HELP! I'M BEING HELD CAPTIVE IN A WASTEWATER TREATMENT PLANT LABORATORY!!

A HELPFUL GUIDE FOR IN-SERVICE WASTEWATER ANALYSTS

1994 EDITION

BY

CHARLES FREIDLINE, Ph.D.

U. S. ENVIRONMENTAL PROTECTION AGENCY

REGION VII
KANSAS CITY, KANSAS 66115

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CONTENTS

I. INTRODUCTION

In the course of doing laboratory inspections of permittees and contract laboratories throughout Environmental Protection Agency (EPA), Region VII, I have noticed that there are some technique errors which occur again and again in the laboratories visited. These errors significantly affect the result of analyses reported on Discharge Monitoring Reports. Many of you that I have visited have expressed a desire for help. You feel trapped with no easy way to find out what your problems are, much less how to solve them. This paper offers help with some of the most common errors and points of confusion, hopefully in simple terms.

For certain topics a somewhat more detailed discussion is given in later sections. Since about 90 percent of permittees are doing only analyses for DO, BOD_5, total suspended solids, pH, and ammonia nitrogen, specific comments here mostly concern these parameters. However, many of the techniques discussed apply to other analyses too, including even the preparation of samples and standards for analysis of metals by atomic absorption spectrophotometry or inductively coupled atomic plasma emission.

Please take the time to thoroughly read this guide, asking yourself, "Am I doing these procedures correctly?" If you find you have some of the problems listed here, you are not alone, as these are common problems. Please work on the solutions to any problems you may have so that you can have renewed confidence in your results.

You should pay close attention to Section III and start now to set up a program of systematic checks on the reliability of your analyses.

In the 13 years since I wrote the first edition of this booklet, the same year EPA started the DMR-QA Studies, the quality of analyses in Region VII have improved dramatically. This is a tribute to your capabilities and cooperation!

II. SOLUTIONS TO THE MOST COMMON LABORATORY PROBLEMS IN REGION VII (AND ANY OTHER REGION)

A. BEING SURE

PROBLEM: Not being sure your analyses are correct.

SOLUTION: Set up a regular program to be sure your analytical results are correct. Being sure (quality assurance) is discussed in section III of this "Guide".

B. RECORDING AND SAVING DATA

PROBLEM: Inadequate recording of primary data and associated information such as the time the sample was collected and time the analysis was run.

SOLUTION: Prepare a bench sheet(s) (see Appendix B for an example) for your facility and use it rigorously, recording all the information. It is particularly important to record such data as:

1. Weight of the filter pad or crucible empty and after the solids are collected, and volume filtered (TSS).

2. Initial and final dissolved oxygen values, volume of sample used (BOD).

3. Values for any control samples used for quality assurance (making sure).

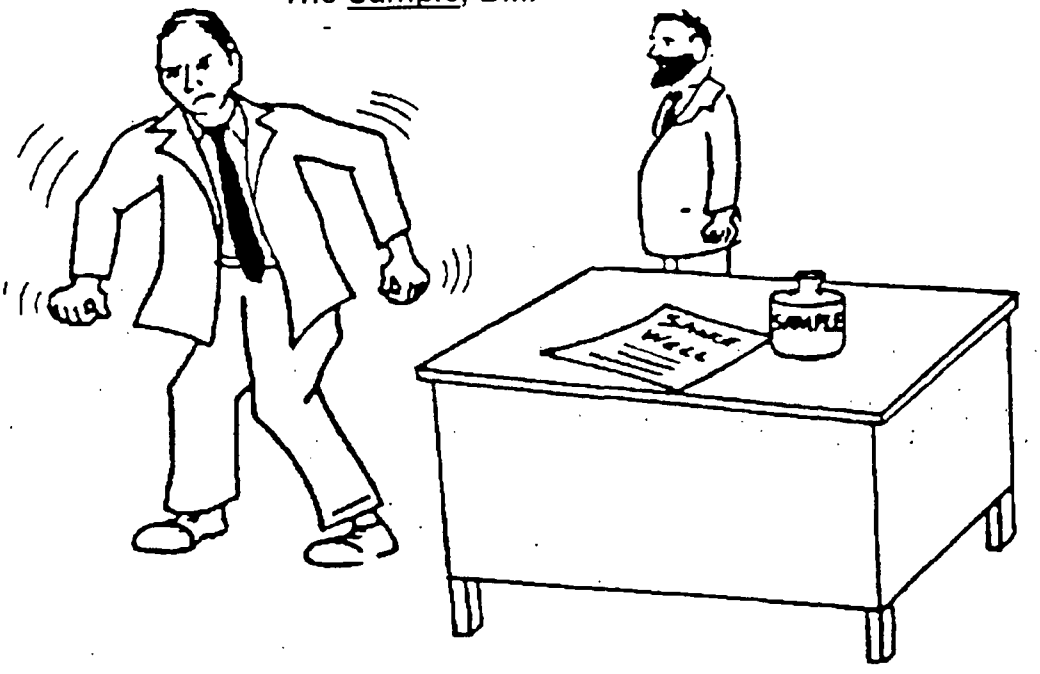
A sample bench sheet will be found in Appendix B. If more room is needed, each type of analysis can be expanded to a full page, or multiple pages can be used. Obviously, similar bench sheets can be constructed for ANY analysis. If control samples are used, they can be given a permanent place on the bench sheet. Method references should be to the method YOU are using. In the example, the most recent Edition of Standard Methods (18th) is referenced, and the page and procedure numbers are somewhat different from earlier editions.

C. SAMPLE HANDLING

PROBLEM: Not shaking a sample adequately just before measuring out a part of your sample (EXTREMELY important for suspended solids and BOD analyses).

SOLUTION: Get into the habit of vigorously shaking or stirring the sample immediately before taking a portion for analysis. This is <u>critical</u> for suspended solids analysis because settling of the solids can cause gross errors. The suspended solids sample must be measured out rapidly immediately after vigorously shaking or stirring to prevent settling. Shaking is also very important for the sample taken for BOD analysis and valuable for most other analyses <u>EXCEPT</u> dissolved oxygen. When pipetting is needed, continuously stir the sample with a magnetic stirrer or the pipet after shaking.

I Think It Means To Shake
The Sample, Bill!



D. ANALYTICAL BALANCE TECHNIQUE

(1) **PROBLEM:** Balance does not have adequate sensitivity for the job.

SOLUTION: Suspended solids and Oil and Grease analyses require a sensitivity and accuracy of 0.0001 grams (0.1 mg) for the normal size samples. Buy the proper balance!

(2) **PROBLEM:** Not routinely checking the balance zero.

SOLUTION: Make it a habit to check the balance zero before each series of weighings. Balance zero can change rapidly with changes in room temperatures, or if the balance is moved or bumped. Balance zero is also a check to be sure the balance is working properly. If it will not zero, check to see that weight dials and vernier are at zero, the balance level (make sure the bubble is in the center of the bubble level) and the weighing pan clean. If your balance has a taring feature, be sure you have really explored the entire range of the zero knob. If it still will not zero, the balance repairman should be called.

(3) **PROBLEM:** Weighing chamber not clean

SOLUTION: Chemicals should be weighed only in solid containers, such as beakers. A filter pad should be supported by a planchet (aluminum dish) or other container. A camel's

hair brush can be used to clean up any solids spilled in the balance. The pan can be removed and the chamber washed and dried using paper towels, if needed. Dirt or chemicals in the weighing chamber can cause contamination and apparent weight changes of your sample as well as corrosion of your very expensive balance.

(4) PROBLEM: Dirty Pan

SOLUTION: Loose solids can be brushed off with a camel's hair brush. If necessary, the pan can be removed, washed, rinsed with distilled water and dried thoroughly. Prevent future problems by practicing good weighing techniques [see (3)]. Dirt on a pan is a serious problem and can cause erratic weighings at the milligram level due to dirt falling off or sticking to the weighing container. Be sure any surfaces you lay your weighing container on are dust free.

(5) PROBLEM: Objects to be weighed are handled directly. Small amounts of grease, moisture or salt transferred from your fingers can add up to 0.1 mg quantities.

SOLUTION: Use strips of lintless paper, clean tongs or forceps to handle objects to be weighed on the analytical balance.

E. TOTAL SUSPENDED SOLIDS

(1) PROBLEM: Filter discs not prewashed to remove soluble impurities and lose fibers.

SOLUTION: The discs should be put into Gooch crucibles or into the filter holder and rinsed three times with distilled water, while pulling vacuum. Be careful not to tear the disc while rinsing or transferring.

(2) PROBLEM: Filter discs not completely dried.

SOLUTION: The prewashed discs, and the solids and discs after filtering the sample are to be dried at 103-105°C until two successive drying periods give weights that agree within 0.5 mg. In other words, both the tare and gross weights

must be "constant weights" to ensure that all water is removed.

(3) PROBLEM: Filter discs from a membrane filter holder or Buchner funnel are taken off and put directly on racks in drying oven. (The disc often sticks to the hot racks, and fiber is lost when it is pulled loose, changing the weight. Also, solids are easily lost when the disc is not supported).

SOLUTION: An aluminum weighing dish or Planchet should be weighed with the filter disc and used as a support for it in all operations except the actual filtration. If you are using filtering (Gooch) crucibles, these supply the needed support.

(4) PROBLEM: Incorrect filter disc

SOLUTION: Use a 0.45 μm porosity glass fiber filter disc such as Millipore AP-40, Reeves Angel 934AH, Gelman Type A/E, or equivalent.

CAUTION: RIDDLE

QUESTION: What kind of solids analysis do you do on those Performance Evaluation samples that EPA sends you where they know the right answer?

ANSWER: Totally suspenseful solids!

F. pH MEASUREMENT

(1) PROBLEM: Not standardizing often enough.

SOLUTION: The pH meter should be standardized <u>at least</u> once every day of use. This should be done more often if many samples are run, or considerable time passes between uses. On some instruments the standardize knob is called "calibrate". Check your instrument manual if your instrument is confusing.

(2) PROBLEM: Not standardizing properly with TWO buffers and then using a third buffer as a control check.

SOLUTION: Start by adjusting the standardize knob (called "calibrate" on some instruments) with a pH=7 buffer. Each day of use, the slope or calibrate function (often built into temperature compensator, see your instrument manual) on the meter should also be set using a second buffer (above pH = 7.00 if the samples are likely to fall in that range, or below pH = 7.00 if the samples are more likely to fall in that range). A third buffer (usually about pH=4 or 10) is then used to check the functioning of the meter. If your meter has an automatic temperature compensator probe, it should be adjusted whenever the slope adjustment is made.

The pH meter should now be put in the "read pH" mode and a pH=10.0 buffer should read 9.9 to 10.1 (if you have calibrated with pH=7.0 and pH=4.0 buffers). If yours reads that close, everything is working correctly.

For digital meters, follow manufacturers instructions for "Two-buffer calibration".

(3) PROBLEM: Not rinsing the electrode(s) before each measurement.

SOLUTION: The electrode(s) must be rinsed thoroughly with distilled water (a squirt bottle is useful) before they are inserted into each sample. This is especially critical at the beginning of a series of measurements after the electrodes have been stored in buffer, or calibrated with buffer. Just a <u>drop</u> of buffer solution can greatly alter the pH of an unbuffered sample!

G. PIPETTING ERRORS

(1) PROBLEM: Incorrect use of your pipet.

SOLUTION: There are several types of pipets in common use. The identification and use of each type is given in Section IV. Familiarize yourself with the procedures for the pipets you use.

(2) PROBLEM: Mouth pipetting (or starting siphons with the mouth)

SOLUTION: Use a pipet bulb or filler. Mouth pipetting is a serious safety problem in any laboratory situation due to toxicity, corrosiveness, etc. In the wastewater analysis laboratory it also exposes you to water-borne pathogens that can make you sick. Exposure can occur even if you do not get the <u>liquid</u> in your mouth. An analyst at a treatment plant I recently visited was just recovering from Shigella, a water-borne disease. Generally good hygiene (washing hands, keeping laboratory items and food

separate, not smoking or eating in the laboratory, and using bulbs) is an occupational necessity.

(3) PROBLEM: Dipping pipets into standard solutions.

SOLUTION: The solution should be shaken well and poured into a clean, dry beaker. The pipet can be dipped into the beaker of solution, rinsed three times by pulling a small amount into the pipet and using it to rinse the walls, each time discarding the rinse solution to waste. Then the pipet is filled with standard from the beaker, the liquid meniscus adjusted to the mark, and the liquid dispensed. Remaining standard in the beaker is discarded when all pipetting is finished, **NEVER** returned to the stock bottle. Dipping the pipet into the standard solution can cause both dilution and contamination. These effects are cumulative and can cause significant errors.

(4) PROBLEM: Dipping contaminated pipets into non-standardized stock solutions (such as manganous sulfate, sulfuric acid, etc.)

SOLUTION: In general, it is best never to dip a pipet into the stock bottle of any solution. The same technique suggested for standard solutions can be used, although it is inconvenient. More practical approaches are:

(a) Use separate small dropper bottles with approximate calibration marks on the droppers to dispense the appropriate amounts. These solution volumes do not need to be exact. The dropper bottles are refilled from the stock bottles.

(b) Use separate clean pipets in each stock bottle (less desirable).

(c) Use automatic pipets or burets for each solution (a very expensive approach). The point of each of these techniques is to avoid contamination, especially over a period of time.

For many reagents, "powder pillows" with premeasured amounts of the solids are available. These can solve the contamination problem and have much better shelf lives than solutions. Be sure the solids completely dissolve in the solution.

H. DISSOLVED OXYGEN METER CALIBRATION

(1). PROBLEM: Dissolved oxygen meter not calibrated properly

SOLUTION: The preferred method for calibration is to place the probe in a BOD bottle of aerated nutrient water matched to one standardized using a Winkler titration. The calibration knob is used to set the meter on the value of the oxygen concentration determined by the careful titration.

There are two objections to the air calibration method often used:

(a) It uses different conditions than the sample measurement

(b) It does not in practice agree well with the Winkler method. Air calibration is not forbidden in the EPA methods approvals in 40 CFR 136, but I would encourage you to use the Winkler Method for calibration. If you do use air calibration, it is <u>essential</u> membranes be kept fresh, as the difference between the methods becomes greatest with older membranes.

The "saturated oxygen method" is rarely used because it suffers from the difficulty of achieving true saturation.

(2). PROBLEM: Not matching the pairs of bottles of dissolved oxygen used in the Winkler titration adequately to be certain they are the same.

SOLUTION: Nutrient water is best added through a siphon hose with a glass tube long enough to reach the bottom of the bottle. Fill from the bottom up, slowly lifting the tube while keeping it under water. The procedure is important whether you are just matching two bottles to calibrate a D.O. meter, or if you are doing all your analyses by the Winkler method.

I. AMMONIA-NITROGEN TEST

(1) PROBLEM: The sample is not being pre-distilled

SOLUTION: EPA rules state that wastewater samples for ammonia analysis must be predistilled. This removes many of the impurities present in wastewater and gives a reasonably pure ammonia solution. For distillation, a pH=9.5 borate buffer is added to the sample to release the ammonia. The ammonia (a volatile base) is distilled into boric acid which reacts with the ammonia to make it into the ammonium ion (no longer volatile). The finish of the analysis can be done by any of the following:

(a) Nessler reaction, color readout on colorimeter or spectrophotometer.

(b) Titration of the ammonium borate with acid (acidimetric titration).

(c) Use of the ammonia electrode.

If you can <u>prove</u> by doing the analysis by the Nessler procedure or the ammonia electrode with and without distillation over an extended period of time that you can get the same results under <u>all conditions</u> with your samples, you can do it without distillation. The acidimetric titration <u>always</u> requires distillation. Be sure to keep all your data on hand to prove it to any EPA/State inspector who might ask to see it. If you cannot produce the information showing the distilled values are the same as the non-distilled, it is not legal to skip the distillation step. Domestic wastewater is NOT a good candidate for an exception!

(2) PROBLEM: Using a visual comparometer (color wheel) to read out the Nessler color.

SOLUTION: A spectrophotometer or colorimeter must be used for the readout. These instruments are capable of the necessary wavelength selectivity to avoid including impurities in the measurement. It also avoids the subjective color matching which can cause significant disagreement in concentration between two observers. Color wheels are not acceptable for other wastewater analyses either.

J. SAMPLING

(1) PROBLEM: Not using the sampling method called for in your permit.

SOLUTION: Check your permit for the type of sampling required for each parameter. The most common types of sampling methods are:

(a) 24-hour composite - Samples must be taken at least once every four hours around the clock. One-shift plants with this requirement either

need a man to come out and take night samples or need to obtain an automatic sampler. The best practice for collecting composite samples is to purchase and use an automatic sampler. These make the job easier and give a sample more representative of your discharge.

Generally, night samples will be more likely to be within permit limits than day samples, and it is to your advantage to include them!

(b) Grab samples - single sample taken directly. Note that pH, dissolved oxygen and temperature are always run on a grab sample.

(2) PROBLEM: Not icing or refrigerating samples that are not analyzed immediately, especially composites.

SOLUTION: Preservation of a sample requires that it be kept cold (4°C, 40°F) until analyzed. In winter this is usually automatic for compositors kept outside. When daytime temperatures get above about 50°F (10°C), or water temperatures get above 5°C, it is time to ice or refrigerate your sample.

(3) PROBLEM: Collecting samples at locations that do not give a true picture of your effluent discharge.

SOLUTION: Effluent samples must be obtained at a point representative of the water actually being discharged. Normally this should be just after the effluent has passed through a discharge weir or flume while the water is moving rapidly and is well-mixed. Sampling hoses lying on the bottom of still areas before or after discharge are not likely to represent the true effluent.

WASTEWATER TREATMENT PLANTS HAVE NOW BEEN AROUND FOR MOST OF A CENTURY. I PREDICT THE RISE OF NEW BIRD SPECIES TO FIND THEIR SPECIAL NICHES AROUND WASTEWATER TREATMENT PLANTS. SOME FUTURE "GUIDE TO BIRDS" MAY DESCRIBE THEM THIS WAY:

1. BROWN SLUDGIN: This species is completely brown and individuals are known best for their habit of diving head-first into activated sludge tanks. Some ornithologists, after observing them following a heavy rain, believe their natural color may not really be brown.

2. FILTER FLYCATCHER: Trickling filters have long been known as the habitat of filter flies. These birds have adapted to using filter flies as an almost exclusive diet. To encourage the habits of these useful birds, ecologically minded operators will be sure that any covered filter domes have access holes large enough for them to enter, but small enough to exclude their predators (3-4"in diameter).

III. HOW TO BE SURE YOUR ANALYTICAL RESULTS ARE CORRECT

A. The General Idea

All of us like to know we are right. It is especially important to know and be able to prove you are right when doing analyses that affect your permit compliance status. At present, very few labs have established any routine method of being sure. Words that are frequently used for a program of being sure are:

(1) Quality Assurance is a regular program using the analyses of known amounts of a substance to BE SURE your analyses give good results.

(2) Quality Control is the daily use of proper techniques, equipment calibrations, etc., to ensure good results

A simple detailed quality assurance program geared to small laboratories doing NPDES tests is not easy to find. The following discussion is intended to give you enough detail so you can set up a routine quality assurance program for your laboratory. There are four general procedures that you should start doing regularly for each parameter you analyze:

(a) Run a blank: A blank is usually distilled water carried through all the steps of your analysis just like it was a sample. Clearly, the result should be very close to zero. A significant positive result tells you there is a problem with the water, reagents or technique. Not all parameters have blanks, e.g. pH.

(b) Run at least duplicate (two or more of each) samples on all analyses required by your permit: The analysis of duplicate samples gives an idea of how consistent your results are. The sample is analyzed at least twice. If everything is being done the same way each time, the results should be reasonably close to each other. If you are doing large numbers of samples on the same day, at least one of every ten should be run in duplicate. If your sample load is not large, ALL samples should be run in duplicate.

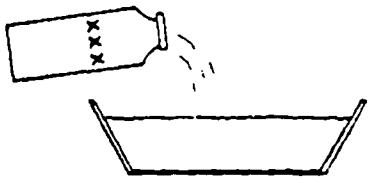
(c) Run a standard (preferably in duplicate): A standard is a carefully measured amount of the material for analysis dissolved in an accurately known volume of solution. This solution is analyzed just like a sample, and the results obtained are compared with the known concentration. Known samples for analysis can be occasionally ordered from EPA. Please limit your requests to one or two sets of samples each year. Call or write:

Michelle Shirley
The Bionetics Corporation
16 Triangle Dr.
Cincinatti, OH 45246

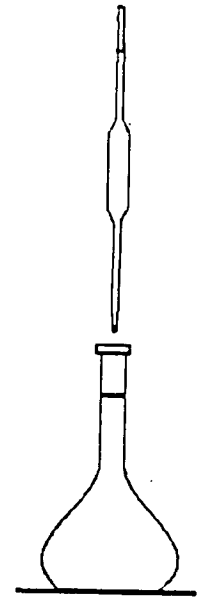
(513) 771-0448

Please specify which analyses you do. Several companies now sell similar standards, and some have routine programs to check your results. These samples are not a substitute for your own program for being sure.

(d) Run a spiked sample along with the sample: Have you heard of "spiking the punch?" Spiking a sample is very similar, except that one carefully measures volume of the spiking standard and the sample so the amount of standard added to the sample is accurately known. Then both the sample and the spiked sample are analyzed. The DIFFERENCE between them should equal the concentration calculated for the spiking standard from the total volume of sample used.



SPIKING THE PUNCH



SPIKING THE SAMPLE

B. HOW IT CAN BE DONE

(1) DISSOLVED OXYGEN (D.O):

(a) Meter and Probe: The D.O. Meter should be calibrated in oxygenated water analyzed for D.O. by the Winkler method. Approximate checks can be made by then:

1 Measuring the oxygen in air and comparing the meter reading with the concentration of oxygen from the manufacturer's tables for the barometric pressure in your laboratory. These will differ some, but will normally fall within about 0.2 - 0.4 ppm (mg/L) from the Winkler standardized readings if your membranes are fresh. See the manufacturer's manual for measurement conditions.

2 Measuring the oxygen in water carefully saturated with oxygen and comparing the readings with the theoretical solubility of oxygen in water at that temperature. This method depends on

reaching complete saturation. A reasonable way to prepare a saturated oxygen in water solution is to take water a few degrees cooler than 20 °C (for example, 15°C). Bubble air through it for at least 15 minutes or shake it in an oversized container for at least two minutes, then let it stand in the 20°C incubator long enough to reach a uniform temperature. The solution will now have too much oxygen in it for the temperature of 20°C. Shake it vigorously for two minutes, releasing the pressure occasionally to remove the excess oxygen. The concentration of oxygen in water at 20°C is given in Standard Methods, 18th Ed., p. 4-101t under method 4500-O-C.

Using the uncorrected barometric pressure (just as it is read off of the barometer), calculate the solubility (see note below table) of oxygen at the temperature of your nutrient water. This concentration can be used to check the D.O. Meter value, and one can also titrate the saturated solution using the Winkler Method. All three values should agree within ± 0.4 mg/L if all the procedures have been done adequately.

(b) Winkler Method: The saturation method (B.1, a.2) just given can be used as an approximate check of the Winkler Method.

It is important that the standard titrant (sodium thiosulfate) be standardized against primary standard potassium biniodate or potassium iodate (See Standard Methods, 18th Ed., method 4500-O-C, p 4-100.). When preserved with sodium hydroxide the sodium thiosulfate solution is fairly stable. It should be re-standardized about once per three months. Phenylarsine oxide (PAO) is more stable, but should be checked occasionally.

CAUTION: ORGANIC ARSENIC COMPOUNDS (such as PAO) HAVE HIGH TOXICITY. WASH HANDS, DISPOSE OF PROPERLY.

ANOTHER BIRD:

3. LAGLOON: (can be pronounced either lag'loon or la gloon') These algae feeders can be see floating gracefully on aerated lagoons, their distinctly green bottom half is believed to be the best identification marking. Their rather eerie call sounds remarkably like the whine of an aeration pump, and ends with a distinct gurgling sound.

(2) BIOCHEMICAL OXYGEN DEMAND (BOD_5)

(a) Introduction: The use of a "standard" mixture of glucose-glutamic acid for a standard check is suggested in Standard Methods, 18th Edition, Method 5210B, page 5-3. It is necessary to seed these standard samples to provide the micro-organisms necessary for the BOD, so the procedure given will include the seeding process.

A simple way to use the "standard" mixture is as a spiking solution in the sample. In this way the sample provides an accurately known seed.

(b) Preparation of Glucose-Glutamic Acid "Standard": Dry some reagent grade glucose and reagent grade glutamic acid for one hour at 103°C. Weigh 150.0 mg (.1500g) of each (use beakers as weighing containers) and transfer them carefully through a funnel into a 1 liter volumetric flask. Rinse the beaker and funnel well with distilled water to get all the solids into the flask. Add two pellets of sodium hydroxide and shake to dissolve. Dilute carefully to the

```
CAUTION:  Sodium hydroxide BURNS the skin!!!
Use gloves or forceps to handle.
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mark. After mixing, transfer to a one-liter bottle (preferably brown) for storage. This solution will keep several months in the refrigerator. (Date it. Remake after one year or when bad results are obtained, whichever comes first.)

(c) Analysis of the Standard: A BOD can be run directly on 5.00 mL (volumetric pipet) of this solution as suggested in Standard Methods, 18th Ed., Method 5210B, page 5-3. Expected results are discussed in BOD Example 1. It is necessary to seed the sample and run a BOD on the seed.

(d) Seeding BOD Samples: Choose as seed a fairly low BOD sample such as river water, unchlorinated effluent from your plant, or filtered raw influent. The point is to choose a seed with plenty of bacteria but not a high BOD value.

Add about 3.0 mL seed* to each 300 mL BOD bottle. An alternative is to add 10.0 mL seed solution for each liter of dilution water and then use it all within 30 minutes after seeding. Make up the dilutions of the standard using the seeded dilution water. The BOD of the solution used as seed should be determined the same way you usually determine BOD on similar samples and a seed blank correction used that is calculated from the BOD of the seed. The calculation section below will illustrate this.

* You may need to use more seed if your values do not agree well with each other. If your seed has too high a BOD, you may need to use less. Generally use about 1/10 of your lowest seed dilution WHICH GIVES A USEABLE DEPLETION (greater than 2 mg/L change in D.O.) to seed.

EXAMPLE: if 5% (15 mL), 10% (30 mL) and 15% (45mL) are useable dilutions to calculate the seed BOD, 1.5 mL (or round to 2 mL) would be a good amount to add to 300 mL of the sample to be seeded, or 6 mL to each liter of nutrient water.

(3) BIOCHEMICAL OXYGEN DEMAND CALCULATIONS FOR SEEDED SAMPLES:

(a) Calculation of the seed blank correction:

$$B = \frac{(BOD_{seed}) \times (V_{seed})}{V_{soln}} = SEED\ \ BLANK\ \ CORRECTION$$

WHERE:

BOD_{seed} = BOD of seed you determined by your usual method

V_{seed} = Volume of seed solution added to sample or nutrient water, mL

V_{soln} = Total volume of sample or nutrient water made up, mL

B = Seed blank correction

(b) Calculation of the BOD of the standard (or any seeded sample):

$$BOD_{std} = \frac{(D_1 - D_2 - B) \times (V_{bottle})}{V_{std}}$$

WHERE:

D_1 = Initial D.O. of diluted sample, mg/L

D_2 = Final D.O. of incubated diluted sample, mg/L

V_{bottle} = Capacity of BOD bottle (or cylinder, if used), mL

V_{std} = Volume of standard (or any seeded sample) added to BOD bottle (or cylinder), mL.

BOD EXAMPLE NUMBER 1

TABLE 1-A: BOD of Your Sample Used For Seed

Seed Solution (Plant Effluent, use your normal BOD Procedure)

V_{sample}	D_1	D_2	D_1-D_2	BOD_{seed}
10	7.6	7.0	0.6	Too little D.O. change[+]
50	7.4	4.8	2.6	15.6
100	7.2	2.3	4.9	14.7

NOTE: VALID SAMPLES MUST DEPLETE AT LEAST 2 mg/L, AND THE FINAL D.O. MUST BE AT LEAST 1 mg/L.

Average seed BOD = 15.1 (Avg. of 15.6 and 14.7).

a. A 2.0 liter (2000 mL)[*] bottle of nutrient water was seeded by adding 20.0 mL of this effluent.

b. A series of three 300 mL BOD bottles were set by adding 5.0 mL of standard glucose-glutamic acid solution to each and filling with this seeded nutrient water

c. The results obtained are tabulated below:

TABLE 1-B: Data and Calculation of the BOD ofthe Standard

V_{std}	D_1	D_2	D_1-D_2	BOD_{std}	Deviation from average
5.0	7.6	3.7	3.9	225	14
5.0	7.5	4.0	3.5	201	10
5.0	7.5	3.9	3.6	207	4

Average BOD = 211 Average Deviation = 9

How the calculations were done:

Seed Blank Correction = B

$$B = \frac{(BOD_{seed}) \times (V_{seed})}{V_{soln}} = \frac{15.1 \times 20.0}{2000} = 0.15$$

For each bottle of standard incubated in this example:

$$BOD_{std} = \frac{(D_1 - D_2 - 0.15) \times (300)}{5.0}$$

(where B = 0.15)

BOD EXAMPLE CALCULATION:

$$BOD_{std1} = \frac{(7.6-3.7-0.15) \times (300)}{5.0} = 225 \text{ (first bottle)}$$

The AVERAGE of the BOD's of the standard is found by adding up all the BOD_{std} values and dividing by the number of values as follows for our EXAMPLE 1:

$$Average\ BOD_{std} = \frac{(225+201+207)}{3} = 211\ mg/L$$

The AVERAGE DEVIATION is calculated by adding up the ABSOLUTE differences (subtract small number from the larger number) between the average BOD and each individual value:

$$AVG\ DEV = \frac{(225-211)+(211-201)+(211-207)}{3} = 9\ mg/L$$

Notice that the average deviation (9 mg/L) in our example is close to the standard deviation (10 mg/L) obtained for a single laboratory long-term study listed in 5210B on page 5-6 in Standard Methods, 18th Edition. While the standard deviation is a better statistical indicator, it is more complicated, but the average deviation gives a reasonable approximation. Also, the average BOD value obtained in this single laboratory study was 204, within 10 of our value (211).

In a large interlaboratory study with all kinds of conditions, the average BOD value and standard deviation was 198 ± 30.5. If your values for the glutamic acid-glucose standard lie within that range, your values are probably o.k.

NOTE TO MATHEMATICIANS AND CHEMISTS: See Appendix A for a discussion of standard deviation. In this example, standard deviation was 12.5 mg/L.

(c) Analysis of Spiked Samples:

Spiked samples have a known amount of the glucose-glutamic acid standard added to a regular BOD sample. The BODs of the sample by itself and the spiked sample are determined, and the DIFFERENCE between them is the BOD of the glucose-glutamic acid standard. This is a good test of your techniques as well as the quality of the seed.

BOD EXAMPLE 2: SPIKING A PLANT SAMPLE

Spiked Sample Results for a Trickling Filter
Plant Effluent with Approximately BOD = 20 mg/L

VOLUME SAMPLE	VOLUME STAND	INIT DO D_1	FIN DO D_2	*ΔDO SAMPLE	ΔDO STD SPIKE	BOD SAMPLE	BOD STD SPIKE
0	0	8.8	8.8	0		0	
30	0	8.7	6.8	1.9		ΔD<2	
45	0	8.5	5.4	3.1		21	
60	0	8.3	4.3	4.0		20	
				ΔDO MIXTURE			
30	3.0	8.7	4.6	4.1	4.1-1.9= 2.2		220
45	3.0	8.5	3.3	5.2	5.2-3.1= 2.1		210
60	3.0	8.3	2.0	6.3	6.3-4.0= 2.3		230

* NOTE: Δ means "difference," or one subtracted from the other.

How the calculations were done:

This example assumes 300 mL BOD bottles. For another size, substitute the actual volume for "300".

$$BOD_{sample} = \frac{300(D_1 - D_2)}{V_{sample}(mL)} = \frac{300(\Delta DO_{sample})}{V_{sample}(mL)}$$

$$BOD_{std} = \frac{300(\Delta DO_{std})}{V_{std}} = \frac{300(\Delta DO_{mix} - \Delta DO_{sample})}{V_{std}}$$

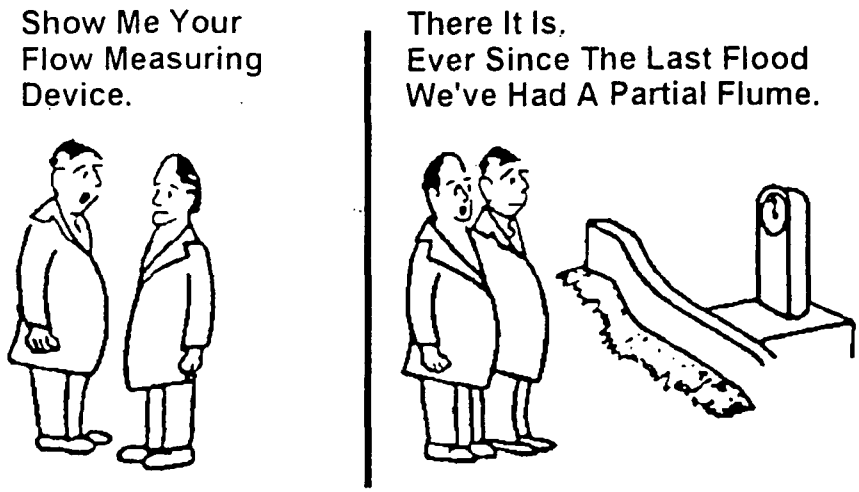
Find the average of the BODs of the standard by adding up all the $BODs_{std}$ and dividing by the number of $BODs_{std}$.

$$Average\ BOD_{std} = \frac{(220+210+230)}{3} = 220\ mg/L$$

As a test of the agreement between your values the average deviation can again be calculated as we did in the previous example:

$$\textit{Average Deviation} = \frac{(220-220)+(220-210)+(230-220)}{3} = 7\ mg/L$$

Notice that our values of 220 mg/L ± 7 adequately agree with the value of 198 ± 30 for the pure spiking standard. In Standard Methods, 14th edition, page 548 a table suggested spiked trickling filter samples would run higher, to about 225, but would give good precision (small standard deviation).



(4) <u>pH MEASUREMENT</u>

The three buffer procedure outlined in the "Common Problems" section is both a calibration and quality assurance procedure. Be sure to **RECORD** the true and measured value of the pH of the third buffer.

Additional buffers can be used as further checks.

(5) <u>TOTAL SUSPENDED SOLIDS-(NON-FILTERABLE RESIDUE)</u>

A perfect standard for the suspended solids in wastewater is difficult to find.

Possible standards must have the following characteristics:

a. Be insoluble in water
b. Not pass through the filter medium
c. Disperse well in water
d. Not be volatile at 105°C
e. Have a density not too much higher than water

f. Be wettable by water

Because of the difficulty in finding and preparing such substances, the use of prepared quality assurance samples from EPA or a commercial standards company like SPEX or Environmental Research Associates (ERA) is recommended. EPA standards can be ordered from:

Michelle Shirley
The Bionetics Corporation
16 Triangle Dr.
Cincinatti, OH 45146

(513) 771-0448

Follow the instructions sent with the samples for preparing the standard solution, and analyze it by your usual procedure for suspended solids. Your results should be within about 10% of the known value included with the sample. Good analyses are generally low; high values suggest problems with the weighings or inadequate drying.

(6) AMMONIA-NITROGEN

(a) General Principles

Ammonium Chloride is an ideal standard for the ammonia-nitrogen test. For accuracy, it is usually best to make a solution more concentrated than desired and dilute it accurately using a volumetric pipet and a volumetric flask. For example, if your samples run in the 1.0 - 10.0 mg/L range, prepare a stock solution of about 100 mg/L nitrogen using ammonium chloride. Dilutions of this solution can then be used both as a standard to run through the entire procedure, and as calibration standards for a spectrophotometer if the Nessler procedure is used. The EPA "Methods For Chemical Analysis of Water and Wastes" (1979) gives directions for a specific stock solution and a series of calibration standards that can be made from it, or you can use the series given in (b):

(b) Preparation of the Standard Solutions

To make up your own series with directly stated concentrations, do the following:

	General Procedure	Specific Example
1.	Decide on stock solution concentration (about 10 times your sample concentration).	If samples run about 5 mg/L Nitrogen, make stock 50 mg/L Nitrogen
2.	Multiply concentration of Nitrogen desired by 3.821 to get the weight of ammonium chloride needed per liter.	Ammonium chloride = 3.821 x 50 mg/L = 191.0 mg/L
3.	Move decimal point three places left to get grams.	191.0 = .1910 g.
4.	Weigh out that many grams of ammonium chloride.	Weigh out .1910 grams ammonium chloride in a beaker on the analytical balance.
5.	Using distilled water in a wash bottle, rinse all the ammonium chloride into a 1.000 liter volumetric flask.	Wash all .1910 grams ammonium chloride from beaker into 1.000 liter volumetric flask
6.	Add enough water to dissolve all the solid. Then dilute to the mark and mix for <u>two minutes</u> by inverting the flask every few seconds.	
7.	This solution is your stock solution. Calculate the <u>exact</u> concentration of nitrogen, C_N, by:	
	$C_{N_{stock}} = \frac{Wt.\ Ammon\ Chlor(mg)}{(3.821)(Vol\ [L])}$	$C_{N_{stock}} = \frac{191.0\ mg}{(3.831)(1.00[L])}$
8.	Solutions for analysis are made by diluting the stock solution using a pipet and volumetric flask.	10.0 mL of the above stock are pipetted into a 100.0 mL volumetric flask, diluted to the mark, and mixed well.
	$C_{N_{std}} = \frac{(V_{stock})(C_{N_{stock}})}{V_{std}}$	$C_{N_{std}} = \frac{(10.0\ mL)(50.0\ mg/mL)}{100.0\ mL} =$
		= 5.00 mg/mL ammonia-nitrogen

(c). Using the Standards

1. As a quality assurance check standard:

The standard is analyzed just like a sample, but you know the real value. Carry a measured amount of standard through the distillation and finish steps just like a sample. Do not forget that the maximum ammonia-nitrogen concentration that can be determined directly by the Nessler Method is 2 mg/L. For greater concentrations, accurate volumetric dilution must be made to bring the concentration into range.

2. To calibrate the spectrophotometer:

A calibration curve can be made for a spectrophotometer by diluting the stock (or an intermediate dilution) to make a range of solutions with ammonia-nitrogen concentrations up to 2 mg/L. These can be directly Nesslerized without distillation.

EXAMPLE: Using the 5.00 mg/L solution prepared in the previous example, the following dilutions can be made using pipets and volumetric flasks:

PREPARATION OF STANDARD SOLUTIONS

VOLUME 5.0 mg/L STOCK, mL	VOLUMETRIC FLASK VOLUME, mL	CONCENTRATION OF STANDARD SOLUTION, mg/L
10	100.0	0.50
20	100.0	1.00
25	100.0	1.25
30	100.0	1.50
40	100.0	2.00

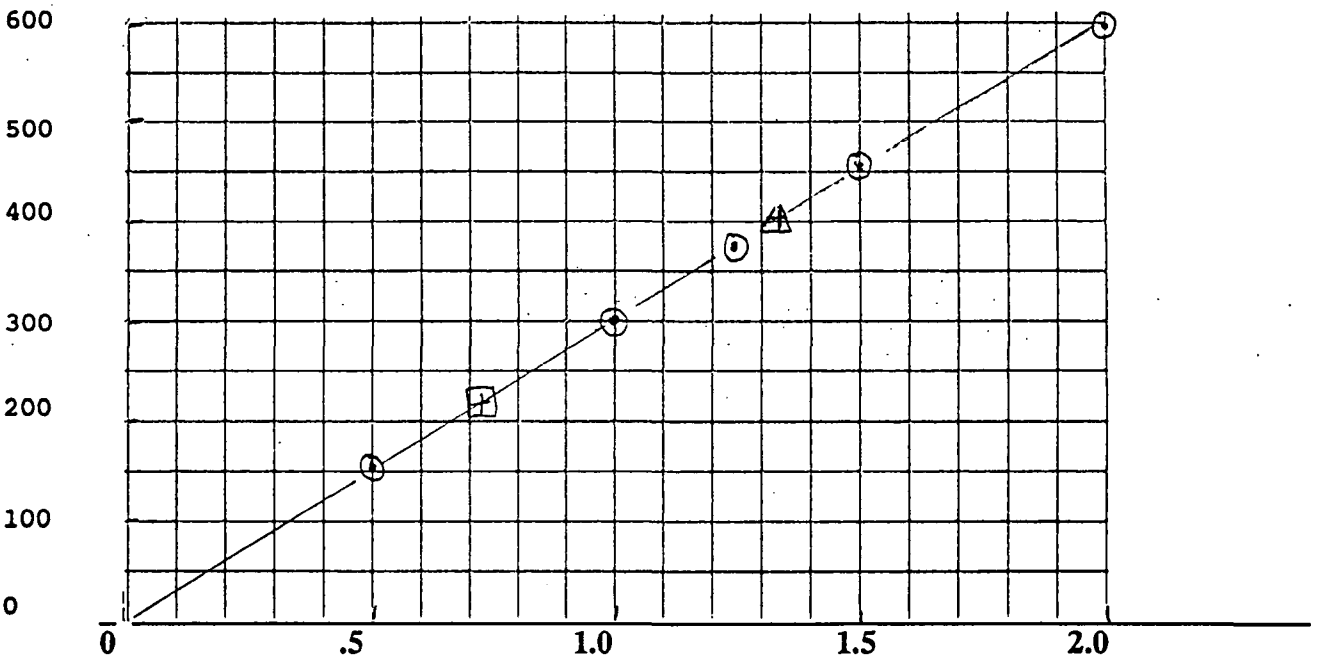
Nesslerize the standards just like you would do for your samples and measure the absorbance of each. Then prepare a graph of absorbance versus concentration of ammonia-nitrogen. The concentration of your unknown samples and quality assurance check standard can then be read off the graph once you have measured their absorbance with your spectrophotometer.

AMMONIA NITROGEN EXAMPLE:

Assume you measured the following absorbances for your Nesslerized standards and your quality assurance check standard and your unknown sample:

CONCENTRATION OF STANDARD	ABSORBANCE AT 425 nm
0.50	0.152
1.00	0.301
1.25	0.373
1.50	0.453
2.00	0.594
QA 1.33 mg/L	0.397
UNKNOWN SAMPLE	0.220

The graph would be made as follows where values for the standards are shown by circles, and the value for the sample is shown by a square. The concentration of the Nesslerized sample would be read as 0.72 mg/L.



Note the QA sample fits the data from the standards even though it was treated like a sample. This is a good indication your method is accurate and working. A SPIKED sample could also be run.

7. OIL AND GREASE

(a) Preparation of a Standard Oil Mixture:

Make a 50-50 mixture by weight of a mineral oil and vegetable oil.

Mineral Oil - from drugstore, or get a
NON-DETERGENT motor oil

Vegetable Oil - such as Wesson Oil

As an example, one could mix 10 grams of mineral oil with 10 grams of the vegetable oil. The weights are not critical for this solution.

Standard Methods, 18th Ed, page 5-26 gives similar mixtures.

(b) Preparation of a Stock Oil in Water Solution

Weigh exactly 1.000 g (1000 mg) of the oil mixture in a 120 mL (4 Oz) or 240 mL (8 oz.) bottle. Pipet 100.0 mL distilled water into the bottle, cap and shake vigorously to form a good emulsion. Each 1.00 mL of this solution supplies 10.0 mg. of oil

$$\frac{1000\ mg}{100\ mL} = 10\ mg/mL$$

You can make the stock solution more concentrated or more dilute by weighing out more or less oil.

For any weight of oil added to any volume of water:

$$\frac{\text{No. of mg Oil}}{\text{No. of mL Water}} = \text{mg/mL of Oil.}$$

(Each mL stock solution pipetted delivers this many mg oil.)

Shake the stock solution VIGOROUSLY just before using.

NOTE TO CHEMISTS: The extra volume of oil is about .80 mL ($density_{oil}$ = about .8) and can be included in the equation above for total volume, but it is really negligible in this analysis (< 1%).

(c) Standard or Spiked Sample for Analysis

To make a standard solution for direct analysis, measure and add 350-800 mL distilled water (amount depends on size of your separatory funnel and normal sample size) to an oil and grease sample bottle. Carefully pipet a definite amount of the stock solution into the collection bottle. If you are using the stock standard from part b), each mL of stock standard will give you 10 mg oil and grease in the volume of water used. For any volume stock standard, 10 mg/mL X V(mL) = mg oil. Analyze this solution and compare the amount you added with the results of your analysis.

For a spiked sample, you measure about 350-800 mL of your effluent instead of distilled water. Pipet the stock standard as before to prepare your spiked sample. Now analyze separately the spiked sample and your pure effluent. The DIFFERENCE between the results should equal the mg. of oil added from the spiking solution.

DESCRIPTIONS OF SOME MORE NEW BIRDS:

4. DITCH DUCK: These water fowl can often be seen riding the agitation brush in an oxidation ditch up into the air and then splashing down on the other side. There is no evidence they feed in the oxidation ditch, and its attraction may be completely recreational!

5. BOTTOMLESS PITABOLINK: These remarkable birds produce no effluent of their own, completely metabolizing all their food to carbon dioxide, water and nitrogen. Where large flocks have formed at wastewater treatment plants, suspended solids and BOD of the plant effluents have dropped to less than 1 mg/L! Attempts are being made to breed flocks of these useful birds for other treatment plants.

IV. PIPET TECHNIQUES

A. INTRODUCTION

There are a surprising number of pipet styles available and in use in wastewater treatment plants. You need to be familiar with the proper use of the kinds you have, and use them in the intended manner in order to obtain accurate results. This section will first outline general techniques used for all pipets, and then will discuss the specific types of pipets.

B. GENERAL TECHNIQUES

1. Pipets are NEVER dipped into bottles of standards, and only under special conditions into the stock bottles of other solutions, because of the danger of contamination or dilution. After carefully shaking the bottle of stock solution,

pour a small amount into a clean dry beaker. The pipet will be dipped into the beaker.

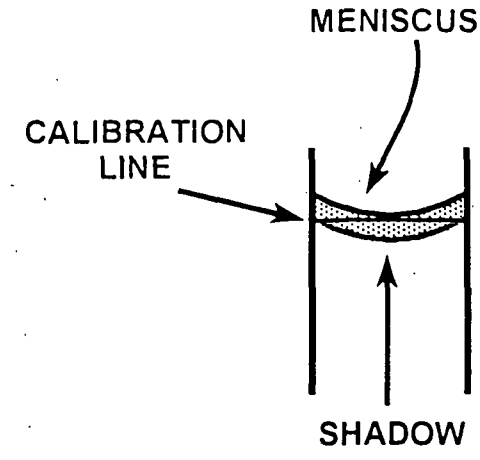
2. Pipets are rinsed with the solution to be measured. Using a bulb, carefully draw a little of the solution up into the pipet (about 10 percent of its capacity). Hold the pipet sideways and roll it so the solution wets all the walls. Drain the pipet into a waste container.

At this time check the wet pipet for beads of water between calibration lines or calibration line and tip. Beads are due to grease in the pipet and must be cleaned out of the pipet. Various cleaning solutions are available to remove grease, and need to be used. See any chemical company catalog for detergents or cleaning solutions that remove grease from volumetric ware.

For standards or high purity solutions, repeat the process of rinsing with the solution to be used two more times. These additional rinses can be omitted if your pipet was clean and **DRY** at the start. Any remaining solution in the beaker is discarded, and enough fresh solution added to the beaker to fill the pipet.

3. Pipets are filled and read while <u>straight</u> up and down. Using the bulb, fill the pipet to above the appropriate mark. Remove the bulb, and using the index finger of your major hand, leak enough air to allow the liquid to escape until the bottom of the meniscus is on the top of the line. What you do with the tip depends on the type of pipet (discussed below under each specific type).

What is a meniscus??



Put a piece of black paper (or paper blacked in with pen) behind your pipet at the water level in the neck. The shadow will mostly disappear under these conditions. Now move the black paper up and down. The shadow will move, but the true meniscus will not. Bring the bottom of the meniscus down until it just sits on top of the calibration line. If you have trouble holding the liquid at the right place, practice with a buret first.

C. SPECIFIC TYPES OF PIPETS AND THEIR USE

1. Volumetric (Transfer) Pipet

a. These have a single calibration line and look like this

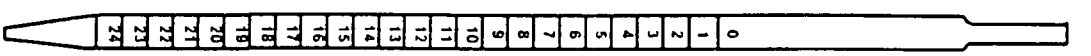
b. The tips are usually fine and this type of pipet should not be used for solutions where larger floating solids must be transferred.

c. The tip of the pipet should be held against the wall of a waste container while bringing the meniscus down to the calibration line.

d. After bringing to the mark, the solution is allowed to flow freely into the container you are transferring to with the tip against the inside wall of the container.

e. Leave the pipet tipped to the container for several seconds after flow has stopped.

2. Serological, MOHR or Measuring Pipets



SERIOLOGICAL, MOHR OR MEASURING PIPETS

a. After bringing the meniscus to the appropriate mark, touch the tip to the wall of a waste container.

b. Allow to drain into the container you want solution in without touching tip to container.

c. Drain to final mark and then touch tip to inside wall of container you want your solution in.

3. Serological pipet with one or two ground-glass or etched bands on mouthpiece, or two colored bands.

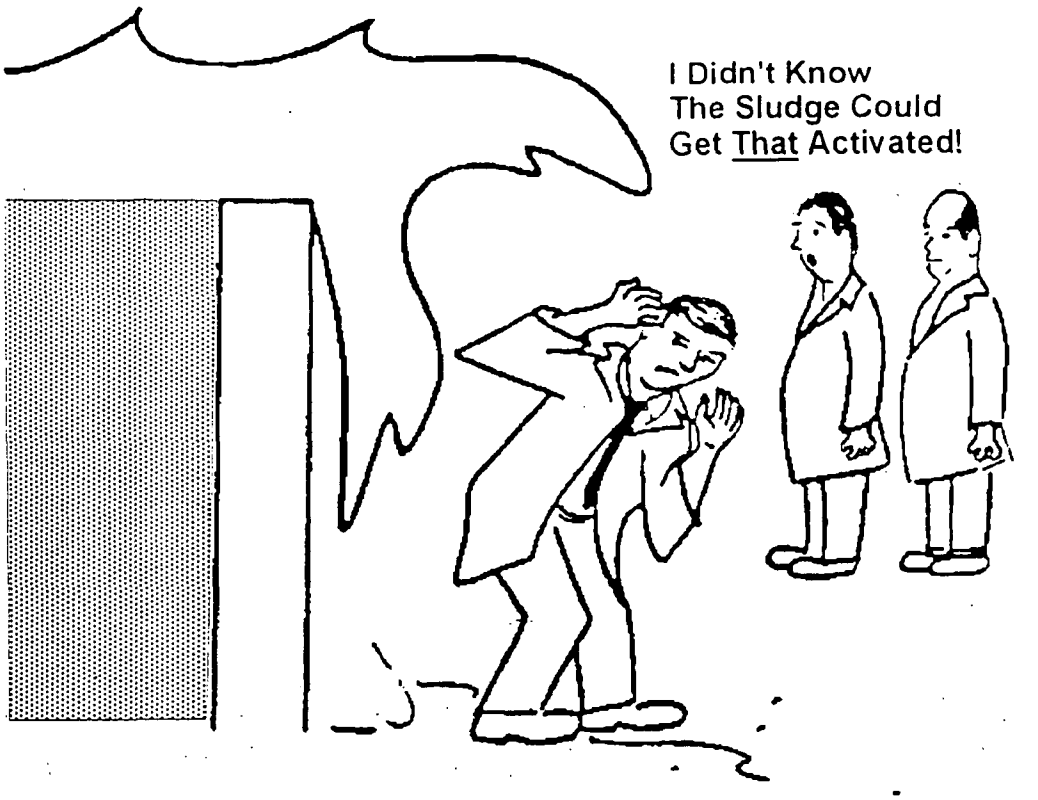
Treat just like the regular serological pipet except when it is completely drained, blow it out with your bulb.

4. **Dual Purpose Pipets**

These have separate lines for:

TD - Calibrated to deliver the stated volume. Use just like equivalent pipets above.

TC - Calibrated to contain the stated volume. Would be more accurate where it is necessary to wash out a pipet - such as during suspended solids analysis where the solids sticking to side must be washed down.



The mention of brand names in this document does not imply endorsement by the U.S. Environmental Protection Agency.

APPENDIX A

NOTES TO MATHEMATICIANS AND CHEMISTS

1. In the standard mathematical symbolism the average BOD is:

$$\overline{X} = \frac{\Sigma x_i}{n}$$

WHERE:

$\overline{X}$ = Average BOD (mean)
x_i = Individual BOD values
n = Number of BOD values

2. The average deviation is written

$$\overline{d} = \frac{\Sigma(\overline{x}-x_i)}{n}$$

The standard deviation is a better statistical indicator and is defined by the function.

$$s = \frac{\sqrt{\Sigma(\overline{x}-x_i)^2}}{n-1}$$

APPENDIX B: SAMPLE BENCH SHEET.

TILLIBOTZAM LABORATORIES
ANALYSTS: Murgatroid Tillibotzam
Dripworth R. Wetwater

DATE & TIME SAMPLE COLLECTED: PERSON COLLECTING:

DATE & TIME SAMPLE ANALYZED: BOD: INITIALS OF ANALYST:
TSS: pH:

BIOCHEMICAL OXYGEN DEMAND-BOD OR CARBONACEOUS BOD:

METHOD REFERENCE: EPA method 410-1, Methods for Chemical Analysis of Water and Wastes, 1979. Or Standard Methods 5210, p. 5-2, 18th Ed, 1992.

SAMPLE LOCATION &/OR CLIENT	BOTTLE NUMBER	SAMPLE VOLUME	INIT. D.O.	FINAL D.O.	CHANGE IN D.O.	BOD
BLANK 1						
BLANK 2						
QA SAMPLE						

BOD = Δ(D.O.,mg/L)x 300mL/SAMPLE VOL(mL) = mg/L

TOTAL SUSPENDED SOLIDS:

METHOD REFERENCE: EPA method 160.2, Methods for Chemical Analysis of Water and Wastes, 1979. Or Standard Methods 2540D, p 2-54, 18th Ed, 1992.

SAMPLE LOCATION &/OR CLIENT	SAMPLE NUMBER	SAMPLE VOLUME FILT'D	WT PAD +SOLID	WT PAD	WT. OF SOLIDS	TSS

TSS = WT. SOLIDS(g)X(1000 mg/g x 1000 mL/L)/SAMPLE VOL(mL) = mg/L

pH:

METHOD REFERENCE: EPA method 150.1, Methods for Chemical Analysis of Water and Wastes, 1979. Or Standard Methods 4500, p.4-65, 18th Ed., 1992.

SAMPLE LOCATION &/OR CLIENT	pH
CONTROL BUFFER (TRUE pH =)	

APPENDIX C: LIST OF APPROVED INORGANIC TEST PROCEDURES FOR COMMON ANALYTES SELECTED FROM 40CFR136.3 DATABASE, JAN, 1994

PAAMETER, UNITS AND METHOD	REFERENCE (METHOD NUMBER OR PAGE)				
	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
1. Acidity, as $CaCO_3$, mg/L: Electrometric endpoint or phenolphthalein endpoint	305.1	2310 B(4a)	D1067-92		
2. Alkalinity, as $CaCO_3$, mg/L: Electrometric or Colorimetric titration to pH 4.5, manual or automated	310.1				
3. Aluminum--Total[4], mg/L; Digestion[4] followed by:					
AA direct aspiration[36]	202.1	3111 D		I-3051-85	
AA furnace	202.2	3113 B			
Inductively Coupled Plasma/Atomic Emission Spectrometry (ICP/AES)[36]	[5]200.7	3120 B			
Direct Current Plasma (DCP)[36]			D4190-82(88)		Note 34.
Colorimetric (Eriochrome cyanine R)		3500-Al D			
4. Ammonia (as N), mg/L:					
Manual distillation (at pH 9.5)[6] followed by:	350.2	4500-NH_3B			973.49.[3]
Nesslerization	350.2	4500-NH_3C	D1426-89(A)	I-3520-85	973.49.[3]
Titration	350.2	4500-NH_3E			
Electrode	350.3	4500-NH_3F or G	D1426-89(B)		
Automated phenate, or	350.1	4500-NH_3H		I-4523-85	
Automated electrode					Note 7.
5. Arsenic--Total[4], mg/L:					
Digestion[4] followed by:	206.5				
AA gaseous hydride	206.3	314 B 4.d	D2972-88(B)	I-3062-85	
AA furnace	206.2	3113 B	D2972-88(C)		
ICP/AES[36] or	[5]200.7	3120 B			
Colorimetric (SDDC)	206.4	3500-As C	D2972-88(A)	I-3060-85	
9. Biochemical oxygen demand (BOD_5), mg/L:					
Dissolved Oxygen Depletion	405.1	5210 B		I-1578-78[8]	973.44[3] p.17[9]
12. Cadmium--Total[4], mg/L; Digestion[4] followed by:					
AA direct aspiration[36]	213.1	3111 B or C	D3557-90(A or B)	I-3135-85 or I-3136-85[9].	974.27[3] p.37
AA furnace	213.2	3113 B	D3557-90(D)		
ICP/AES[36]	[5]200.7	3120 B		I-1472-85	
DCP[36]			D4190-82(88)		Note 34.
Voltametry[11], or			D3557-90(C)		

	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
Colorimetric (Dithizone)		3500-Cd D			
14. Carbonaceous biochemical oxygen demand ($CBOD_5$), mg/L[12]:					
Dissolved Oxygen Depletion with nitrification inhibitor		5210 B			
15. Chemical oxygen demand (COD), mg/L; Titrimetric, or	410.1				
Spectrophotometric, manual or automated	410.4	5220 D	D1252-88(B)	I-3561-85	Notes 13 or 14.
16. Chloride, mg/L:					
Titrimetric (silver nitrate)		4500-Cl B	D512-89(B)	I-1183-85	
Or (Mercuric nitrate)	325.3	4500-Cl C	D512-89(A)	I-1184-85	973.51[3]
Colorimetric, manual or Automated (Ferricyanide)	325.1 or 325.2	4500-Cl E		I-1187-85 I-2187-85	
17. Chlorine--Total residual, mg/L; Titrimetric:					
Amperometric direct	330.1	4500-Cl D	D1253-86(92)		
[redacted]metric direct	330.3	4500-Cl B			
Black titration either end-point[15] or	330.2	4500-Cl C			
DPD-FAS	330.4	4500-Cl F			
Spectrophotometric, DPD Or Electrode	330.5	4500-Cl G			Note 16.
18. Chromium VI dissolved, mg/L; 0.45 micron filtration followed by:					
AA chelation-extraction or	218.4	3111 C		I-1232-85	
Colorimetric (Diphenylcarbazide)		3500-Cr D	D1687-92(A)	I-1230-85	
19. Chromium--Total[4], mg/L; Digestion[4] followed by:					
AA direct aspiration[36]	218.1	3111 B	D1687-92(B)	I-3236-85	974.27[3]
AA chelation-extraction	218.3	3111 C			
AA furnace	218.2	3113 B	D1687-92(C)		
ICP/AES[36]	[5]200.7	3120 B			
DCP[36], or			D4190-82(88)		Note 34.
Colorimetric (Diphenylcarbazide)		3500-Cr D			
20. Cobalt--Total[4], mg/L; Digestion[4] followed by:					
[redacted]irect aspiration	219.1	3111 B or C	D3558-90(A or B)	I-3239-85	p.37[9].

	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
AA furnace	219.2	3113 B	D3558-90(C)		
ICP/AES	[5]200.7	3120 B			
DCP			D4190-82(88)		Note 34.
12. Copper--Total[4], mg/L; Digestion[4] followed by:					
AA direct aspiration[36]	220.1	3111 B or C	D1688-90(A or B)	I-3270-85 or I-3271-85[9].	974.27[3] p.37
AA furnace	220.2	3113 B	D1688-90(C)		
ICP/AES[36]	[5]200.7	3120 B			
DCP[36], or			D4190-82(88)		Note 34.
Colorimetric (Neocuproine) or		3500-Cu D			
(Bicinchoninate)		or E			Note 19.
13. Cyanide--Total, mg/L:					
Manual distillation with $MgCl_2$ followed by		4500-CN C			
Titrimetric, or		4500-CN D			p. 22.[9]
Spectrophotometric, manual[31] or		4500-CN E	D2036-91(A)	I-3300-85	
Automoted[20]	335.3				
	335.3				
14. Cyanide amenable to chlorination, mg/L:					
Manual distillation with $MgCl_2$ followed by titrimetric or Spectrophotometric	335.1	4500-CN G	D2036-91(B)		
15. Fluoride--Total, mg/L:					
Manual distillation[6] followed by		4500-F-B			
Electrode, manual or Automated	340.2	4500-F-C	D1179-88(B)	I-4327-85	
Colorimetric (SPADNS)	340.1	4500-F-D	D1179-88(A)		
Or Automated complexone or	340.3	4500-F-E			
17. Hardness--Total, as $CaCO_3$, mg/L:					
Automated colorimetric	130.1				
Titrimetric (EDTA), or Ca plus Mg as their carbonates, by inductively coupled plasma or AA direct aspiration. (See Parameters 13 and 33)	130.2	2340 C	D1126-86(92)	I-1338-85	973.52B[3]
18. Hydrogen ion (pH), pH units:					
Electrometric measurement, or	150.1	4500-H^{+B}	D1293-84(90) (A or B)	I-1586-85	973.41[3]
Automated electrode					Note 21.
20. Iron--Total[4], mg/L; Digestion[4] followed					

	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
by:					
AA direct aspiration[36]	236.1	3111 B or C	D1068-90(A or B)	I-3381-85	974.27[3]
AA furnace	236.2	3113 B	D1068-90(C)		
ICP/AES[36]	[5]200.7	3120 B			
DCP[36], or			D4190-82(88)		Note 34.
Colorimetric (Phenanthroline)		3500-Fe D	D1068-90(D)		Note 22.
31. Kjeldahl Nitrogen--Total, (as N), mg/L:					
Digestion and distillation followed by	351.3	4500-NH_3B or C	D3590-89(A)		
Titration	351.3	4500-NH_3E	D3590-89(A)		973.48[3]
Nesslerization	351.3	4500-NH_3C	D3590-89(A)		
Electrode	351.3	4500-NH_3F or G			
Automated phenate colorimetric	351.1			I-4551-78[8]	
Semi-automated block digestor colorimetric, or	351.2		D3590-89(B)		
Manual or block digestor Potentiometric	351.4		D359-89(A)		
[illegible]ead--Total[4], mg/L; [illegible]estion[4] followed by:					
AA direct aspiration[36]	239.1	3111 B or C	D3559-90(A or B)	I-3399-85	974.27[3]
AA furnace	239.2	3113 B	D3559-90(D)		
ICP/AES[36]	[5]200.7	3120 B			
DCP[36]			D4190-82(88)		Note 34.
Voltametry[11], or			D3559-90(C)		
Colorimtric (Dithizone)		3500-Pb D			
35. Mercury--Total[4], mg/L:					
Cold vapor, manual or Automated	245.1 245.2	3112 B	D3223-91	I-3462-85	977.22[3]
37. Nickel--Total[4], mg/L; Digestion[4], followed by:					
AA direct aspiration	249.1	3111 B or C	D1886-90(A or B)	I-3499-85	
AA furnace	249.2	3113 B	D1886-90(C)		
ICP/AES[36]	[5]200.7	3120 B			
DCP[36], or			D4190-82(88)		Note 34.
Colorimetric (heptoxime)		3500-Ni D			
38. Nitrate (as N), mg/L:					
Colorimetric (Brucine sulfate), or Ni-[illegible]ate-nitrite N minus [illegible]rite N (See	352.1				973.5[3] 419D[17] p.28[9]

parameters 39 and 40)	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
39. Nitrate-nitrite (as N), mg/L:					
Cadmium reduction, Manual or	353.3	4500-NO_3E	D3867-90(B)		
Automated, or	353.2	4500-NO_3F	D3867-90(A)	I-4545-85	
Automated hydrazine	353.1	4500-NO_3H			
40. Nitrite (as N), mg/L; Spectrophotometric:					
Manual or	354.1				Note 25.
Automated (Diazotization)		4500-NO_2			
41. Oil and grease--Total recoverable, mg/L:					
Gravimetric (extraction)	413.1	5520 B			
42. Organic carbon--Total (TOC), mg/L:					
Combustion or oxidation	17 415.1	5310 B, C, or D	D2579-85(A or B)		973.47[3] p.14[24]
43. Total Kjeldahl N (Parameter 31) minus ammonia N (Parameter 4)					
44. Orthophosphate (as P), mg/L Ascorbic acid method:					
Automated, or	365.1	4500-P F		I-4601-85	973.56[3]
Manual single reagent	365.2	4500-P E	D515-88(A)		973.55[3]
Manual two reagent	365.3				
46. Oxygen, dissolved, mg/L:					
Winkler (Azide modification), or	360.2	4500-O C	D888-92(A)	I-1575-78[8]	973.45B[3]
Electrode	360.1	4500-O-G	D888-92(B)	I-1576-78[8]	
48. Phenols, mg/L:					
Manual distillation[26]	420.1				Note 27.
Followed by:					
Colorimetric (4AAP) manual, or	420.1				Note 27.
Automated[19]	420.2				
50. Phosphorus--Total, mg/L:					
Persulfate digestion followed by	365.2	4500-P B,5			973.55[3]
Manual or	365.2 or				
Automated ascorbic acid reduction	365.1	4500-P F		I4600-85	973.56[3]
Semi-automated block digestor	365.4		D515-88(B)		

	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
55. Residue--nonfilterable (TSS), mg/L: Gravimetric, 103-105° post washing of residue	160.2	2540 D		I-3765-85	
56. Residue--settleable, mg/L: Volumetric, (Imhoff cone), or gravimetric	160.5	2540 F			
50. Selenium--Total[4], mg/L; Digestion[4] followed by: AA furnace	270.2	3113 B			
ICP/AES,[36] or	[5]200.7	3120 B			
AA gaseous hydride		3114 B	D3859-88(A	I-3667-85	
54. Specific conductance, micromhos/cm at 25°C: Wheatstone bridge	120.1	2510 B	D1125-91(A	I-1780-85	973.40[3]
55. Sulfate (as SO_4^{-2}), mg/L: Automated colorimetric (barium chloranilate)	375.1				
Gravimetric	375.3	4500-SO_4^{-2}-2C or D			925.54[3]
Turbidimetric, or	375.4		D516-90		426C[30]
[redacted]ulfide (as S), mg/L: Titrimetric (iodine), or	376.1	4500-S^{-2}E		I-3840-85	
Colorimetric (methylene blue)	376.2	4500-S^{-2}D			
57. Sulfite (as SO_3^{-2}), mg/L: Titrimetric (iodine-iodate)	377.1	4500-SO_3^{-2} 2B			
58. Surfactants, mg/L: Colorimetric (methylene blue)	425.1	5540 C	D2330-88		
59. Temperature, °C: Thermometric	170.1	2550 B			Note 32.
71. Tin--Total,[4] mg/L; Digestion[4] followed by: AA direct aspiration	282.1	3111 B		I-3850-78[8]	
AA furnace, or	282.2	3113 B			
ICP/AES	[5]200.7				
73. Turbidity, NTU: Nephelometric	180.1	2130 B	D1889-88(A	I-3860-85	
75. Zinc--Total[4], mg/L; Digestion[4], followed [redacted]rect aspiration[36]	289.1	3111 B or	D1691-90	1-3900-85	974.27[3], 37[9]

	EPA[1,35]	Std. Methods 18th Ed.	ASTM	USGS[2]	Other
AA furnace	289.2				
ICP/AES[36]	[5]200.7	3120 B			
DCP,[36] or			D4190-82(88)		Note 34.
Colorimetric (Dithizone)		3500-Zn E			
or					
(Zincon)		3500-Zn F			Note 33.

FOOTNOTES:

1. 'Methods for Chemical Analysis of Water and Wastes', Environmental Protection Agency, Environmental Monitoring Systems Laboratory-Cincinnati (EMSL-CI), EPA-600/4-79-020, Revised March 1983 and 1979 where applicable.

2. Fishman, M.J., et al, 'Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments,' U.S. Department of the Interior, Techniques of Water--Resource Investigations of the U.S. Geological Survey, Denver, CO, Revised 1989, unless otherwise stated.

3. 'Official Methods of Analysis of the Association of Official Analytical Chemists,' methods manual, 15th ed. (1990).

4. For the determination of total metals the sample is not filtered before processing. A digestion procedure is required to solubilize suspended material and to destroy possible organic-metal complexes. Two digestion procedures are given in 'Methods for Chemical Analysis of Water and Wastes, 1979 and 1983'. One (section 4.1.3), is a vigorous digestion using nitric acid. A less vigorous digestion using nitric and hydrochloric acids (section 4.1.4) is preferred; however, the analyst should be cautioned that this mild digestion may not suffic for all samples types. Particularly, if a colorimetric procedure is to be employed, it is necessary to ensure that all organo-metallic bonds be broken so that the metal is in a reactive state. In those situations, the vigorous digestion is to be preferred making certain that at no time does the sample go to dryness. Samples containing large amounts of organic materials may also benefit by this vigorous digestion, however, vigorous digestion with concentrated nitric acid will convert antimony and tin to insoluble oxides and render them unavailable for analysis. Use of ICP/AES as well as determinations for certain elements such as antimony, arsenic, the noble metals, mercury, selenium, silver, tin, and titanium require a modified sample digestion procedure and in all cases the method write-up should be consulted for specific instructions and/or cautions. Note: If the digestion procedure for direct aspiration AA included in one of the other approved references is different than the above, the EPA procedure must be used.

Dissolved metals are defined as those constituents which will pass through a 0.45 micron membrane filter. Following filtration of the sample, the referenced procedure for total metals must be followed. Sample digestion of the filtrate for dissolved metals (or digestion of the original sample solution for total metals) may be omitted for AA (direct aspiration or graphite furnace) and ICP analyses, provided the sample solution to be analyzed meets the following criteria:

a. Has a low COD (<20),
b. Is visibly transparent with a turbidity measurement of 1 NTU or less,
c. Is colorless with no perceptible odor, and
d. Is of one liquid phase and free of particulate or suspended matter following acidification.

5. The full text of Method 200.7, 'Inductively Coupled Plasma Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes,' is given at Appendix C of this Part 136.

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

Ammonia, Automated Electrode Method, Industrial Method Number 379-75 WE, dated February 19, , Bran & Luebbe (Technicon) Auto Analyzer II, Bran & Luebbe Analyzing Technologies, Inc., sford, N.Y. 10523.

8. The approved method is that cited in 'Methods for Determination of Inorganic Substances in Water and Fluvial Sediments', USGS TWRI, Book 5, Chapter A1 (1979).

9. American National Standard on Photographic Processing Effluents, Apr. 2, 1975. Available from ANSI, 1430 Broadway, New York, NY 10018.

11. The use of normal and differential pulse voltage ramps to increase sensitivity and resolution is acceptable.

12. Carbonaceous biochemical oxygen demand ($CBOD_5$) must not be confused with the traditional BOD_5 test which measures 'total BOD'. The addition of the nitrification inhibitor is not a procedural option, but must be included to report the $CBOD_5$ parameter. A discharger whose permit requires reporting the traditional BOD_5 may not use a nitrification inhibitor in the procedure for reporting the results. Only when a discharger's permit specifically states $CBOD_5$s required can the permittee report data using the nitrification inhibitor.

13. OIC Chemical Oxygen Demand Method, Oceanography International Corporation, 1978, 512 West Loop, P.O. Box 2980, College Station, TX 77840.

14. Chemical Oxygen Demand, Method 8000, Hach Handbook of Water Analysis, 1979, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

15. The back titration method will be used to resolve controversy.

16. Orion Research Instruction Manual, Residual Chlorine Electrode Model 97-70, 1977, Orion Research Incorporated, 840 Memorial Drive, Cambridge, MA 02138. The calibration graph for the Orion residual chlorine method must be derived using a reagent blank and three standard ions, containing 0.2, 1.0, and 5.0 ml 0.00281 N potassium iodate/100 ml solution, ctively.

17. The approved method is that cited in Standard Methods for the Examination of Water and Wastewater, 14th Edition, 1976.

19. Copper, Biocinchoinate Method, Method 8506, Hach Handbook of Water Analysis, 1979, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

20. After the manual distillation is completed, the autoanalyzer manifolds in EPA Methods 335.3 (cyanide) or 420.2 (phenols) are simplified by connecting the re-sample line directly to the sampler. When using the manifold setup shown in Method 335.3, the buffer 6.2 should be replaced with the buffer 7.6 found in Method 335.2.

21. Hydrogen ion (pH) Automated Electrode Method, Industrial Method Number 378-75WA, October 1976, Bran & Luebbe (Technicon) Autoanalyzer II. Bran & Luebbe Analyzing Technologies, Inc., Elmsford, N.Y. 10523.

22. Iron, 1,10-Phenanthroline Method, Method 8008, 1980, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

24. Wershaw, R.L., et al, 'Methods for Analysis of Organic Substances in Water,' Techniques of Water-Resources Investigation of the U.S. Geological Survey, Book 5, Chapter A3, (1972 Revised 1987) p. 14.

25. Nitrogen, Nitrite, Method 8507, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

26. Just prior to distillation, adjust the sulfuric-acid-preserved sample to pH 4 with 1+9 NaOH.

27. The approved method is cited in Standard Methods for the Examination of Water and wastewater, 14th Edition. The colorimetric reaction is conducted at a pH of 10.0±0.2. The ved methods are given on pp. 576-81 of the 14th Edition: Method 510A for distillation,

Method 510B for the manual colorimetric procedure, or Method 510C for the manual spectrophotometric procedure.

30. The approved method is that cited in Standard Methods for the Examination of Water and Wastewater, 15th Edition.

31. EPA Methods 335.2 and 335.3 require the NaOH absorber solution final concentration to be adjusted to 0.25 N before colorimetric determination of total cyanide.

32. Stevens, H. H., Ficke, J. F., and Smoot, G. F., 'Water Temperature--Influential Factors, Field Measurement and Data Presentation', Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, Chapter D1, 1975.

33. Zinc, Zincon Method, Method 8009, Hach Handbook of Water Analysis, 1979, pages 2-231 and 2-333, Hach Chemical Company, Loveland, CO 80537.

34. 'Direct Current Plasma (DCP) Optical Emission Spectrometric Method for Trade Elemental Analysis of Water and Wastes, Method AES0029,' 1986--Revised 1991, Applied Research Laboratories, Inc., 24911 Avenue Stanford, Valencia, CA 91355.

35. Precision and recovery statements for the atomic absorption direct aspiration and graphite furnace methods, and for the spectrophotometric SDDC method for arsenic are provided in Appendix D of this part titled, 'Precision and Recovery Statements for Methods for Measuring Metals'.

36. 'Closed Vessel Microwave Digestion of Wastewater Samples for Determination of Metals', CEM Corporation, P.O. Box 200, Matthews, NC 28106-0200, April 16, 1992. Available from the CEM Corporation.
