope:

Distillation is a required preliminary step to the Nesslerization Method (4500 NH3 C) for the evaluation of wastewater samples. The ammonia released in the distillation process is captured in a solution of boric acid and can be determined colorimetrically by nesslerization using a spectrophotometer . The samples are buffered at a pH of 9.5 with a borate buffer solution to decrease hydrolysis of cyanates and organic nitrogen compounds. This is a colorimetric test in which an increase in color is proportional to an increase in the concentration of ammonia (NH3-N).

Sample Handling, Preservation and Preparation:

Distillation is performed on 24 hour, 8 hour composites samples and/or grab samples of influent and/or effluent wastewater. If the samples are not tested immediately after distillation, they are place in plastic or glass containers and stored in the sample refrigerator.

Composite samples during compositing are kept refrigerated near 4 degree Celsius. After collection samples are stored in a refrigerator near 4 degrees Celsius.

Upon collection, samples are dechlorinated using either sodium thiosulfate or sodium sulfite. Samples that are to be distilled immediately following collection are not preserved otherwise 1 ml of 1 + 1 H_2SO_4 is added per liter of sample (or an amount equivalent for the sample volume) so that a pH of less than 2 is achieved.

Distillation Apparatus and Equipment:

1. Several borosilicate glass flasks of 500 ml capacity are attached to vertical water cooled condensers with the outlet tips submerged below the surface of the receiving solution (boric acid).

2. Electromantle heating units with temperature adjustments capable of causing the sample to boil.

3. pH meter or litmus paper

4. Boiling chips

5. Receiving flasks

Reagents:

Ammonia free water - distilled water passed through a Barnstead Easy Pure ion-exchange system

and prepared as needed. Ammonia free water is used for all reagents, standards, laboratory control samples and blanks.

Borate buffer solution - 88 mls of 0.1N NaOH solution and 4.75 g of sodium tetraborate ($Na_2B_4O_7 * 10\ H_2O$) is added to 500 ml of ammonia free water, then diluted to1 liter.

Boric acid solution - 20 g of boric acid diluted to 1 liter.

0.4% Thymol Blue indicating solution

1 + 1 Sulfuric Acid solution - Equal parts of Sulfuric Acid and reagent grade water are added together stored in a glass container. **Safety Note: Acid into water!**

Sodium Thiosulfate (dechlorinating agent) - dissolve 0.35 g of $Na_2S_2O_3 * 5\ H_2O$ into 50 ml of reagent grade water and dilute to 100 ml. Add 0.5 ml to sample to remove 1 mg/L of residual chlorine.

Sodium hydroxide solution:

0.1N NaOH - 4.0 g of NaOH diluted to 1 liter with reagent grade water.
5N NaOH - 200 g of NaOH diluted to l liter with reagent grade water.

Equipment Preparation:

Adjust a 250 ml volume of distilled water to a pH of 9.5 with 5N sodium hydroxide solution. Place into distillation unit along with some boiling chips and 12 mls of borate buffer solution. This mixture is used to steam out the distillation apparatus to eliminate traces of ammonia.

Procedure:

An adequate amount of sample volume (approximately 1 L) is checked for a residual chlorine and an appropriate amount of thiosulfate solution is added for dechlorination. Add 0.1 ml to sample to remove 0.2 mg/L of residual chlorine.

12 ml of borate buffer solution, 2-3 drops of 0.4% thymol blue indicating solution and boiling chips are added to a 200 ml dechlorinated sample in a distillation unit. The sample should turn blue if pH is in the 9.5 range. If not, the sample needs to be adjusted to that pH range with the appropriate (5N) sodium hydroxide solution using a pH meter. Turn on heating mantle to an appropriate temperature and distill off into a 50 ml boric acid solution to a total volume of 200 ml. The tip of condenser needs to be submerged into the receiving solution. During the last minute or two of distillation, lower the receiving solution as to cleanse the condenser and delivery tube.

Quality Control:

Distillation should include: a blank, laboratory control sample, duplicate, spike duplicate spike and a 10 ppm ammonia standard.

Refer to the Quality Manual for additional QA/QC requirements and information.

Standards:

Using a Class A pipet, 10 ml of 1000 ppm stock ammonia standard is diluted to 500 ml with reagent grade water in a volumetric flask. Added 1 ml of 1+1 Sulfuric Acid solution, then further dilute to 1 L. (10 ppm final concentration)

Refer to Ammonia, Nesslerization Method 4500-NH3 C. following this section.

Notes:

Standard, LCS and all QA/QC should be distilled off as a sample.

Determine the ammonia concentration by the Nesslerization Method *Standard Methods 18th ed.* 4500-NH3 C.

Ammonia, Nesslerization Method of (*Standard Methods 18th ed.* 4500-NH3 C.)

Scope:

It has been established by the Authority that this method, following distillation, is applicable for the determination of ammonia in it's wastewater influent and effluent samples. A spectrophotometer with a light path of 5 cm set at a wavelength of 425 nm can determine ammonia concentration between 0.1 ppm and 5.0 ppm. Absorbance is measured and conforms to Beer's Law having a linear curve.

Upon addition of the Nessler's Reagent, a light yellow color in the sample is indicative of a low ammonia concentration. Color increase is proportional with ammonia concentration.

The calculated detection limits are 0.05 ppm. The lowest concentration that the Authority will report is < 0.1 ppm. The upper limit for this curve is 5.0 ppm. Sample concentrations above this amount need to be diluted to a concentration from mid-range to the upper concentration of the curve.

A minimum of a 4 point standard curve will be ran, not including the blank. The correlation coefficient (r value) shall be no less than 0.9980.

Sample Handling, Preservation and Preparation:

Analysis is performed on 24 hour, 8 hour composites samples and/or grab samples of influent and/or effluent wastewater. If the samples are not tested immediately following distillation, they are stored in the sample refrigerator at 4 degrees Celsius.

Composite samples during compositing are kept refrigerated near 4 degree Celsius. After collection samples are stored in a refrigerator near 4 degrees Celsius.

Upon collection, samples are dechlorinated using either sodium thiosulfate or sodium sulfite. Samples that are to be distilled immediately following collection are not preserved otherwise 1 ml of 1 + 1 H2SO4 is added per liter of sample (or an amount equivalent for the sample volume) so that a pH of less than 2 is achieved and the sample is distilled and analyzed at a later time within 2 weeks of sample collection or sooner if required by regulation.

Standards are prepared at the same temperature and reaction time (approximately 10 minutes) as used for the samples.

Standards:

Preparation of the standards are as follows:

mls of 10 ppm Standard	Diluted to (mls)	Final concentration (ppm)
1	100	0.1
3	100	0.3
5	100	0.5
5	50	1.0
10	50	2.0
15	50	3.0
20	50	4.0
25	50	5.0

CCV preparation:

mls of 10 ppm Standard	Diluted to (mls)	Final concentration (ppm)
4	100	0.4
45	100	4.5

A 0.4 ppm and 4.5 ppm standards are used as a continuing calibration verification (CCV). CCVs are to be alternated between high and low. Any CCV is acceptable to use if the low CCV is in the lower 20% of the curve but not more than 5 times the lowest quantitation level and the high CCV is within the upper 20% of the curve.

Procedure:

Let samples and standards sit out at room temperature for approximately 1 hour.
Make up standards in the concentration range starting at 0.1 ppm to 5.0 ppm. Use 5 different concentration for the curve.
Warm up spectrophotometer for 10 minutes. Set at 425 nm wavelength.
Zero spectrophotometer with distilled water according to manufacture's directions located on the instrument itself.
Set spectrophotometer to the Absorbance reading.
Run the standard curve starting with the lowest concentration through the highest.
Use a 50.0 ml sample or a portion diluted with reagent grade water.
Add a few drops of 5N sodium hydroxide solution to neutralize the boric acid solution.
Add 1 ml of Nessler Reagent.
Run the LCS, blank and samples with all QA/QC.
Run a CCV and a blank every 10 samples and at the end of the analysis.
Mix thoroughly and let stand 10 minutes for color development.
Record absorbance readings.
Using absorbance versus concentration, calculate the curve correlation coefficient (R value).
r = 0.9980 or better is acceptable.
Calculate and report concentration of NH_3-N from curve on data sheet.

Example analysis run:

Blank
0.1 ppm standard
0.5 ppm standard
1.0 ppm standard
2.0 ppm standard
3.0 ppm standard
5.0 ppm standard
LCS
Reagent Blank (Boric Acid)
CCV (0.20 ppm)
Effluent sample 1
Effluent sample 2
Effluent sample 3
Effluent sample 3 duplicate
Effluent sample 3 spike
Effluent sample 3 duplicate spike
Influent sample 1
Influent sample 2
Influent sample 3
CCV (4.5 ppm)
Blank

Apparatus:

Spectrophotometer - Spectronic 20D+

pH meter

Class A pipet: 1 ml.

50 ml beakers

50 ml graduate cylinders

Reagents:

Ammonia free distilled water (Reagent grade)

Nessler Reagent - purchased

5N sodium hydroxide solution - 20 g of NaOH diluted to l00 ml with reagent grade water.

Stock ammonium solution - Dissolve 3.819 g anhydrous NH_4Cl, dried at 100 degrees Celsius, in ammonia free distilled water, and dilute to 1 L.; 1.00 ml = 1.00 mg N = 1.22 mg NH_3.

Standard ammonium solution - Dilute 10.ml of stock ammonium solution to 1 L with reagent grade water. 1 ml = 10 ppm N = 12.2 ppm NH_3.

QA/QC:

Refer to page 31 of the Laboratory Quality Manual.
Control limits are ±3 times the standard deviation for CCVs.
Control limits for laboratory control samples (LCS) are set by the manufacture. Refer to their documentation for limits.
Duplicate samples with values beyond 3.27 times the average value are considered "Not Acceptable".
Spike concentrations (refer to page 30 of the Laboratory Quality Manual).

Corrective Actions:

Refer to page 31 of the Quality Manual.
Data outside the limits is considered "Not Acceptable" and the analyst is to flag the data , check for errors and take corrective action.
QA/QC results that are more than ± 2 times the standard deviation but less than ±3 times are acceptable but the analyst should check for error.

Reporting of Results:

Refer to the Quality Manual.

Equations:

mg NH_3-N/L = C x A/B x D/E

A = total volume (50 ml)
B = sample volume used
C = concentration from curve
D = sample volume originally distilled
E = distillate volume collected including boric acid solution

The ratio D/E only applies if the original sample volume and the distilled sample volume are not the same.

mg NH_3-N/L = Concentration of Sample x (Total Volume ÷ Sample Volume used)
= Concentration of Sample x (50 ml ÷ Sample Volume used)

Dilutions for Standards:
(Concentration of A) x (Volume of A) = (Concentration of B) x (Volume of B)

Total Suspended Solids Dried at 103 - 105 °C (*Standard Methods 18th ed.* 2540 D.)

Scope:

A well-mixed composite sample of wastewater influent or effluent is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. This method is appropriate for the examination of wastewater.

Interferences:

Exclude large floating particles or submerged gathered masses of nonhomogeneous materials from the sample if it is not representative of that sample. Limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure the removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

Sample Handling, Preservation and Preparation:

Analysis is performed on 24 hour or 8 hour composites samples of influent and effluent wastewater. If the samples are not tested within 1 hour after sampling, they are placed in plastic containers that have leak-proof tops and stored in the sample refrigerator at 4 degrees Celsius. All samples will be analyzed within 24 hours of collection.

Reagents:

Distilled water

Apparatus:

Drying oven, for operation at 103 to 105°C.
Analytical balance, 10 to 200 g capacity, capable of weighing up to 0.0001 g (0.1 mg).
Filtration apparatus (vacuum pump with reservoir) or aspirator.
Gooch crucibles, 25-ml to 40-ml capacity, each with their own unique identification.
Desiccator, provided with a desiccant containing a color indicator of moisture concentration.
Graduated cylinders; 25-ml, 50-ml, 100-ml, 250-ml, 500-ml.
Glass-fiber filter disks, 2.4 cm to 4.7 cm; Whatman grade 934AH, Gelman type A/E, Millipore type AP40, or equivalent.
Suction Flask, 250-ml.
Dish tongs
Forceps, smoothed-tip

Preparation of glass-fiber filter disk:

The glass-fiber filter disk is inserted rough side up in the Gooch crucible using the forceps. Discard any filters that are torn or contain holes. Do not handle the glass fiber filters.
Connect to filtration apparatus and turn on vacuum.
Wash disk with three successive 25-ml portions of reagent-grade water.
Continue suction until all traces of water are removed.
Discard rinse filtrate from filter flask.
Remove Gooch crucible and transfer to the drying over for a period of 1 hour.
Remove from drying oven and transfer to the desiccator.
Let crucible cool to room temperature in desiccator.

Procedure:

Select crucible and weigh 3 times on an analytical balance.
Record crucible identification and weights.
Connect to filtration apparatus and begin suction.
Wet the filter with a small volume of reagent-grade water to seat it.
Stir or mix sample thoroughly, remove the appropriate volume into a graduated cylinder and filter. The appropriate volume would be that which would yield between 2.5 and 200 mg or residue.
If filtration time exceeds 10 minutes, decrease the sample volume or use a larger filter to ensure a representative sample can be filtered.
Rinse with three successive 10-ml volumes of reagent-grade water, allowing complete drainage between washings and continue suction for about 2 minutes after filtration is complete.
Samples with high dissolved solids may require additional washings.
Remove the crucible from the filtration apparatus and transfer to the drying oven for at least 1 hour at 103 to 105°C.
After drying is complete, transfer to the desiccator and allow to cool to room temperature.
Select crucible and re-weigh 3 times on an analytical balance.
Record crucible identification and weights.

Alternatively, the crucible, if dried over a minimum period of 16 hours, can be weighed one time instead of 3.

Calculation:

$$\text{mg total suspended solids/L} = \frac{(Wf - Wi) \times 1000}{\text{sample volume, ml}}$$

where: Wf = the final weight of the crucible, mg
Wi = the initial weight of the crucible, mg.

Each sample will be analyze with two crucibles with equivalent volumes. The average of the two results will be reported.

QA/QC:

Two crucibles per sample.
One sample must be run in duplicate (total of 4 crucibles).
Run a "Method Blank".
A reference weight (20 g) in the range of the weight of the samples and crucibles must be weighed on the analytical balance prior to running samples to check it's precision and accuracy.

Acceptance Criteria: The method blank result must be below the reportable detection limit of the method. In this case, it is less than (<) 3 ppm. Report to management if results are unacceptable.

Refer to the Quality Manual

The laboratory will keep a calibration certificate demonstrating the traceability to NIST standards and the weights must be ASTM type 1, 2, or 3 (Class S or S-1). Reference weights will be re-certified every 5 years and documented.

pH, Electrometric Method (*Standard Methods 18th ed.* 4500-H^+ B.)

Introduction:

pH is a mathematical expression of the intensity of the acid or alkaline condition of a sample. The term "pH" is defined as the negative logarithm of the hydrogen ion concentration, and is generally represented with a scale from 0 to 14 pH units.

Water is made up of both hydrogen ions (H+) and hydroxyl ions (OH−). Because water is made up of these two ions, any solution which contains water (called an aqueous solution) always has both hydrogen ions and hydroxyl ions. The pH scale is a means of showing which ion has the greater intensity.

At a pH of 7.0, the intensities of hydrogen and hydroxyl ions are equal and solution is said to be pH neutral. When the pH is less than 7.0, the intensity of the hydrogen ions is greater than that of the hydroxyl ions and the solution is said to be acidic. When the pH is greater than 7.0, the hydroxyl ions have the greater intensity and the solution is referred to as alkaline or basic.

The pH measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and reference cell using NIST buffers having assigned values.

pH monitoring and control is important is the operation of wastewater treatment plants. This method is applicable for the analysis of wastewater.

Sampling:

Only grab samples may be taken for pH measurement. It is very important that the samples be well mixed and representative of the plant flow. Each sampling point should have it's own sample collection devices and container in order to prevent cross-contamination.

Preservation:

The pH must be measured as soon after collection as possible. There is not approved method of sample preservation for pH samples. If the time lag between sample collection and testing exceeds 30 minutes, the samples should be discarded and fresh samples collected.

Sample Containers:

Polyethylene, polypropylene or glass.

Sample Container Preparation:

All sample collection containers and bottles should be cleaned thoroughly with soap and water on a regular basis and rinsed well. A final rinsing should be 3 times with distilled water and allowed to dry.

Equipment:

pH meter, readable to at least 0.1 pH unit with temperature probe.

Beakers

Electrode, combination

Magnetic stirrer and stirring bars.

Reagents:

pH buffer solutions: pH of 4, 7 & 10

Calibration:

1. Turn on pH meter and allow instrument to warm up.
2. Calibrate according to manufacturers instruction found in the laboratory.
3. Insert pH probe into a 7.00 pH buffer solution. Record mV and temperature.
4. Remove pH probe from the buffer solution, rinse with distilled water and carefully blot the probe dry.
5. Place probe into a 10.00 pH buffer solution. Record mV and temperature.
6. Remove pH probe from the buffer solution, rinse with distilled water and carefully blot the probe dry.
7. Place probe into a 4.00 pH buffer solution. Record mV and temperature.
8. Check calibration with a laboratory control sample (LCS).

Procedure:

Turn on pH meter and allow instrument to warm up.
Remove pH probe from buffer solution.
Rinse pH probe with distilled water and blot the probe dry.
Insert probe into a beaker containing the sample along with a stirring bar.
Turn on magnetic stirrer and stir sample at a moderate rate.
Record pH reading and temperature.
After usage, turn pH meter on “standby”.

QA/QC:

Check calibration with a fresh 7.00 buffer solution daily.
Run a laboratory control sample.
Duplicate samples should be run on every 10th sample.
Laboratory bench sheets must be maintained that document the date and time the sample was collected, the analyst, method number, result and temperature of sample.
Refer to the Quality Manual.

Interferences:

The glass electrode is relatively free from interferences due to color, turbidity, colloidal matter, oxidants, reductants or high salinity, except for a sodium error at a high pH. This error at a pH above 10 may be reduced by using a special “low sodium error” electrode. It is not typical for the treatment plant to analyze samples above the pH of 10.

Since both ph, itself, as well as the measurement of pH are temperature dependent, it is important that temperature compensation be performed on all samples and that the temperatures of buffers and samples be as similar as possible.

Notes:

Record temperature along with pH.
Check the pH probe for cracks and the appropriate amount of filling solution.
Calibration should be a 3 point calibration.
Replace buffer solution daily.
Do not use expired buffer solutions.
Discard used buffer solutions.
Watch for erratic results.

Reporting of Data:

Report pH values to the nearest 0.1 pH unit.
Record temperature of samples with pH values.

Dissolved Oxygen, Membrane Electrode Method (*Standard Methods 18th ed. 4500-O G.)*

This method is applicable for the determination of the amount of dissolved (or free) oxygen present in water or wastewater. Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. An accuracy of ±0.1 mg DO/L and a precision of ±0.05 mg DO/L can be obtained.

The membrane electrode procedure utilizes a meter and electrode, and is based on the rate at which oxygen molecules diffuse (or pass through) a membrane covering a set of electrodes. The oxygen molecules react with an internal filling solution to develop a small electrical charge between the electrodes which can be read on a meter. The readings on the meter correspond directly to the amount of DO present in the sample.

Sampling:

Samples are to be analyzed immediately.
Samples will be grab samples.
Samples must be representative. Sampling location where mixing is through and the wastewater is uniform.
Sampling should be done at the same time of each day.

Sample Containers:

Grab samples in glass 300-ml BOD bottles with stoppers.
BOD bottles should be washed thoroughly with soap and water, rinse well with tap water, then a final rinse (3x) with distilled water.

Equipment & Apparatus:

DO meter
DO electrode (probe)
Electrode membrane kit
BOD bottles, 300-ml

Reagents:

The addition of an excess of sodium sulfite (Na2SO3), and a trace of cobalt chloride (CoCl2) to

distilled water in a full 300-ml BOD bottle will yield a sample with zero DO.

Calibration:

The probe must be air calibrated before each use.
Shake of excess water/condensation from the probe.
Wait 5 minutes.
Check barometer for atmospheric pressure reading (mm Hg).
Off of chart, find the calibration value for the pressure reading.
Check temperature (°C) of air in BOD bottle containing 50-ml of distilled water.
Off of chart, find the value for DO at 100% humidity at that temperature.
Multiple ppm DO value time pressure value to find value to calibrate the meter to.
Pressure calibration value x ppm DO value = value to calibrate the meter to.

To enter the value into the DO meter:
Turn the knob of the DO meter to "CALIBRATE".
Push "SKIP", the meter thin will show "CALIBRATE IN MG/L?".
Push "CONFIRM", the meter will show "ENTER CAL VALUE" "LAST = 8.xx MG/L"
Push "⇑" or "⇓"and hold button until the calibration value is shown.
Push "CONFIRM", the meter will show "CALIBRATED TO 8.xx".
Turn the knob to O2/TEMP. Calibration is complete.
Refer to manufacturer's instructions located in the laboratory.

Atmospheric Pressure Chart:

mm Hg	Calibration value	mm Hg	Calibration value
768	1.010	745	0.980
764	1.005	741	0.975
760	1.000	737	0.970
756	0.995	734	0.965
752	0.990	730	0.960
748	0.985		

DO/Temperature Chart:

Temp. °C	ppm DO	Temp. °C	ppm DO
18.0	9.50	24.0	8.50
18.5	9.40	24.5	8.45
19.0	9.30	25.0	8.40
19.5	9.25	25.5	8.30
20.0	9.20	26.0	8.20
20.5	9.10	26.5	8.15
21.0	9.00	27.0	8.10
21.5	8.90	27.5	8.00

22.0	8.80	28.0	7.90
22.5	8.75	28.5	7.85
23.0	8.70	29.0	7.80
23.5	8.60		

Reporting Data:

There are no calculations for this method. The DO concentration is read directly from the meter.

QA/QC:

A laboratory control sample and duplicate sample is run each analysis or every 10 samples, whichever is more frequent.

Refer to the Quality Manual.

Interferences:

There are very few substances which will interfere with the DO method when utilizing the electrometric meter. Salinity caused by dissolved inorganic salts (such as industrial or manufacturing processes) can influence the probe's readings. Reactive compounds and gases (like hydrogen sulfide and other sulfur compounds) can interfere with the reading by reducing probe sensitivity. Chlorine residual can create a positive interference.

Biochemical Oxygen Demand, BOD5 (*Standard Methods 18th ed. 5210 B.)*

In the presence of free oxygen, aerobic bacteria use the organic matter found in wastewater as "food". The BOD test is an estimate of the "food" available in the sample. The more "food" present in the waste, the more Dissolved Oxygen (DO) will be required. The BOD test measures the strength of the wastewater by measuring the amount of oxygen used by the bacteria as they stabilize the organic matter under controlled conditions of time and temperature.

The BOD test is used to measure waste loads to treatment plants, determine plant efficiency (in terms of BOD removal) , and control plant processes. It is also used to determine the effects of discharges on receiving waters. A major disadvantage of the BOD test is the amount of time (5 days) required to obtain the results.

In many biological treatment plants, the facility effluent contains large numbers of nitrifying organisms which are developed during the treatment process. These organisms can exert an oxygen demand as they convert nitrogenous compounds (ammonia and organic nitrogen) to more stable forms (nitrites and nitrates). At least part of this oxygen demand is normally measured in a five day BOD.

Sometimes it is advantageous to measure just the oxygen demand exerted by organic (carbonaceous) compounds, excluding the oxygen demand exerted by the nitrogenous compounds. To accomplish this, the nitrifying organisms can be inhibited from using oxygen by the addition of a nitrification inhibitor to the samples. The result is termed "Carbonaceous Biochemical Oxygen Demand", or CBOD. This method is appropriate for the analysis of wastewater.

An aliquot of sample is placed into a BOD bottle containing aerated dilution water. The DO content is determined and recorded and the bottle is incubated in the dark of five days at 20°C. At the end of the five days, the final DO content is determined and the difference between the final DO reading and the initial DO reading is calculated. The decrease in DO is corrected for sample dilution and represents the biochemical oxygen demand of the sample.

Sampling:

Samples can be either grab or composite. Composite samples are taken of influent and effluent wastewater at the treatment plant as specified by the NPDES permit. Samples are taken at points where they are well-mixed and proportional to the amount of the flow.

Sample Preservation:

Testing should be started as quickly as possible. During compositing, samples will be stored at or near 4°C in plastic containers. These containers will be of adequate volume in proportion to the plant flow and sample aliquot volume. Samples may be kept for no more than 48 hours before beginning the BOD test. The 48 hours starts when the very first aliquot of a composite is collected.

Sample storage containers should be cleaned thoroughly between samples. It is recommended that they be acid cleaned on a regular basis to prevent residue buildup which occurs over time. Each sampling point should have its own storage container. Containers should be clean and dry before a new set of composite samples are stored in them.

Pretreatment of Samples:

Samples with extreme pH values and samples containing disinfectants such as residual chlorine must be treated prior to testing.

Samples which have pH values higher than 7.5 or lower than 6.5 must be neutralized to a pH between 6.5 and 7.5 before this test is performed. Neutralized samples must be seeded for the BOD test.

Reagents:

Phosphate buffer solution:	Dissolve 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 * 7H_2O$, and 1.7 g NH_4Cl in about 500 ml distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.
Magnesium sulfate solution:	Dissolve 22.5 g $MgSO_4 * 7H_2O$ in distilled water and dilute to 1 L.
Calcium chloride solution:	Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.
Ferric chloride solution:	Dissolve 0.25 g $FeCl_3 * 6H_2O$ in distilled water and dilute to 1L.
Sodium hydroxide solution:	Dissolve 40 g NaOH in distilled water and dilute to 1 L. (1N concentration)
Sulfuric acid solution:	Add 28 ml to 1 L of distilled water and mix. (1 N concentration)
Sodium sulfite solution:	Dissolve 0.1575 g Na_2SO_3 in 100 ml distilled water. This solution is not stable. Prepare daily.
Nitrification inhibitor:	2-chloro-6-(trichloro methyl) pyridine. Bought from Fisher Scientific or Hach.

Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 hour. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use. This can also be purchased from Fisher Scientific.

Ammonia chloride solution: Dissolve 1.15 g NH_4Cl in about 500 ml of distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/ml.

BOD Seed: Commercially bought

Preparation of Dilution Water:

Using a 3 L or larger bottle, add 1 ml of each phosphate buffer, magnesium chloride, calcium chloride and ferric chloride solutions per liter of distilled, deionized water. The pH of dilution the water should be around 7.00. Adjust with 1N NaOH or H_2SO_4 solutions if necessary. Saturate with DO before using by vigorously shaking the dilution water bottle or by aerating. The DO should be approximately 8 mg/L at room temperature. Store at 20°C in a dark area such as the BOD incubator or in a cabinet.

It is very important that the distilled water used for dilution water be of high grade and free from contaminants such as copper and chlorine which could inhibit the growth of bacteria.

Equipment:

BOD bottles, 300 ml with stoppers and water seal caps.

DO meter with probe.

Incubator: air, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent possibility of photosynthetic production of DO.

Graduated cylinders, various capacities.

Pipets, various capacities

Pipet bulb

Magnetic stirrer and stirring bars

pH meter and probe

Dilution water bottle, 3L or larger

Burette graduated to 0.1 ml

250 ml Erlenmeyer flask

Determination of Sample Size:

The BOD test relies on a measurable depletion of DO over a 5 day period of time. Because most samples of wastewater will have a BOD higher than the amount of oxygen available in the BOD bottle during the incubation period, the samples must be diluted. This dilution is done by adding dilution water to the sample in the BOD bottle. If the sample is not diluted, the biological activity of the microorganisms will use up the DO in the BOD bottle before the five day incubation time is up. If the final DO is too low, the BOD cannot be determined. There is no way of knowing at what point during the five days the BOD reached zero.

Once a general range for the BOD of a sample has been determined, the dilutions can be established which will ensure that at least one dilution will meet the criteria for valid BOD results. Generally, for the FTMSA treatment plant, the effluent samples can be analyzed at 100, 150, 199 ml aliquots. Influent samples can be analyzed at 3, 6, 9 & 12 ml aliquots during normal flow conditions. During wet weather conditions, due to infiltration and inflow, smaller dilutions will be required.

The criteria for most valid results states that the DO depletion at the end of five days incubation should be at least 2.0 mg/L and the residual DO at least 1.0 mg/L. The formulas to calculate the minimum and maximum estimated dilutions are as follows:

ml sample added to BOD bottle = (Minimum allowable depletion, mg/L x Volume of BOD bottle, ml) ÷ Estimated BOD, mg/L

(2 mg/L x 300 ml) ÷ Estimated BOD, mg/L

and

ml sample added to BOD bottle = (Maximum allowable depletion, mg/L x Volume of BOD bottle, ml) ÷ Estimated BOD, mg/L

(7 mg/L x 300 ml) ÷ Estimated BOD, mg/L

Since the BOD value used is only an estimate, and the BOD bottles do not always have a volume of exactly 300 ml, several bottles with different volumes of sample are set up to ensure that the test requirements are met.

Those sample dilutions which deplete less than 2 mg/L, or have a final DO of less than 1 mg/L would not be used in the calculation of the average sample BOD.

Procedure for Neutralizing Samples:

1. Pour 50 ml of sample into a 100 ml beaker.
2. Measure the pH of the sample using a pH meter. If the pH is out of the range of 6.5 to 7.5 continue with steps 3-6, otherwise perform the BOD test on the untreated sample.
3. Add 1 N sulfuric acid if the sample is alkaline, or 1 N sodium hydroxide if the sample is acidic, until the pH reaches 7.0.
4. Calculate the amount of sulfuric acid or sodium hydroxide needed to neutralize 1000 ml of the sample.
5. Add the calculated amount of acid or base to the sample.
6. Repeat steps 1-5 until the pH test shows 7.0.

Calculation:

Calculate the amount of 1 N sodium hydroxide or 1 N sulfuric acid needed to neutralize the sample to pH 7.0 using the following calculation:

ml needed = (ml acid or base used x ml total test sample) ÷ ml sample portion used for neutralization.

To Prevent Interference from Chlorine:

Any samples containing residual chlorine must be pretreated to remove chlorine before the test is run. This is done by adding sodium sulfite to the sample. Samples which are dechlorinated must be seeded for the BOD test.

Procedure for Dechlorinating Samples:

1. To a 250 ml Erlenmeyer flask, add 100 ml of a well-mixed portion of the sample to be dechlorinated.
2. Add 10 ml of either 1+1 acetic acid solution or 1+50 sulfuric acid solution to the flask and swirl to mix.
3. Add 10 ml of potassium iodide (KI) solution and 1 ml of starch indicator solution. Swirl to mix and let stand for 15 minutes.
4. If a blue color does not appear. There is no chlorine in the sample and it soes not require further treatment prior to the BOD test.
5. If a blue color appears, titrate the treated portion of sample with 0.025 N sodium sulfite ($Na2SO3$) until the blue color first disappears. Record this amount on a laboratory bench sheet.
6. Calculate the amount of sodium sulfite solution needed to dechlorinate the selected BOD sample volume.
7. Add the calculated volume of sodium sulfite to the BOD test procedure, otherwise continue with steps 5-8 until the sample is dechlorinated.
8. Allow the sample to stand for 10 to 20 minutes, then repeat steps 1-3.

9. If no chlorine is detected, continue with the BOD test procedure, otherwise continue with steps 5-8 until the sample is dechlorinated.

Calculation:

Calculate the amount of sodium sulfite needed to dechlorinate the BOD sample using the following equation:

ml Na_2SO_3 needed = (ml Na_2SO_3 used x ml total test sample) ÷ ml sample volume used for dechlorination

Laboratory Procedure:

1. Take out enough BOD bottles for the number of samples to be analyzed along with any QA/QC.
2. Write down BOD bottle and sample identification on a laboratory bench sheet.
3. Add 3.33 mg of nitrification inhibitor to **each** bottle.
4. Completely fill one BOD bottle with dilution water. This is your blank.
5. Into additional BOD bottles, partially filled with dilution water.
6. Add between 2 - 5 ml seed. 4 bottles will be seeded dilution water and the effluent bottles will need to be seeded.
7. Carefully measure out the proper volume of samples.
8. Add dilution water until bottles are completely filled to the top of bottle neck. General rule for FTMSA treatment plant: For influent samples use 3, 6, 9,& 12 ml. For effluent samples use 100, 150, 250 & 295 or 298 ml.
9. Determine the initial DO content on one of each BOD bottle and record data on the laboratory bench sheet.
10. Stopper each bottle taking care to avoid trapping air bubbles inside the bottles as the bottle stoppers are inserted.
11. Cap bottles with plastic BOD bottle caps (for water seal).
12. Place in the BOD incubator at 20°C and incubate for five days.
13. At the end of five days ±3 hours, test the DO content of the incubated bottles.
14. Calculate the BOD for each dilution. The most accurate BOD will be obtained from those dilutions that have a depletion of at least 2 mg/L DO and at least 1.0 mg/L DO residual. If there is more than one dilution that meets these criteria, the BOD results should be averaged to obtained a final BOD value.
15. The dilution water blanks are used only to check the quality of the dilution water. If the quality of the water is good and free from impurities, the depletion of the DO should be less than 0.2 mg/L. In any event, do not use the depletion obtained as a blank correction.
16. Report the results of the nitrification inhibited samples as $CBOD_5$.

Calculations:

To determine the value of the BOD in mg/L, use the following equations:

$$\text{BOD, mg/L} = [(\text{Initial DO} - \text{Final DO}) \times 300] \div \text{ml sample used}$$

or

$$\text{BOD, mg/L} = [(\text{Initial DO} - \text{Final DO} - \text{Seed DO}) \times 300] \div \text{ml sample used}$$

Whenever a sample is dechlorinated, it must be seeded. If the sample is seeded, a correction factor must be calculated to determine the effects that the seed material has on the DO depletion. A number of BOD's must be run on the seed material to determine the seed correction factor.

Interferences:

Since the BOD test is dependent on biological activity, the major interferences will be those substances which inhibit the growth of the microorganisms. These will include chlorine, caustic alkalinity or acidity, mineral acids, and heavy metals such as copper, zinc, chromium and lead.

Excessive nitrites can interfere with the BOD determination. Growth of algae in the presence of light can cause problems by actually increasing DO of the samples before testing, which must be removed by deaeration.

A common problem encountered in BOD testing results from building up in the BOD and dilution water bottles. To prevent this, all glassware should be acid cleaned on a regular basis.

Samples Supersaturated with DO:

Samples are to be brought up to about 20°C in a partially filled bottle while agitating by vigorous shaking for approximately one minute to eliminate supersaturation.

Typical FTMSA BOD Sample Run:

Blank (2)
4 seeded bottles (2, 3, 4 or 5 ml)
Effluent samples (100, 150, 199 ml)
Duplicate effluent samples (same dilutions as effluent samples)
Influent samples (3, 6, 9 &12 ml)
Glucose-Glutamic Acid check (4 bottles, 6 ml each)

Seed Correction Procedure:

The BOD test relies on the presence of healthy organisms. If the samples tested contain materials which could kill or injure the microorganisms (such as chlorine, high or low pH, toxic materials), the condition must be corrected an healthy active organisms added. This process is known as seeding.

The appropriate pretreatment steps (dechlorination, pH adjustment, acclimation, etc.) should be performed prior to preparation of the sample dilutions for this test.

Preparation of Seed Material:

Select a material to be used for seeding which will have a BOD of at least 180 mg/L. This will help ensure that the seed correction meets the 0.6 mg/L minimum specified in "Standard Methods". Commercially available seed is used by FTMSA. As an alternative, domestic sewage sludge placed in a suitable container and incubated at 20°C for 24-36 hours can be used if it has sufficient BOD. If not, small quantities of digester supernatant can be used to increase the potency of the seed material used for the test. The seed correction should not exceed 1.0 mg/L BOD, therefore care should be taken not to use too strong a seed material. The key to a good seed correction is a relatively stable seed material which produces a good seed correction in every test situation.

Using the commercially available seed, add between 2 and 5 ml to each BOD bottle excluding the blank. The volume of seed add to each BOD bottle needs to be consistent for that batch. The seed correction will be the same for all bottles containing seed in that batch.

Seed Correction:

Set up 4 BOD bottles, add a consistent volume of seed (2-5 ml), fill the bottle to capacity, stopper and cap.

Seed correction = $\sum$(initial seed DO - final seed DO) ÷ 4

in other words,

take the initial seed DO and subtract out the final seed DO for each seed bottle, then take the average.

This average will be subtracted out from each seeded BOD sample.

Determination of Sample BOD:

The calculated seed correction is subtracted from the DO depletion in the determination of the BOD from each valid sample dilution. It should be noted that there are two criteria specified in "Standard Methods which should be checked before the seed correction is used to determine the sample BOD. Those sample dilutions meeting these criteria should yield the most valid results. These criteria are as follows:

1. The sample dilutions should deplete at least 2.0 mg/L of DO after five days incubation at 20°C.

2. The sample dilutions should have a final DO of at least 1.0 mg/L after five days incubation at 20°C.

The BOD, using the seed correction, should be calculated for the sample dilutions which meet bot criteria. If more than one sample dilution meets the criteria, the final BOD should be an average of the individual BOD results for the sample dilutions. If none of the sample dilutions meet both of the criteria, the one dilution which comes closest should be used to calculate the final BOD of the sample.

If this is the case, a notation should be made on the sample bench sheet that potentially invalid data has been used to determine the noted value. Sample dilution volumes should be carefully selected to ensure that at least one dilution meets both criteria.

BOD mg/L = (DO depletion - Seed correction) x 300 ÷ ml of sample

QA/QC:

A batch shall include a blank, duplicate sample, laboratory control sample (LCS) and glucose-glutamic acid check.

Results of duplicate samples should not differ by more than 10%.

Dilution water blanks should not show a depletion of more than 0.2 mg/L DO.

Nitrification inhibitor is used in all BOD bottles in a batch except in the blank since the Authority is reporting CBOD.

Six ml of a glucose-glutamic acid solutions in a 300 ml BOD bottle should yield 198 ±30.5 mg/L BOD after five days incubation at 20°C. This solution must be seeded since the solution does not contain any microorganisms.

While precision is sometimes though with the BOD test, a check of dilution water quality, seed effectiveness, and analytical technique can be made using a glucose-glutamic acid solution. A 2% dilution (6 ml per 300 ml BOD bottle) should yield 198 ± 30.5 mg/L BOD, after five days incubation at 20°C. To ensure valid results for this “Standard” check, the glucose-glutamic acid dilutions must be seeded since the solution is essentially sterile and does not contain any microorganisms.

CBOD result are almost always lower that BOD results. For a highly nitrified effluent sample, the difference can be as great as 50%.

Refer to the Quality Manual for control limits.

Chlorine (Cl2) Residual (*Standard Methods 18th ed. 4500-Cl G. DPD Colorimetric Method.)*

The chlorine residual test is used to determine the total amount of chlorine present as a residual (the amount of chlorine present after the demand has been satisfied). Too much chlorine can kill the aquatic life in the receiving waters. This method is applicable for the analysis of wastewater as outlined in the Federal Register 40 CFR Part 136.

This method requires compensation for color and turbidity by using color and turbidity blanks.

Sampling:

Chlorine in water solutions is not stable. As a result, its concentration in samples decreases rapidly. Exposure to sunlight or other strong light, air, or agitation will further reduce the quantity of chlorine present in solutions. Samples to be analyzed for chlorine cannot be stored or preserved. Test must be started immediately after sampling. Therefore, samples taken for the chlorine residual test must be grab samples only and excessive agitation must be avoided.

Sample Containers:

It is not necessary to use special sample containers for the chlorine residual test. An appropriate container would be a BOD bottle or plastic bottle of appropriate volume.

All sample collection containers should be thoroughly cleaned on a regular basis with soap and water and rinsed well with distilled water.

Sensitivity:

This method has a minimum detectable concentration of 0.010 mg/L under ideal conditions. The Authority is currently running a minimum detectable concentration of 0.04 mg/L.

Apparatus:

Spectrophotometer, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.

Glassware: Spectrophotometer cells, 100 ml beakers and various sizes of pipets, graduated

cylinders and volumetric flasks.

Reagents:

Phosphate buffer: Dissolve 24 g anhydrous Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 ml distilled water in which 800 mg disodium ethylenediamime tetraacetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water.

N, N-Diethyl-p-phenylenediamine (DPD) indicator solution: Dissolve 1 g DPD oxalate, or 1.5 g DPD sulfate pentahydrate, or 1.1 g anhydrous DPD sulfate in reagent grade water containing 8 ml 1 + 3 H_2SO_4 and 200 mg EDTA. Make up to 1 liter, store in a brown glass-stoppered bottle in the dark, and discard when discolored.

Note: The buffer and indicator sulfate are available commercially as a combined reagent in stable powder form.

Chlorine solutions: Prepare standards in the range of 0.04 to 4.0 mg/L from 1000 mg/L potassium permanganate solution ($KMnO_4$).

Stock chlorine solution: Dissolve 891 mg of $KMnO_4$ into 500 ml of reagent grade water and dilute to one liter. This solution has a concentration of 1000 ppm

10 ppm chlorine solution: 10 mls of stock (1000 ppm) chlorine solutions and dilute to 1 liter.

Working chlorine solutions:

Prepare the following working standards using a volumetric pipet and volumetric flasks:

mls of 10 ppm standard	total volume (mls)	standard concentration (ppm)
1	250	0.04
1	200	0.05
1	100	0.10
2	100	0.20
5	100	0.50
10	100	1.00
20	100	2.00

30	100	3.00
40	100	4.00

Prepare the following calibration verification standards using a volumetric pipet and volumetric flasks:

mls of 10 ppm standard	total volume (mls)	standard concentration (ppm)
2	100	0.20
35	100	3.5

A 0.2 ppm and 3.5 ppm standards are used as a continuing calibration verification (CCV). CCVs are to be alternated between high and low. Alternatively, use a CCV at the lower 20% of the curve and a CCV at the upper 20% of the curve.

Calculations:

(Concentration of A) x (Volume of A) = (Concentration of B) x (Volume of B)

Example analysis run:

Blank
0.04 ppm standard
0.05 ppm standard
0.10 ppm standard
0.20 ppm standard
0.50 ppm standard
1.0 ppm standard
2.0 ppm standard
3.0 ppm standard
4.0 ppm standard
LCS
Blank
CCV (0.20 ppm)
Effluent sample
Effluent sample duplicate
Effluent sample spike
Effluent sample duplicate spike
CCV (3.5)
Blank

Procedure:

Warm up spectrophotometer for 10 minutes. Set at 515 nm wavelength.
Zero spectrophotometer with distilled water according to manufacture's directions located on the instrument itself.
Set spectrophotometer to the Absorbance reading.
Make up standards in the concentration range starting at 0.04 ppm to 4.0 ppm. Use a minimum of 5 different concentration for the curve.
Run the standard curve starting with the lowest concentration (blank) through the highest.
Calculate correlation coefficient, r value. This value should be equal to or greater than 0.9990
Take 100 mls of sample, add 5 mls of both phosphate buffer solution, 5 mls of DPD reagent and mix. The buffer and indicator sulfate are available commercially as a combined reagent in stable powder form and can be used in place of the above.
Read absorbance and record on data sheet.
Read absorbance for each sample without any reagents added.
Subtract out the absorbance without reagent from the absorbance with reagent. This will be the absorbance to use to find the concentration from the curve. This is a correction for turbidity and color.
From calibration curve find the concentration (plot %T vs A)
Report anything under 0.04 mg/L as ND (non-detected).
Any sample over the highest standard concentration need to be diluted with reagent grade water and rerun. Multiply the concentration by the amount of the dilution.
Run duplicates, spikes, CCVs and blanks every ten samples.
Run a CCV and blank at the end of the run.

Quality Control:
Refer to page 31 of the Laboratory Quality Manual.
Control limits are ±3 times the standard deviation for CCVs.
Control limits for laboratory control samples (LCS) are set by the manufacture. Refer to their documentation for limits.
Duplicate samples with values beyond 3.27 times the average value are considered "Not Acceptable".
Spike concentrations (refer to page 30 of the Laboratory Quality Manual).

Corrective Actions:

Refer to page 31 of the Quality Manual.
Data outside the limits is considered "Not Acceptable" and the analyst is to flag the data , check for errors and take corrective action.
QA/QC results that are more than ± 2 times the standard deviation but less than ±3 times are acceptable but the analyst should check for error.

Formula:

Absorbance with reagents - Absorbance without reagents = Corrected Absorbance

Fecal Coliforms, (*Standard Methods 18th ed. 9222 D. Membrane Filter Technique)*

Fecal coliform bacteria are non-disease causing organisms which are found in the intestinal tract of all warm-blooded animals. Each discharge of body wastes contains large amounts of these organisms. The presence of the fecal coliform bacteria in a stream or lake indicates the presence of human or animal wastes. The number of fecal coliform bacteria present is a good indicator of the amount of pollution present in the water.

Most waterborne disease-causing organisms originate in human or animal bodies and are discharged as part of body wastes. Due to the relatively small numbers of disease-causing organism, it is very difficult to isolate and identify specific disease-causing bacteria. Since fecal coliform bacteria originate in the same location, they are used as an indicator of possible disease hazards in a body of water. The presence of very few fecal coliform bacteria would indicate that a water source probably contains no disease-producing organisms, while the presence of large numbers of fecal coliform bacteria could indicate a very high probability that the water source could contain disease-producing organisms.

Both the membrane filter (MF) procedure and the multiple tube fermentation (MPN) are US EPA approve procedures for the analysis of wastewater.

Reagents:

Reagent grade water

All chemicals used mused be ACS reagent grade or equivalent.

M-FC media is purchased commercially prepared through Fisher Scientific or other sources.

Stock buffer solution: Dissolve 34 g of potassium dihydrogen phosphate (KH2PO4) in 500 ml of distilled water. Adjust the pH to 7.2 with 1 N sodium hydroxide (NaOH). Dilute to 1 L with distilled water.

Magnesium chloride solution: Dissolve 38 g of magnesium chloride (MgCl2) in 1 L of distilled water.

Dilution water: Add 1.25 ml of stock buffer solution and 5.0 ml of magnesium chloride solution to l liter of distilled water. Dispense buffered dilution water into the dilution bottle and autoclave at 121°C for 15 minutes at 15 psi.

Sodium thiosulfate (1 N), Dissolve 2.5 g $Na_2S_2O_3$ in 100 ml distilled water.

Apparatus:

Culture dishes; disposable, sterile, plastic, tight-fitting with absorbent pads, 0.47 mm, Millipore PD10047S5 or equivalent

Petri pads; 0.45 um, 47mm, Millipore HAWG0475S3 or equivalent.

Filtration units: A filter-holding assembly constructed of a glass consists of a seamless funnel fastened to a base by a locking device or held into place by a magnetic force. This design should permit the membrane filter to be held securely on the porous plate of the receptacle without mechanical damage and allow all fluid to pass through the membrane during filtration.

Filtering flask, 250 ml

Vacuum pump and a water aspirator for back-up.

Incubator: water bath or heat-sink incubator with a temperature tolerance of 44.5 ± 0.2°C. A thermometer will also be needed for temperature monitoring.

Alcohol torch with methanol.

Forceps, smoothed tiped

Autoclave for sterilization of filtration apparatus.

Graduated cylinder, 100 ml

Pipet, 10 ml volume

Sample bottles; autoclavable glass or plastic bottles of 100 ml volume or greater.

Microscope with light source, to determine colony counts on membrane filters. Magnification of 10x to 15x.

Preparation of Glassware:

All glassware used for bacteriological testing must be thoroughly cleaned using a suitable

detergent and hot water. The glassware should be rinsed with hot water to remove all traces of residual from the detergent and finally rinsed with distilled water.

It is recommended to use a detergent that is certified to meet bacteriological standards, or at a minimum, rinse all glassware after washing with 2 tap water rinses followed by 5 distilled water rinses.

Autoclave Sterilization:

Sample bottles, dilution water, glassware and filtration units are sterilized by autoclaving at 121°C for 15 minutes at 15 psi. The caps or stoppers of glassware sterilized in the autoclave should be partially loosened to prevent pressure buildup inside the containers.

Sampling:

Samples are grab samples collected in bottles as described above. Add 0.1 ml of sodium thiosulfate solution to the bottles prior to autoclaving for samples that contain chlorine. Samples are to be representative of the flow for that day in which they are taken.

Samples are to be analyzed immediately after collection. If testing cannot be started within one hour of sampling, the sample should be iced or refrigerated at 4°C.

The maximum recommended holding time for fecal coliform samples from wastewater is 6 hours. If storage is necessary, the temperature and holding time need to be recorded as part of the test data.

Procedure:

Assemble the filtration unit. The filtration unit should be sterile at the start of each filtration series.

Light the alcohol torch and sterilize the forceps by flaming before each use.

Place a membrane filter on the filtration unit using the forceps.

Apply vacuum to 100 ml of distilled water and filter the entire volume through the membrane filter. (blank).

Remove membrane filter using the sterilized forceps and carefully place onto a petri pad containing 2 ml of MFC broth (w/rosolic acid) using a rolling motion to avoid catching air bubbles under the filter. Label the top of each petri dish with sample identification and sample volume.

Continue procedure with samples. Each sample will need to be analyzed at 3 different volumes: 10 ml, 50 ml & 100 ml. or volumes in a similar range depending on the sample. *All volumes are analyze in duplicate.* The sample size and/or necessary serial dilution should be selected to grow 20-60 fecal coliform colonies after incubation.

Rinse the funnel assembly and membrane filter with three 20-30 ml portions of sterile buffered dilution water. Allow the entire volume of each portion to pass through the filter before adding the next portion.

Place the petri dishes in the incubator for a period of 24 ± 2 hrs. @ 44.5°C.

At the end of the incubation period, remove the petri dishes and count the blue colored colonies on the surface of the filter.

Counting Colonies:

Upon completion of the incubation period, the surface of the filter will have growths of both fecal and non-fecal coliform bacterial colonies. The rosolic acid present in the MFC media will normally reduce the number of non-fecal coliform colonies to a minimum. The fecal coliform colonies will appear blue in color, while the non-fecal coliform colonies with appear gray or cream colored.

When counting the colonies, the entire surface of the filter should be scanned using a 10x - 15x binocular, wide field dissecting microscope. Colonies may be counted by scanning across one row and back across the next, etc. This should ensure that all areas of the filter are observed.

The desired range of colonies for the most valid fecal coliform determination is 20 to 60 colonies per filter. If multiple sample dilutions are used for the test, counts for each filter should be recorded on the laboratory data sheet.

Filters which show a growth over the entire surface of the filter with no individually identifiable colonies should be recorded as TNTC (too numerous to count).

Calculation of Colonies:

Report the fecal coliform density as the number of colonies per 100 mls of sample.

For samples with one or more volumes with colony counts in the range of 20 to 60 colonies, the correct daily average calculation is as follows:

Arithmetically average only the samples with colony counts in the acceptable (20 to 60) range.

Example:

Volume	Colony count
10 mls	20
25 mls	51
100 mls	211

Calculate the colony count per 100 ml for each sample in the acceptable range using the following formula:

Colonies/100 ml = (100 ml x colony count) ÷ volume used.

10 ml = (100 x 20) ÷ 10 = 200 colonies

25 ml = (100 x 51) ÷ 25 = 204 colonies

100 ml = (100 x 211) ÷ 100 = 211 colonies

Reject the 100 ml sample since the count is greater than 60 colonies.

Average the results.

(200 + 204) ÷ 2 = 202 colonies per 100 ml.

For samples with colony counts for all volumes less than 20 and greater than zero, the correct daily average calculation is as follows:

Add up the total number of colonies on all plates, multiply by 100 and divide by the total volume used. Calculate the colony count per 100 ml for that sample.

Example:

Volume (ml)	Colony count
10	2
50	9
100	19

The total colony count would be 30 and the total volume would be the 160 ml volume. The result

would be (30 x 100)/160 = 19 colonies per 100 ml.

For samples with all colony counts greater than 60, but still countable (non-TNTC), the calculation is as follows:

Select the count from the smallest volume filtered and calculate the colony count per 100 ml for that sample.

Volume (ml)	Colony count
10	62
50	256
100	393

10 ml = (100 x 62) ÷ 10 = greater than 620 colonies per 100 ml.

The result is reported as "greater than" because all counts were greater than 60. Greater than values are to be avoided by analyzing multiple dilutions. If such results are obtained more than twice a month, the number of dilutions routinely analyzed must be increased. The DMR data associated with these results must be flagged with a statement that includes the number of "greater than" or '"TNTC" occurrences and what corrective measures have been performed to avoid such results in the future. These results must be included in the monthly average (geometric mean) without the "greater than" sign.

QA/QC:

All samples and volumes are ran in duplicate.
Blanks should be run each batch to serve as a sterility check.

Records of positive and negative culture control tests will be established and contain the following information for media:

1. date,
2. media lot number,
3. type of media,
4. identification of positive culture organism (E. Coli),
5. identification of negative culture organism (Enterobacter aerogenes),
6. results,
7. initials of analyst.

The laboratory will verify and record the pH for each manufacture's certified lot of medium to

ensure that it is correct.

Fecal Coliforms (Biosolids) in A-1 Medium (SM 9221 E.)

Introduction:

To qualify as a Class A sludge, FTMSA's sewage sludge is thermally treated (pasteurized) through our TTSS system. The Class A biosolids are monitored for fecal coliform and must have a density of less than 1,000 MPN fecal coliform per gram of total solids on a dry weight basis. The total solids content for each sample must be determined in accordance with procedure SM 2540 G.

Preparation of Glassware:

All glassware used for bacteriological testing must be thoroughly cleaned using a suitable detergent and hot water. The glassware should be rinsed with hot water to remove all traces of residual from the detergent and finally rinsed with distilled water.

It is recommended to use a detergent that is certified to meet bacteriological standards, or at a minimum, rinse all glassware after washing with 2 tap water rinses followed by 5 distilled water rinses.

Autoclave Sterilization:

Sample bottles, dilution water, glassware and mixing units are sterilized by autoclaving at 121°C for 15 minutes at 15 psi. The caps or stoppers of glassware sterilized in the autoclave should be partially loosened to prevent pressure buildup inside the containers.

Sampling:

Reagents:

Reagent grade water

A-1 Medium: purchased commercially prepared through Fisher Scientific or other sources. Mix 31.5 grams of A-1 Medium with 1 liter of reagent grade water, adjust to pH 6.9 ± 0.1 and autoclave at 121°C for 10 minutes at 15 psi. Store in the dark at room temperature for no longer than 7 days. Ignore formation of precipitate.

Stock buffer solution: Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water. Adjust the pH to 7.2 with 1 N sodium hydroxide (NaOH). Dilute to 1 L with distilled water.

Magnesium chloride solution: Dissolve 38 g of magnesium chloride ($MgCl_2$) in 1 L of distilled water.

Dilution water: Add 1.25 ml of stock buffer solution and 5.0 ml of magnesium chloride solution to l liter of distilled water. Dispense buffered dilution water into the dilution bottle and autoclave at 121°C for 15 minutes at 15 psi.

All chemicals used mused be ACS reagent grade or equivalent.

Apparatus:

Blender

Volumetric flask, 250 ml

Incubator: water bath or heat-sink incubator with a temperature tolerance of 44.5 ± 0.2°C. A thermometer will also be needed for temperature monitoring.

Autoclave for sterilization

Graduated cylinder, 100 ml

Pipet, 1 and 10 ml volumes

Sample bottles; autoclavable glass or plastic bottles of 100 ml volume or greater.

Equipment:

Warm Air Incubator

Water Bath

Procedure:

Add 10 mls of A-1 medium to the test tubes containing the smaller inverted tubes.

Put on stainless caps.
Autoclave for 15 min. at 121 degrees.
Let slowly cool to remove air pockets from the inverted tubes.

Collect composite biosolids sample.
Record time, sample location(s), depth(s) which samples were taken, person collecting sample.
Run % solid (SM2540 G).

Weigh out 50.0 ± 0.1 grams of well mixed composite biosolids sample in a sterile dish. Transfer the entire sample to the sterile blender and add 450 mls of sterile buffered dilution water. Cover the blender and turn on high speed for 2 minutes. 1 ml contains 0.10 grams of the original sample (a 1:10 dilution). Neutralize to a pH of 7.0-7.5 by adding 1.0 N hydrochloric acid or 1.0 N sodium hydroxide, if necessary.

Note: Do not suspend bacteria in dilution water for more than 30 minutes at room temperature.

Make serial dilutions:

1:100	10 ml of biosolid sample into 90 ml of dilution water.
1:1,000	10 ml of 1:10 into 90 ml of dilution water.
1:10,000	10 ml of 1:100 into 90 ml of dilution water.
1:100,000	10 ml of 1:1,000 into 90 ml of dilution water.

Use an influent wastewater sample for a positive control.
Use Enterobacter aerogenes (ATCC # 13048) as a negative control. Mix 0.01g into 100ml reagent grade water.

Add 1 ml of each dilution into 5 separate test tubes including the sample mixed in the blender.
Add 1 ml of influent sample into 5 separate test tubes.
Add 1 ml of Enterobacter aerogenes into 5 separate test tubes.
Add 1 ml dilution water into 5 separate test tubes as a blank.

NOTE: Use a 2x broth for the 1:10 dilution. Take 6.3 g of A-1 Medium, add 100 ml reagent grade water and dissolve.

Mix each tube on vibrating stir plate.

Incubate for 3 hours at 35 ± 0.5°C in warm air incubator and record time in and out. Then transfer tubes to the water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hrs.

Interpretation:

Gas production in inverted tube equates to a positive reaction indicating coliforms of fecal origin.
No gas bubbles equates to a negative reaction.

Count the number of positive and negative tubes for each dilution. Record.

Calculation:

The estimated density of fecal coliform bacteria is calculated in terms of most probable number (MPN). Calculate MPN from the number of positive A-1 medium tubes. MPN/g total solids (dry weight) is calculated in 3 steps:

8. Selection of significant dilutions
9. Calculation of MPN/ml (wet weight)
10. Conversion to MPN/g total solids (dry weight)

Selection of Significant Dilutions:

Only three of the four dilutions will be used to estimate the MPN. Select the three significant dilutions according to the following criteria:

1. Chose the most dilute giving positive results in all five tubes inoculated and the two succeeding more dilute dilutions.

2. If the least dilute tested has less than 5 positive tubes, select it and the two next succeeding higher dilutions.

3. When a positive result occurs in a more dilute sample that the three significant dilutions selected according to the rules above, change the selection to the least dilute that has less than 5 positive results and the next 2 more dilute samples.

4. When the selection rules above have left unselected more dilute samples with positive results, add those more diluted positive results tot the results for the highest selected dilution.

5. If there were not enough more diluted samples tested to select 3 dilutions, then select the next lower dilution.

Calculate MPN/ml (wet weight):

$$\text{MPN/ml} = \frac{\text{MPN}}{\text{Largest volume tested in the dilution series used for MPN determination}}$$

*Refer to Table 4 of EPA Method 1681 for MPN Index

Convert to MPN/g total solids (dry weight):

$$\text{MPN/g (dry weight)} = \frac{\text{MPN/ml (wet weight)}}{\text{\% total solids expressed as a decimal}}$$

Quality Control

Records of positive and negative culture control tests will be established and contain the following information for media:

6. date,
7. media lot number,
8. type of media,
9. identification of positive culture organism (E. Coli),
10. identification of negative culture organism (Enterobacter aerogenes),
11. results,
12. initials of analyst.

The laboratory will verify and record the pH of the medium.

% Total Solids (based on Standard Methods 2540 G)

Introduction:

This method is being used to calculate the % solids of the sludge being process in the treatment plant. A crucible is weighed out and recorded. Balance is tarred, 25 - 50 grams of sludge in weighed out, recorded and dried in an oven at 103 - 105°C overnight. The sample is cooled to room temperature the next day, weighed and recorded. A calculation between the wet and dry weights yields the percent solids contained in the sample.

Apparatus:

Porcelain Evaporating Dishes
Desiccator
Analytical Balance
Drying Oven

Procedure:

Weight out a evaporating dish from the desiccator and record the weight.
Tare the balance and weight out 25 - 50 grams of sludge. Record the weight.
Place into the drying over overnight.
The next day, remove from the drying oven and place into the desiccator to cool.
Record the times and dates the sample(s) was placed in and out of the drying oven.
After cooling, weight the evaporating dish and record the weight.

Calculation:

% total solids = (A - B) x 100 / C

A = dried dish and sludge weight
B = dish weight
C = wet sludge weight

QC

Run samples in duplicate.
Refer to the Quality Manual for duplicate control limits.