Common Deficiencies Found in the Wastewater Treatment Plant Laboratory

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This handout has been prepared based on deficiencies found while performing Performance Audit Inspections (PAI) at wastewater treatment plants for the Tennessee Department of Environment and Conservation in the Division of Water Resources. Suggestions have been made in an effort to assist the operator of a wastewater treatment plant in performing his laboratory duties thus improving the self-monitoring program of the facility.

Every wastewater treatment plant has its own distinctive characteristics and needs. Many of the observations and suggestions provided may not apply to every facility.

Quality assurance and quality control requirements can be found in the 40CFR part 136.7 (promulgated May, 2012), method update rule (MUR). Guidance documents may be found on the Fleming Training Center web page. http://www.tn.gov/environment/article/wr-ftc-waste-water-information

The following are problems associated with records and reports:

1. Failure to keep permits and records at the wastewater treatment plant.
   (NPDES Permit Part 1, 2.1.3 & 1.2.5)
   All permits and records should be kept at the wastewater treatment plant. In most cases this is a requirement in the permits.

2. Failure to keep records for three years. **(NPDES Permit Part 1, 1.2.5)**
   All records and information resulting from the monitoring activities required by the permits are to be retained for a minimum of three (3) years, or longer, if requested by the Division of Water Resources. This includes all worksheets and scrap pieces of paper where calculations for analyses are performed and calibration and maintenance of instrumentation.

3. Uncertainty of what to do with records past the three (3)-year period.
   If this Division has not given instructions to retain them past the three (3)-year period then, the facility can either destroy or archive the records.

4. Inadequate or no worksheets on which to record data. Refer to permit.
   (NPDES Permit Part 1, 1.2.4)

5. Illegible and unorganized data recorded on worksheets.
   (NPDES Permit Part 1, 2.1.3)
   All work should be written in a manner where inspectors are able to interpret the data. Only the EPA approved methods may be used to perform the required analyses.

6. Failure to document calibration and maintenance of equipment.
   (NPDES Permit Part 1, 1.2.5)
   Documentation of calibration and maintenance of equipment should also be maintained.

7. Failure to monitor parameters at the required frequencies. **(NPDES Permit Part 1, 1.2.2)**
   Parameters should be monitored at their required frequencies (according to the permit). Analyses should not be locked into specific days during the week. These days should be changed to ascertain any potential problems that may occur during an entire week.

8. Inability to understand what should be recorded on Monthly Operational Reports (MORs).
   All columns on an MOR should be filled out to the best of the operator's ability.
   (NPDES Permit Part 1, 1.3.1)

9. Uncertainty of whether a computer-generated MOR is permissible or not.
   Should an operator want to submit his data on computer-generated MORs, he should use the same format as the State-provided MORs. The operator should provide both the MOR and the computer-generated MOR to the field office for approval.
   (NPDES Permit Part 1, 1.3.1)

   The initial documentation, even if hand-written, becomes the permanent record and must be maintained. Storage of data and other information generated on computer is acceptable but a very rigorous tracking system with passwords and control level for data must be in place. Also, all records must be backed up on routine schedule and second disc or tape stored at a secure second location. These all have to be in place before electronic data is acceptable.

10. Failure to fill out DMRs correctly.
    The Discharge Monitoring Reports (DMRs) are not being filled out correctly, due to a misleading feature of the pre-printed DMR form. The headings near the top of the sheet labeled. **(NPDES Permit Part 1, 1.3.1)**

| AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS |

    These have caused some misunderstanding as to the information required. The sampling intervals and units typed in the stippled gray portion to the right of each parameter listed should be followed, even where there is a conflict with the pre-printed heading. For example, the entry for "Solids, Total Suspended, Effluent Gross Value", in the fourth column to the right, the monthly average value should be entered because "MO AVG." is typed in the stippled gray area. (The pre-printed column heading "MINIMUM" should be ignored.)

11. Failure to calculate weekly averages correctly.
    Weekly averages should be reported by averaging the data in each week (Sunday through Saturday) of the month, then reporting the maximum value of those averages. If the week is not complete by the end of one month, then that week's data should be reported with the month in which the week ends.

12. Failure to submit DMRs and MORs in a timely manner.

Discharge Monitoring Reports (DMRs) and Monthly Operational Reports (MORs) frequently arrive late. DMRs should be postmarked no later than 15 days after the

completion of the reporting period. (MORs) are to be submitted, in the field office, by the 15th day of the month following data collection. **(NPDES Permit Part 1, 1.3.1)**

13. Failure to have a Quality Control program. **(NPDES Permit Part 1, 1.2.3)**

A Quality Control (QC) program should be initiated by each facility. An acceptable QC program would incorporate both quality control (precise and accurate data, e.g. duplicates) and quality assurance (QA) (How closely the data agrees. Statistics are commonly used.). Blanks are used to indicate contamination, duplicates measure precision, and spike samples measure accuracy. Duplicates should be analyzed in accordance with the method of analyses and 40CFR part 136.7 (May, 2012).

Also, there is a QA section at the beginning of each part of Standard Methods for the Examination of Water and Wastewater (SM) e.g.1020, 2020, 3020, 4020, 5020, 6020, etc.

14. Failure to have an SOP. **(NPDES Permit Part 1, 2.1.4 and 40CFR part 136.7)**

Each facility should also have its own Standard Operations Procedure (SOP). An SOP would provide what is typically done at the laboratory and how operations are accomplished. This would include collection of samples to the actual method used for each laboratory analyses. An SOP should be available upon request and provide reference to EPA approved methods of analyses (e.g. SM and Hach methods).

15. Use of correction fluid to cover transcription errors.

It is unacceptable to use correction fluid to correct record keeping errors. Errors should be crossed out with a single line, initialed and the correct information entered.

THE FOLLOWING ARE PROBLEMS ASSOCIATED WITH THE LABORATORY.

General Laboratory Considerations

1. Failure to follow approved laboratory procedures. **(NPDES Permit Part 1, 1.2.3)**

The **40 *CFR* Part 136** provides the approved test procedures for the analysis of pollutants under the **Clean Water Act.**

Other equivalent, acceptable and approved laboratory procedures are available (e.g. Hach Company products. See their EPA compliant methods

for WW (wastewater) on the Hach website http://www.hach.com/epa). Any QA/QC must meet the requirements set forth in the 40CFR part 136.7. Not complying will make the self-monitoring data questionable and in some cases invalid; thus, resulting in violations.

2. Improperly marking containers.

   If samples from more than one wastewater treatment plant are analyzed, containers should be marked distinctly to avoid any confusion between samples.

3. Using expired chemicals in analyses.

   Chemicals should not be used beyond the expiration date. Doing so will place sample data in jeopardy of not being legally defensible. Purchase according to need. It won't be a bargain if the reagents expire before they are opened.

   Uncertainty of when reagents have expired.

   Mark the date received on the bottles and/or contact the manufacturer regarding expiration dates for the reagents.

4. Uncertainty on whether to purchase prepared reagents or prepare in-house reagents.

   Some reagents such as sodium thiosulfate, 10%, used for preservation and chlorine removal for bacteriological sample collection, can be prepared at the plant with minimal cost. Although prepared reagents tend to be costly they also can save valuable time.

5. Failure to properly label in-house prepared reagents.

   The in-house prepared reagents should be labeled with date, time, initials, and possible health hazards. A log of the reagents prepared should be kept. They should also be in compliance with OSHA regulations 29 CFR 1910.1200(f)(1)

6. Storing food and drink in ovens or refrigerators where samples are kept.

   Food and drink should not be stored in refrigerators or ovens that also contain chemicals and wastewater samples. Chemicals should be kept away from foods, as it is an unnecessary safety hazard. Some chemicals can be absorbed in food.

7. Failure to document dates, times and initials for collection and analyses. **(NPDES Permit Part 1, 1.2.4)**

   The dates and times that the analyses were performed should be documented as should the name of the person who collected and analyzed the samples. This is especially important when transporting samples from one facility to another for analysis. The method of analyses should also be documented. This can either be referenced to the SOP or written directly on the daily worksheet. If the method can be found in SM, the method number should be followed by the SM committee's approval date. This can be found in the footnote in introductory section A of the method (e.g. 4500-NH_3 D-1997).

8. Failure to adequately clean glassware.

   Glassware should be washed thoroughly in a tub of soapy water (or dish washer) using phosphate-free detergent (preferably lab grade), rinsed well with tap water and a final rinse with distilled water. Just rinsing the glassware is not considered cleaning.
   Think … representative sample for the lab 24 hours.

9. Failure to maintain a log for equipment. **(NPDES Permit Part 1, 2.1.5)**

   A log should also be kept to record the temperatures of the ovens, water bath and incubator. It's advantageous to place the log near the equipment being monitored, but can also be recorded on the daily worksheet. This log should include the date, time, temperature and the initials of whomever is doing the checking.

The following are problems frequently found with the <u>Settleable Solids (SS)</u> analysis. (SM 2540 F-1997)

Failure to follow EPA approved methodology.

Too frequently, deviation of the SS methodology has been found. The appropriate methodology for SS requires that the sample be shaken well and be poured into an Imhoff cone; be allowed to settle for 45 minutes, and then very slowly stirred (one revolution) to dislodge material adhering to the sides. Do NOT stir fast. The solids in the sample should then be allowed to settle for another 15 minutes then be read to the nearest 0.1 mL/L mark.

Laboratories typically have either the glass or the plastic cones. The lowest marking on the glass cone is the 0.1 mL/L mark. The plastic cones have a screw cap at the tip and don't provide a reliable a reading that low. If the lowest marking is the 0.5 mL/L and the solids level is below this, the one would report <0.5 mL/L. You can't record < 0.1 mL/L if the lowest mark is 0.5 mL/L. If you happen to have a glass cone and a plastic cone, use the glass for the final and the plastic for the raw. Otherwise, you must properly record what you see.

The following are problems frequently found with <u>Dissolved Oxygen (DO)</u> analysis. (SM 4500-O G-2001 and Hach method 10360 for LDO)

1. Failure to collect DO sample properly.

   When collecting the sample be sure to collect the sample with a BOD bottle using the stopper. Fill the BOD bottle to near the top and then stopper immediately to avoid any further contact with the air. This assures that no air bubbles are caught between the stopper and the sample.

2. Failure to properly calibrate the DO meter.

   Air Calibration for the meter is acceptable by the EPA. If the air calibration method is used, the membranes must be kept fresh. Change the membrane at a minimum of once per

month. If air bubbles are visible under the membrane, the membrane must be changed. Follow manufacturer instructions for proper maintenance and calibration.

Winkler method is also acceptable by the EPA and is considered the better of the two methods used to calibrate the DO meter. Three bottles should be filled with distilled water. The first and third bottles should be titrated as required by the EPA approved method to determine the DO. The second bottle is used to adjust the setting on the meter to the averaged DO value found by titration of the other two bottles.

3. Failure to standardize the titrant.

   If the titrant, 0.0375 N sodium thiosulfate is used in the Winkler method, then it must be standardized daily according to the approved methodology.

   Another option is to titrate with a 0.03750N phenylarsine oxide (PAO) solution. This can be purchased pre-standardized. PAO solutions are stable. No further standardization would be necessary.

   Even the luminescent dissolved oxygen (LDO) probe must be calibrated properly. The cover for the probe typically replaced yearly.

   Currently, the barometric pressure and room temperature should be recorded at the same time that the DO meter is calibrated. The standard (or theoretical) DO value obtained from these readings should also be recorded. This can be found on the USGS website. http://water.usgs.gov/software/DOTABLES/ . A table can be tailored to one's specific needs on this website. This standard value and the value used to set the meter must lie within 10% of each other

4. Failure to store DO probes properly.

   DO probes can be stored in a BOD bottle containing at least 1 inch of water. Refer to the operations manual for the DO or LDO probe and meter or contact the YSI service center for assistance regarding proper maintenance of the equipment. Keep this bottle clean.

The following are problems frequently found with the Biochemical Oxygen Demand/Carbonaceous Biochemical Oxygen Demand (BOD/CBOD) analysis (SM 5210 B-2001)

1. Uncertainty of when to seed the sample. (5210B.4.d, 5.d. & 6.d.)

   If an effluent composite sample is collected prior to disinfection, no seeding is required for BOD. However, seeding is required for the CBOD method since the nitrification inhibitor must be added to the sample.

   Seeding is required for both influent and effluent samples for CBOD analysis. "*Comparing Apples to Apples*" as the saying goes.

If an effluent composite sample is collected following disinfection, the sample must be checked for any residual chlorine (if not UV disinfected) prior to setting up the CBOD samples. The samples then must be seeded.

2. Failure to adequately dechlorinate a sample. (5210B.4.b.2.)

   Just allowing the samples to sit for a short period of time in order to dissipate any residual chlorine is not as effective as chemical de-chlorination. Besides, the samples must be set up and analyzed within two hours of collection. Chemical dechlorination also eliminates the chlorine in a shorter period of time. Failure to properly dechlorinate would result in artificially low results.

3. Failure to allow the samples to come to room temperature before making dilutions. (5210B.4.b.4.)

   Since composite samples are kept at $6^{o}C$, or below, the samples should be warmed to $20 \pm 3^{o}C$ (comparable to $68^{o}F$) before making dilutions. This may be accomplished by allowing the samples to come to room temperature (approximately $68^{o}F$) or by setting the sample bottles in a warm water bath.

4. Initial DO values do not meet the method criteria. (5210B.8.b.)

   Samples with initial DO's that are greater than 9.0 mg/l at $20^{o}C$ (e.g. stream samples) are considered supersaturated with oxygen. These samples may be vigorously shaken or aerated with clean, compressed air to bring down to saturation, less than or equal to 9.0 mg/l. (5210 B 4.b.4.) The samples should be brought to room temperature and the initial DO $\leq$9.0 mg/L before setting up dilutions and analyzed within two hours of collection.

5. Introducing contamination into the sample.

   Cross-contamination, which can produce inaccurately high results, can be avoided by properly rinsing the graduated cylinders between measuring sample volumes. Specific graduated cylinders can be delegated for each influent, effluent and stream sample. Clean, Clean, Clean!!!

6. Failure to adequately stir/shake the sample prior to making dilutions.

   Sample should be stirred or shaken thoroughly to obtain a representative sample.

7. Failure to set up the appropriate amount of dilutions. 5210B.5.c.

   At least three dilutions should be set up for both influent and effluent samples appropriate QA/QC measures.

8. Uncertainty of the purpose of the glucose-glutamic acid (GGA) check. 5210B.6.b.

   To check the quality of the seed material and analytical technique, it is recommended that a glucose-glutamic acid check be set up with each set of samples. A typical value of 198 mg/L ± 30.5 mg/L has been found for the GGA concentration. 164 ± 30.7 mg/L (nitrification inhibitor added) is recommended for GGA concentration in CBOD analysis.

A low value could be indicative of a toxic affect caused from trace metals in the dilution water (e.g. copper). It could also indicate that the seed material concentration is weak (relatively inactive).

A high value could be indicative of several factors e.g. contamination from improper rinsing of glassware after cleansing, imprecise measurement of standards, or too much seed material added to GGA.

Should the measurement fall outside the previously given range, the tests should be reported and flagged as to the findings. The problem should then be investigated in order to determine and eliminate the source.

9. Failure to follow the BOD criteria for reporting results. 5210B.6.a, 7. and 8.b.

   Follow the BOD criteria for reporting the results. EPA approved methodology states that the initial DO must be less than or equal to 9.0 mg/l. The samples must deplete at least 2.0 mg/L DO and must leave 1.0 mg/L final DO. Results from dilutions that do not meet these criteria are considered invalid and should be discarded.

   The blank oxygen depletion should be 0.2 mg/L or less as required by EPA approved methodology. Otherwise, an investigation should be initiated to determine the cause. Check for soap residue due to improper rinsing. The results still can be reported but must be marked that the blank is outside the guidelines. The blank oxygen depletion is NOT subtracted from the sample depletion. (5210 B. 7.a.3.)

   Be wary of the existence of toxicity trending.

10. Uncertainty of which nitrification inhibitor to use.

    There are two nitrification inhibitors available for use in the CBOD analysis method, **2-Chloro-6 (Trichloromethyl) Pyridine** and **Nitrification Inhibitor, Formula 2533™**. The biggest difference between the two is that Formula 2533™ is more soluble than the other, thereby yielding better results.

    The dispenser cap for the nitrification inhibitor is well worth the money.

11. Failure to properly monitor the temperature of composite samplers. (40CFR part 136, table II)

    The temperature of the composite samplers are not generally monitored or maintained at the required temperature of $\leq 6^{o}C$. During the collection of composite samples, the temperature of the samplers must maintain temperatures at $\leq 6^{o}C$ or below (but above freezing $0^{o}C$).

12. Failure to properly monitor the temperature of the BOD incubator. (5210 B. 5.h.)

    The BOD incubator temperature must be maintained at $20^{o} \pm 1^{o}C$. A log should be kept for each piece of equipment to record the date, time, temperature, and the initials of whoever was checking it. It is just as easy to record the temperature on the daily worksheet.

    Check and record the temperature using a *calibrated* thermometer placed in water inside the incubator. Do not use the digital thermometer found on top of the incubator.

The following are problems frequently found with the <u>pH</u> analysis.
(SM 4500-H B.-2000, revised 2011)

1. Failure to compensate for temperature.

   Since pH is temperature dependent, an automatic temperature compensator (ATC) probe must be use or the temperature must be measured and manually set on the instrument. This temperature of the samples should be recorded.

2. Failure to properly store the pH electrode.

   Store electrodes according to manufacturer instructions! Orion states using pH 7 buffer with 1 gram of KCl (potassium chloride).

3. Using expired pH standards.

   Do not use expired pH standards. Plan to buy what can be used before the expiration date.

4. Failure to properly calibrate the meter.

   A two-point calibration is a required. A two-point calibration brackets the normal pH range found at the plant. It provides a line on which the best possible data point for the sample can be found. If the sample falls outside the calibrated range, the instrument should be recalibrated.

   If the probe that you use has the capability of performing a 3-point calibration, follow manufacturer procedures. This is a great way to ensure that the samples will be bracketed by the calibration buffers.

5. Failure to use fresh standard solutions for calibration of the pH meter.

   Prepare fresh standard solutions for each daily use for calibration. If stock bottles are used, be sure to cap the bottle after pouring out an amount used for standardizing.
   Never pour used reagent back into the stock bottle. This is a big source of contamination. This method could jeopardize the integrity of the sample, which is directly related to the calibration of the meter.

6. Improper calibration procedure used in calibrating the pH meter.

   Start by adjusting the intercept knob with a pH 7 buffer (the isopotential point). The slope or calibrate function on the meter should be set with a second buffer. This buffer depends

on the desired range, either above pH 7 (e.g. pH 10) for the higher pH range, or below pH 7 (e.g. pH 4) for the lower pH range.

There are pH meters that provide a 3-point calibration allowing the use of pH buffers 7, 4 and 10. Follow the manufacturer directions.

7. Failure to rinse the electrode prior to reading the sample.
   Be sure to rinse the electrode well after standardizing the meter and prior to reading the pH of the sample. Pat the probe dry with a soft cloth or tissue to remove the liquid.
   This minimizes cross-contamination.

8. Failure to stir the sample.
   Stirring the sample at a rate of about one revolution/second is necessary in obtaining a quick and accurate reading.

9. Failure to run the sample immediately after collection.
   Don't collect the pH sample until the meter has been calibrated. The analysis of the samples should be run immediately. 40CFR part 136, table II states that the holding time is within 15 minutes of collection.

10. Failure to have a backup probe.
    An extra pH probe should be available in the event that the current probe malfunctions.

11. Uncertainty of how to maintain an electrode.
    The electrolyte should be added to the new electrode, through a filler hole in the side, when it is ready for use. After the electrolyte has been added, it should not be used for at least one hour. Be sure to rinse off any excess electrolyte and pat dry the electrode.
    Do not rub the electrode vigorously because it could create a static charge in the probe. This will assure that none of the electrolyte is introduced into the sample.

    Be sure to take the filler-hole cap off when taking readings for quicker response times. This cap may be replaced when not in use to prevent evaporation of the electrolyte.

The following are problems frequently found with the <u>Total Suspended Solids (TSS)</u> analysis. (SM 2540 D-1997)

1. Failure to adequately shake the sample.
   The biggest problem with the TSS is that operators are not shaking the sample well enough prior to analysis. This is necessary to obtain a representative sample.

2. Uncertainty of why results are too **high**. (When comparing results to another lab's results.)
   Frequently, sample volumes are too high for the amount of solids in the sample. This requires longer filtration periods for elimination of the liquid. Prolonged filtration times

   may produce high results due to excessive solids on the clogged filter. By reducing the

sample size, more satisfactory results should be obtained.

A constant weight must be demonstrated prior to reporting the results. This is to ensure that all the moisture has been eliminated. If this is not accomplished then erroneously high results may be obtained. The re-drying, cooling, desiccating and weighing cycle is

required unless shown otherwise. The weight change must be less than 4% of the previous weight or 0.5 mg, whichever is less. (2540 D.3.c.)

The drying oven should be kept clean to minimize contamination of samples.

3. Uncertainty of why results are too **low**. (When comparing results to another lab's results.)
   Solids can be lost under the filter paper when pouring in the sample. Wetting the filter paper with distilled water prior to pouring the sample will lessen the possibility of the solids being lost under the filter rather than collected on the filter.

   Filtered samples should not be placed directly on the oven rack. The filters could stick to the rack and lose fibers, which could change the weight of the sample. They could also gain contaminants or spill the filter contents. Aluminum weighing dishes are recommended.

4. Lack of good laboratory technique.
   Do not use fingers to pick up crucible or filter. The oil on your fingers adds weight. Use tongs instead.

5. Uncertainty of when to change the desiccant.
   Make sure that the color indicator desiccant is changed when the color turns to pink.

   An option would be to obtain the non-color indicator and mix it with the color indicator desiccant. This would incorporate cost with performance.

6. Failure to properly monitor the temperature of the drying oven.
   The oven should be maintained at a temperature between 103 - 105^{o}C at all times.

7. Failure to perform quality control procedures (SM 2020I and II tables)
   A duplicate should be performed once every 20th sample.

   A distilled water blank should be analyzed with every 20th sample for quality assurance.

The following are problems frequently found with the <u>Total Chlorine Residual</u> analysis. (Hach method 8167)

1. Failure to calibrate the instrument prior to use (or check the calibration for Hach Pocket Colorimeters).

   The Instrument calibration should be checked prior to each use. Chlorine or $KMnO_4$ standards may be used. There are several companies that manufacture pre-made standards. The chlorine standards are typically added to samples that have already been analyzed (standard addition method). See Hach method of analysis.

2. Uncertainty of the proper method to use.

   The permit limit must be considered when deciding the appropriate method to use. The low limits presently being given require the lowest possible detection limits that a method can provide. DPD, electrode and amperometric methods are currently the methods that provide the lowest detection limits available. Current permits state the following:

   > *"Total residual chlorine (TRC) monitoring shall be applicable when chlorine, bromine, or any other oxidants are added. The acceptable methods for analysis of TRC are any methods specified in Title 40 CFR, Part 136 as amended. The method detection level (MDL) for TRC shall not exceed 0.05 mg/l unless the permittee demonstrates that its MDL is higher. The permittee shall retain the documentation that justifies the higher MDL and have it available for review upon request. In cases where the permit limit is less that the MDL, the reporting of TRC at less than the MDL shall be interpreted to constitute compliance with the permit."*

   Iron, manganese, nitrates and organic mercaptans can interfere with DPD readings giving a false positive for chlorine. Some facilities are unable to use the colorimeter due to these interferences.

   The amperometric method requires greater skill than the colorimetric method. However, less interference is found.

3. Uncertainty of what the lowest reportable value should be.

   The amperometric method requires greater skill than the colorimetric method but there are fewer problems with these interferences.

   The current instrument detection limit is 0.05 mg/L. Many permit limits lie below this value. Many operators have been reporting <0.1 mg/L. This was the old detection limit and should not be used anymore. If the value in the permit limit is within the capability of the instrument, it is preferable that the operators report the actual value that is obtained.

   However, MUR now requires that the method detection limit (MDL) must now be determined by the laboratory. However, follow permit requirements until the permit language changes.

The following are problems frequently found with the <u>Ammonia as Nitrogen (NH_3-N)</u> analysis. (SM 4500-NH_3 – 1997)

1. Failure to properly preserve samples. (4500-NH_3 A.3.)

If samples are to be analyzed with within 24 hours of collection, refrigerate until removed for analysis. If the analysis cannot be performed within this time period, the samples must be preserved by acidifying to pH and store refrigerated to $\leq 6^{o}C$ for 28 days (40CFR part 136, table II).

Oh, don't forget that this table can also provide guidance in which to store samples should a problem arises at the facility and the analysis cannot be performed.

2. Failure to adequately shake the sample.
   Thoroughly shaking the sample is necessary to obtain a more representative sampling.

3. Failure to distill samples.
   The analytical method should be consulted regarding the need for distillation. If this method is unclear, distillation is required. Distillation is required to remove impurities that would interfere with the analyses and produce erroneous results. (40CFR part 136 table 1B, footnote 6) It requires distillation for all methods unless comparability has been shown. "Manual Distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies."
   Comparable is defined as <20 RPD (relative percent difference). Since the likelihood of the distilled and undistilled samples will meet this requirement, it recommended that a sample be distilled and compared with the undistilled sample, once per year.

   If distillation is required, guidance can be found in the 40CFR part 136 table 1B, footnote 6.

   Did you say Ammonia-free water? Hopefully, someone has found something out there to assist in this matter. Personally, I've had difficulty coming up with something. The EPA 350.2 method provides guidance

   EPA Method 350.2
   ……
   6.0 Reagents
   6.1 Distilled water should be free of ammonia. Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.
   NOTE 1: All solutions must be made with ammonia-free water.

   Remember, not only does the air that we breathe have oxygen; it also has nitrogen which is a component of ammonia. So, when you distill the water minimize the exposure to the air as it is distilled off.

4. Failure to properly calibrate the instrument.

Instrument calibration is required prior to running the analysis. At least two standards should be used to bracket the sample. If the sample falls outside this range, then the sample can be diluted or new standards prepared.

The following are problems frequently found with ***Fecal Coliform* analysis. Some of this information may also apply to the *E.coli* analyses (Hach m-coli Blue method of analyses - comparable to EPA 1603)**

1. Failure to use the approved method.

   Only use approved methods outlined in the 40CFR part 136 table 1A. Don't get this confused with total Coliform.

2. Failure to use a microscope with the appropriate magnification.

   A microscope with magnification of 10X to 15X is required to obtain a valid colony count. This magnification is necessary to determine the presence of small colonies in a sample that might otherwise be missed.

3. Failure to monitor the temperature of the water bath.

   Maintain the temperature of the water bath at a uniform and constant temperature of $44.5 \pm 0.2^{o}C$ at all times. *E.coli* incubator temperature should be maintained at $35 \pm 0.5^{o}C$.

   The thermometer must be graduated in 1/10 of ^{o}C which is needed for the $\pm 0.2\ ^{o}C$ and **kept** clean**.**

4. Failure to follow proper sterilization procedures.

   0.1 mL of a 10% sodium thiosulfate solution should be added to each sample bottle prior to sterilization. Do not use the preservative N-10 (0.1005-0.0995) sodium thiosulfate. This is potentially harmful to the fecal coliform culture since this is stronger than the 10% (approximately 0.03N) solution stated in the EPA approved procedures.

   The sterilization procedure should be done for 15 - 20 minutes at $121^{o}C$ ($250^{o}F$).

   If you have to use a pressure cooker as an autoclave, do not close the petcock until steam comes out of the ports. When the sterilization period is complete, turn steam supply off; allow glassware to slowly cool before removing. The pressure cooker is only to be used as a **temporary** method of sterilization. The Autoclave is preferable.

5. Failure to properly collect the sample.

   While collecting the sample in the sterilized bottle, care must be taken to eliminate other sources of contamination (e.g. unsterilized dippers used to pour the sample into the sterilized sample bottles).

6. Uncertainty whether to use distilled water in place of buffered dilution water.

Do **NOT** use distilled water in place of the buffered dilution water. EPA approved methodology requires the use of buffered dilution water in the procedures. The method shows you how to prepare this.

7. Failure to prepare the appropriate number of dilutions.

   Many operators are preparing only one dilution. Prepare three dilutions of the sample in order to obtain a 20 - 60-colony count. Do the same to obtain 20 - 80-colony counts for *E.coli*.

8. Performing analysis with faulty equipment.

   Avoid using a leaking filter apparatus. This could give erroneously low results.

9. Failure to perform quality control ("Quick Reference for *E.coli* Analysis) http://www.tn.gov/assets/entities/environment/attachments/wwt_quick_reference_e_coli.pdf

   It is recommended that a positive control sample be set up at least once per month as a quality control check (e.g. 1 mL of effluent prior to chlorination).

   Perform duplicate samples at least once every 10 samples.

10. Failure to perform analyses due to high flow conditions.

    Sampling during high flow events occasionally has been avoided by the operator due to frequently obtaining TNTC (Too Numerous To Count) data. (This is a violation of the permit since representative sampling is required at a specified frequency.) At several plants this occurrence is the norm rather than the exception. Operators who have worked at these plants for a number of years can probably guess as to the appropriate dilutions that could be used to obtain 20 - 60 colony counts. It's better to indicate what actually happens at the plants rather than indicate no problems. These problems would lend proof that there is a need for plant renovations.

11. Failure to report data correctly.

    There are specific guidelines that should be followed when reporting data. TNTC is not to be reported on the MOR. These guidelines are provided upon request or may be found in ***EPA Microbiological Methods for Monitoring the Environment Water and Wastes,*** EPA-600/8-78-017, December 1978.

The following are problems frequently found with the *E.coli* analysis.

1. Failure to use the methods approved for wastewater.

   In the March 2007 40 CFR part 136 the Colilert, Colilert 18 and mColiBlue 24 were approved for *E. coli* analyses. Well, there are others listed as well. But, these particular methods have fought long and hard for "approval".

2. Failure to use appropriate temperature.
   Follow instructions. Don't assume *E. coli* can be used at the same temperature as fecal coliform or visa versa.

3. Failure to count colonies in the appropriate range.
   The appropriate colony count range for Hach's m-Coli Blue method is 20 - 80.
   You are counting the blue colonies only and not the total colonies.

4. The "Quick Reference Guide to *E.coli* Analysis" can be found on the FTC website.
   http://www.tn.gov/environment/article/wr-ftc-waste-water-information

The following are problems frequently found with the IDEXX Colilert analysis.

1. Failure to monitor the incubator temperature at 35 $\pm$ 0.5^{o}C. The thermometer must be graduated in 1/10 of ^{o}C

2. Failure to have a color comparator available to confirm fluorescent blue wells. Yellow wells indicate the presence of total coliforms. The yellow wells that fluoresce under UV light indicate the presence of *Ecoli.*

3. Failure to have the correct Quanta Tray available for use in expected high count flows.
   Trays come in 51-well and 97-well sizes. The 51-well tray allows MPN counts up to 200 per 100 mL of sample. The 97-well tray allows MPN for up to 2419 per 100 mL of sample.

4. Failure to properly dispose of used Quanti trays.
   Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

5. Failure to use **sterile water** for sample dilution.
   Do not use the sterile dilution water that is used in the fecal coliform or m-coli Blue analyses.

6. Failure to perform quality assurance controls.
   Purchase Quanti-Cult control samples (*Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* cultures) to check the quality control of the method.