EPA 910-R-96-001

United States
Environmental Protection
Agency

Region 10
1200 Sixth Avenue
Seattle WA 98101

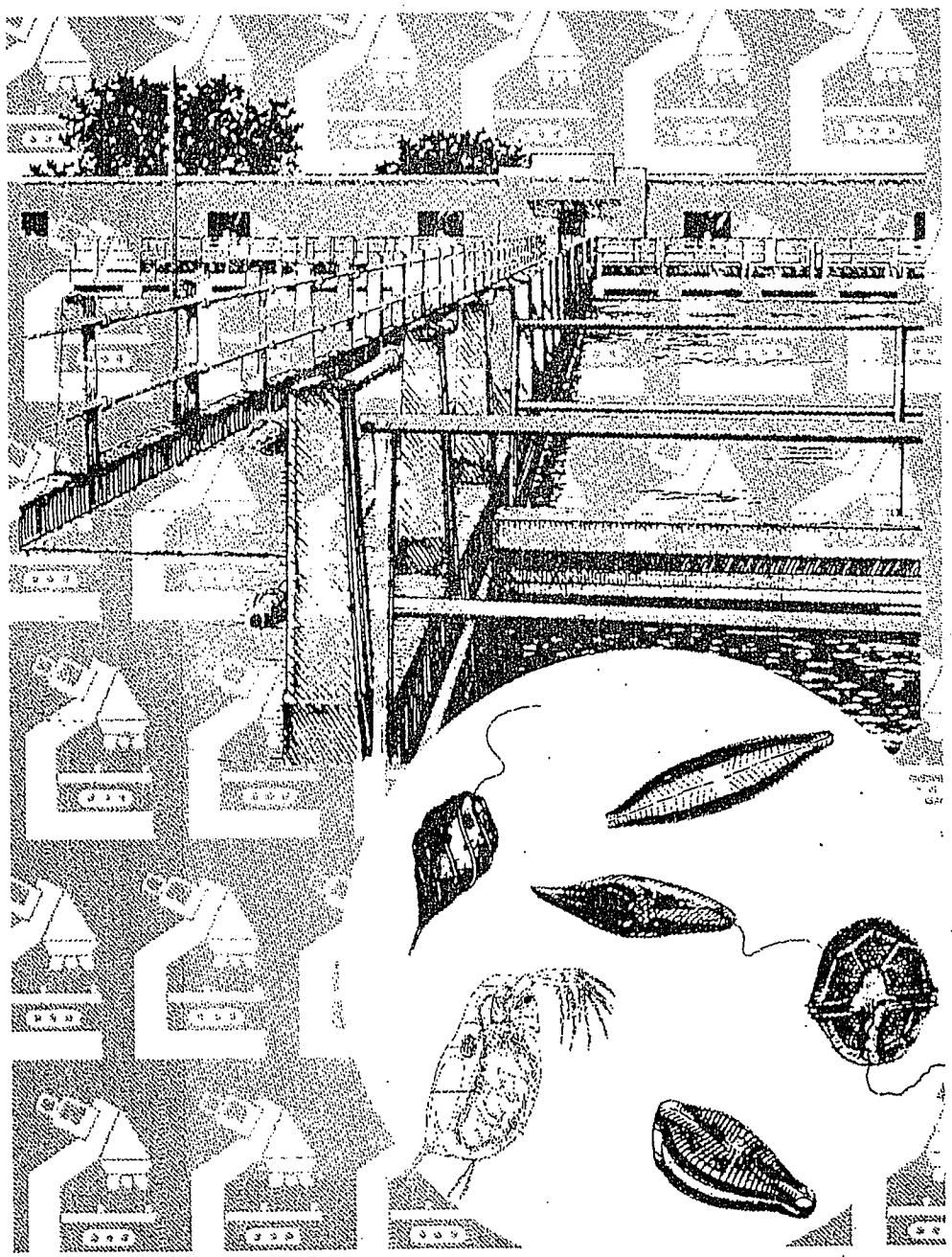
Alaska
Idaho
Oregon
Washington

Office of Environmental Assessment

April 1996

EPA

Microscopic Particulate Analysis (MPA) for Filtration Plant Optimization



Microscopic Particulate Analysis (MPA) For Filtration Plant Optimization

prepared by
Stephanie Harris[1], Carrie Hancock[2], & Jay Vasconcelos[1]

April 1996

[1] *U.S. EPA Manchester Laboratory, 7411 Beach Drive East, Port Orchard, WA 98366*

[2] *CH Diagnostics & Consulting Service, 214 S.E. 19th Street, Loveland, CO 80537*

Acknowledgements

We wish to thank the many microbiologists and others who actively participated in the development and review of the USEPA consensus method entitled **"Microscopic Particulate Analysis (MPA) for Filtration Plant Optimization".**

Dr Susan Boutros
Environmental Associates
1185 East Main
Bradford, PA 16701

Rick Brinkman
250 North 5th St
Grand Junction, CO 81503

Carrie Howe-Carlson
Microsearch Laboratory
2783 Webster Drive
Grand Junction, CO 81503

Dr Jennifer Clancy (Randi McCuin)
Clancy Environmental Consultants
PO Bos 314
St. Albans, VT 05478

Jill Cunningham (Deborah Wayman, Deanna Crump)
Grants Pass Water Lab
558 NE "F"
Grants Pass, OR 07527

Rick Danielson (Craig Johnson)
BioVir Laboratories, Inc.
685 Stone Rd. # 6
Benicia, CA 94510

Michelle Eisenstein
Johnson State College
Chemical Hygiene Office
Johnson, VT 05656

Scott Tighe (Sharon Hallock, Brad Eldred)
Analytical Services, Inc.
PO box 515
Williston, VT 05495

John Rae
2855 Mesa Rd.
Colorado Springs, CO 80904

Dr. Frank Schaefer, III
USEPA/NERL/HERD/BARB
26 W. Martin Luther King Way
Cincinnati, OH 45268

We also wish to thank those who contributed materials and talent to design the cover page.

U.S. EPA Region 10 Graphics Department
BioVir Laboratories, Inc.
Eugene Water and Electric Board

Disclaimer

Mention of any trade names of commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

Microscopic Particulate Analysis (MPA) for Filtration Plant Optimization

Table of Contents

Introduction

With enactment of the 1986 Amendment to the Safe Drinking Water Act (SDWA), the EPA has promulgated new regulations for filtration and disinfection of public water systems using surface water or groundwater under the direct influence of surface water (GWUDI). Those systems identified as surface water or GWUDI must demonstrate a 3 log (99.9 %) removal of Giardia and 4 log removal of virus particles through a combination of filtration and disinfection. Detection of *Giardia* cysts and *Cryptosporidium* oocysts in surface water cannot be used to assess treatment plant performance because of their low concentrations, intermittent occurrence, and limitations of the currently available technology. Because *Giardia* and *Cryptosporidium* may occur at concentrations sufficient to cause disease but not consistently numerous enough to assess filtration performance, surrogates for filtration efficiency have been and continue to be developed.

Performance of water treatment plants can be evaluated by a number of methods, including turbidity, particle counts, and Microscopic Particulate Analysis (MPA). Simultaneous use of more than one evaluation technique may be appropriate, not only for research but also for plant operation. Particle counting and turbidity data must be used with caution because flocculated particles could give false values compared with cyst reduction and overall plant performance. Several investigators have found that MPA can provide information on the effectiveness of water treatment processes for removing particulate matter (1, 2). Electronic particle counting, by itself, does not provide the operator with critical information about the type and number of organisms encountered.

MPA, including particle sizing, is performed on drinking water systems where some form of treatment, chemical or physical, exists between the natural water source and its distribution to the public. This analysis compares the type, size and quantities of bioindicators and particles found in the raw water to those found in the finished, or treated, water. This method can be used to evaluate filtration efficiencies, as log reduction, of conventional filtration systems, as well as the on-site evaluation of alternate filtration technologies.

This method can be used to identify certain groups of microorganisms, 1 to 600 micrometer (μm) in size, which normally only occur in raw water as opposed to finished waters and whose presence, in the finished water, may indicate some breakthrough or growth in the filter beds. These important microorganisms, also called bioindicators, include diatoms, algae, Giardia, coccidia, plant debris, pollen, rotifers, crustaceans, ameba, nematodes and insects/larvae. Comparison of the quantitative numbers of these bioindicators in raw and finished water can also assist in the over-all evaluation of filtration efficiency and may provide information critical to the optimization of the filtration plant beyond simple turbidity reduction or particle counting by instrumentation.

Historically, water treatment professionals have relied on chemical and physical measurements to assess water treatment plant performance; obviously these assessments are not adequate because numerous outbreaks of *Giardia* and *Cryptosporidium* have occurred during periods where the plant met all federally required performance criteria. An eclectic approach using several tools, all of which measure different aspects of plant performance, for the assessment of filtration efficiency along with a thorough understanding of the particular plant design and operation helps avoid the inadequacy of simplistic solutions for explaining the complex interactions of water treatment.

Sample Collection

1.0 General Overview: High Volume Filter (HVF) samples for surface water MPA are collected from the raw water before it enters any phase of water treatment and from the finished water just prior to disinfection and distribution. Evaluation of each filter bed or a composite of the effluent water is optional, and the chosen option should be noted in the final report. However, blending of each filter bed into a composite many prevent identification of individual filter bed inadequacies. The collection site should be selected to avoid stratification of the pipes.

2.0 Sample Equipment and Materials

2.1 Sampling device consists of the following parts (refer to Figure 1 and 2)

- 2.1.1 Six foot inlet hose, preferable disposable, with backflow preventor (Watts No. 8)
- 2.1.2 Pressure regulator (Watts 26A), or equivalent, plus pressure gauge, 0-100 psi
- 2.1.3 Proportionating injector (for chlorinated water), Model 203 B.T. injector, 100-15P-87, or equivalent. (Dema Engineering) (For chlorinated samples only).
- 2.1.4 Commercial Filter model LT-10 filter housing (9499-5015)
- 2.1.5 Water flow meter, readable in gallons or liters.
- 2.1.6 Flow control valve (limiting flow orifice) rated at 1.0 gallon per minute (gpm) for finished water; 0.5 to 1.0 gpm for raw water. (Rationale for this modification is to allow collection for a longer period prior to plugging of the filter in high turbidity waters)
- 2.1.7 Discharge hose
- 2.1.8 Pump, for non-pressurized sources
- 2.1.9 Miscellaneous brass, or PVC, fittings for unit assembly
- 2.1.10 Optional peto tube installed at sampling port is recommended to reduce problems caused by flow dynamics in the pipe

2.2 Sampling Materials

- 2.2.1 Ten inch, 1 μm nominal porosity, polypropylene, yarn-wound, cartridge filter, Commercial Honeycomb filter tube (M39R10A).
- 2.2.2 Whirl pak plastic bags (5.5" x 14") or zip loc heavy duty quality freezer bags
- 2.2.3 Sanitary gloves

3.0 Sample Collection Parameters

Note: Below are recommendations for typical treatment systems. Large, or atypical, systems where retention occurs, may require alteration of these recommendations to provide accurate log reduction values. Moreover, electronic particle count collection sites may vary from MPA collection points. If performing only electronic particle counts, the sample might be more appropriately collected directly from the source, particularly if presedimentation basins are an integral part of the treatment plant. The holding time associated with presedimentation basins, may allow for settling of particulates and may adversely influence log reduction values.

3.1 **Raw surface water** should be sampled prior to chemical addition and after any presedimentation basins (if no chemicals were added prior to presedimentation). The main objective in raw water sampling is to collect a sample representative of the water entering the treatment system; therefore, if recycling operations are practiced, the raw water should be sampled after the recycling input. Such sampling should allow adequate time for mixing of recycling input prior to sampling. If collection at the source is not possible, final report must "qualify" sample

3.2 **Finished water** should be sampled after the filtration system and prior to chlorine addition, if possible. Sodium thiosulfate (final concentration 50 mg/l) is injected into samples that cannot be collected prior to chlorination. Samples are collected prior to post treatment storage to provide a more accurate evaluation of the filtration system. Evaluation of log reduction in large treatment plants with post filtration holding tanks, may be difficult, given the propensity for algal growth in these circumstances.

3.3 **Treatment plant evaluation.** The raw water sampling should be initiated before the finished water sampling. The amount of time elapsed between the beginning of raw sampling and the beginning of finished sampling should be equivalent to the detention time of the system. To accurately assess treatment efficiency, finished water sampling should encompass a full cycle run or for a 24 hour sampling, including at least one backwash in the sampling.

3.4 Pressure over the filter face should be set at 10 psi, using the in-line pressure gauge and meter

4.0 Sample Collection Procedure *(Flow chart, Figure 3)*

4.1 Cleanliness- before each sample collection the hose and filter housing must be washed with hot water containing a mild detergent and bleach solution; rinse with hot water followed by particle free water *(see 7.12)*. If this cannot be done, run a minimum of 50 gallons of sample water through the sampling equipment prior inserting a new filter. Do not touch the filter with bare hands, use sanitary gloves or the plastic cover the filter is wrapped in.

4.2 Connect sampling unit to pressure source and pump in the direction of flow indicated on filter housing. Flush the unit without a filter for 3 - 5 minutes with the source water to be sampled.

4.2.1 Non-Pressurized Sources: Small 1-5 gallon per minute battery operated bilge pumps or electric or gas powered centrifugal pumps may be used. Be sure to put the sample intake in a location where the least amount of bottom sediment will enter into the sampling filter giving a distorted view of the sample. If possible, install the pump downstream (on the effluent end) of the filter to eliminate the potential for cross-contamination of samples. Note: Collect sample as near to intake site as possible. If intake is near the bottom and in fact draws in bottom sediments, then collection here is appropriate.

4.3 Record the date, time of day and gallon reading from the water meter before and after sampling. Document the name and location of each sample point, sampling site (raw or finished) and type of treatment.

4.4 Insert filter in the housing and tighten housing. Make sure "O" ring is in place. Turn water on slowly with the unit in an upright position. Invert unit to make sure all the air within the housing is expelled. When the housing is full of water, return unit to upright position and increase flow up to 3.8 lpm (1 gpm). Measure flow rate by either timing the meter rate or by timing flow rate into a calibrated bucket. Maintain 1 gpm (3.8 Lpm) throughout the sampling period. Exceptions to the 1 gpm rate are given in 5.1. If 0.5 gpm flow control valve is being used follow the directions except that the flow rate will be less and meter may not register so measuring the flow using a calibrated container may be necessary.

4.5 Check reading on pressure gauge. Adjust pressure gauge to 10 psi, if needed..

4.6 Information on the sample volume and water quality parameters should be included on data sheet (See section 5.0)

4.7 When sampling is complete, shut off flow, record stop time and final meter reading or time flow rate into calibrated bucket and average with flow rate measured in 4.4. Subtract the initial reading from the final reading and record the total volume collected.

4.8 Turn off the faucet or pump and disconnect the hose from incoming water source. Maintain the inlet hose level above level of opening on outlet hose to prevent backwashing and loss of particulate matter from the filter. Pour residual water from filter holder into the ziploc (whirlpac) bag.

4.9 Remove the sampling cartridge with the plastic cover or sanitary latex gloves. Do not touch with bare hands. Place filter in the heavy duty quality ziploc (whirlpac) bag and seal.

4.10 With permanent marker record the sample identification, gallons sampled, collection dates and times, collector's name and water quality parameters directly on the bag or on a waterproof label. Place the first bag containing the filter into the labeled ziploc bag. Make sure both bags are sealed to prevent leakage.

4.11 If immediate shipping is not possible, the sample should be stored in a 1 - 5° C refrigerator, until shipping, within limits set forth in 4.14.

4.12 Place freezer cold packs in the shipping container. Place insulating material between the filter and cold packs to prevent HVF sample freezing. Samples that arrive at the laboratory frozen, should be rejected, discarded and resampling requested. Place data sheet containing recorded information in a sealed plastic bag and ship with the filters.

4.13 Ship by overnight delivery service to the analytical laboratory.

4.14 Samples must be processed within 96 hours of initiation of sampling.

5.0 Sample Volumes and Water Quality Parameters

5.1 **Raw water:** Sampling unit should be allowed to run for a 12 to 24 hour period in which time a minimum volume of 100 liters (27 gallons) should be filtered. The ideal volume is the amount equivalent to a complete day of production. If the filter becomes clogged or plugged due to highly turbid waters, terminate sampling and record the volume collected to this point. If the raw water source is known to have high turbidity, the sampling flow rate may be lowered to < 1 gpm to collect a sample over a longer time period thus obtaining a sample more representative of the raw water quality.

5.2 **Finished water:** Minimum 1000 liters or 264 gallons. Collection period should encompass a full cycle run, or for 24 hours, including at least one backwash cycle. Backwash cycles can occur at the initiation of the sampling period. If multiple filter beds are present in the filtration plant, a composite sampling is recommended initially, although later evaluation of individual filter beds is an option. Additionally, if requested, discrete sampling points at ripening, middle of cycle and after backwash may be added to assist with plant operation.

5.3 **Water Quality Parameters:** Measurement of certain water quality parameters should be included in the sample data form for both raw and finished water. Among these should be total and free chlorine residual, temperature, pH, turbidity, and operational parameters of the WTP (pretreatment, filtration, disinfection) and water source. Microbiological testing, such as total and fecal coliform and heterotrophic plate count, are optional.

5.4 **Chlorinated Samples:** Try to sample water prior to any chlorination. If chlorinated water must be sampled, an injector system will need to be installed to add a sodium thiosulfate solution to denature the chlorine. Add sodium thiosulfate solution via the injector system to produce a final concentration of 50 mg/L. Setting the injector system to produce a 1:100 dilution of 0.5% sodium thiosulfate stock solution will result in a final concentration of 50 mg/L. Details on the operation and use of proportioner pumps and injectors can be found in **Standard Methods for the Examination of Water and Wastewater**, Section 9510C, "Virus Concentration from Large Volumes by Adsorption to and Elution from Microporous Filters (Proposed)," 18th ed., 1989, pp. 9-105 to 9-109. Model 203 B.T. injector, 100-15P-87 special tip, Dema Engineering, or equivalent, may be used. Alternatively, a peristaltic pump or electric pump can be used to inject the sodium thiosulfate.

Filter Processing and Analysis *(Fig 3,4)*

6.0 Equipment

6.1 Large capacity centrifuge, refrigerated recommended
6.2 Large capacity swing-bucket rotor (90 °), 1-6 liter/run
6.3 250 mL conical bottom bottles with screw caps or 490 mL glass conical bottles
6.4 15 mL conical graduated centrifugate tubes
6.5 graduated cylinder
6.6 1 - 5° C refrigerator
6.7 50 mL conical graduated centrifuge tubes
6.8 Stomacher lab blender- model 3500 (optional)
6.9 Vortex tube mixer
6.10 Aspiration flask and vacuum source with 0-30 psi gauge
6.11 Pipet aid, syringe or bulb
6.12 Motorized multivolume microliter pipet (Rainin edp plus) or manual equivalent
6.13 Hollow glass tubes (ca 1/4" bore)
6.14 Brightfield, phase contrast, differential interference contrast (DIC) or hoffman modulation optics (HMO) capable microscope equipped with 10, 40 and 100 X objectives. A 35 mm camera or video camera attached, is optional
6.15 Manual or electronic differential counter (10 gang)
6.16 Non-drying immersion oil
6.17 Single place hand held counter
6.18 Palmer-Maloney counting chamber available from Wildlife Supply Company, catalog # 1803-B20, specify glass model. (301 Cass St. Saginaw, MI, 48602)
6.19 Sedgewick-Rafter Counting Chamber (optional)

7.0 Supplies

7.1 Whirl pac bags, 5.5 x 15", sterile, or heavy duty ziploc bags. For filter transportation
7.2 Polypropylene yarn woven filter tubes (M39R10A, Commercial Filter, Lebanon, IN)
7.3 Sanitary gloves
7.4 Pan or tray, stainless steel or glass, autoclavable
7.5 4 liter beakers, autoclavable (glass or plastic)
7.6 Scalpel handles, autoclavable
7.7 Scalpel blades, sterile
7.8 Disposable glass pipets, sterile
7.9 Pasteur pipets, sterile
7.10 10 % buffered formaldehyde, pH 7.0
7.11 Polysorbate 80
7.12 Particle-free water (deionized, distilled or reverse osmosis water, passed through a 0.22 um filter) should contain less than 100 particles/ml (2 μm or larger)
7.13 Clear fingernail polish
7.14 3.5 L capacity Stomacher bags (Seward medical, Tekmar Co)
7.15 2 L beakers
7.16 Non-drying immersion oil

8.0 Processing Reagents

8.1 Wash water (non-sterile)

8.1.1 Sterile Erlenmeyer flask (1,2 or 4 L)

8.1.2 Particle free water (7.12)

8.1.3 Sodium citrate (optional, if iron present)

8.1.4 0.01 % polysorbate 20, add and mix immediately prior to use (optional)

8.1.5 Mix these in the following proportions:

Wash-Water Proportions

	1 L	2 L	4 L
Sodium citrate (Optional)	5.0 g	10.0g	20.0g
0.01 % polysorbate (Optional)	10.0 ml	20.0 ml	40.0 ml
Particle-free water (Quantity Sufficient to make)	1.0 L	2.0 L	4.0 L
Final pH to 5.5 - 7.5			

8.1.6 Use of 0.85 % NaCl or other buffering agent is optional

8.1.7 Record constituents of wash water on laboratory bench sheet

9.0 Particulate Extraction: The filter cartridge is handled aseptically. All glassware and other equipment is mechanically scrubbed, rinsed in particle-free water and autoclaved or chemically sanitized. Sanitary gloves are worn during processing.

9.1 Remove filter from the ziploc/whirl pac bag and place in pan.

9.2 Record the color of the filter and any other notable physical characteristics.

9.3 Rinse the bags with particle-free water. The rinse water is retained in a beaker.

9.4 The filter fibers are cut length-wise to the filter core and separated into a minimum of 6 equal portions. Each portion is washed sequentially in 3 consecutive 1.0 liter volumes of wash water. Begin with the cleanest fibers and proceed to the dirtiest. Washing consists of vigorous kneading and swirling motion. Minimum total wash time of fibers should be 30 minutes. The fibers are wrung out into a collection beaker by placing them in individual interlocking bags which have one corner snipped off to allow for drainage.

9.5 Alternatively, a Stomacher lab blender (model 3500) may be substituted for handwashing. The filter is cut length-wise to the core and after loosening the fibers, place all of the fibers into a single stomacher bag. To insure against bag breakage and sample loss, place the filter fibers in the first stomacher bag into a second stomacher bag. Add 1.75 L of wash water to the fibers. Homogenize for 2- five minute intervals. Between each homogenization period, hand knead the filter material to redistribute the fibers in the bag. Wring the fibers out to express as much of the liquid as possible and place them in another stomacher bag containing 1.25 liters of wash water. Repeat for 2- five minute homogenization periods. Wring the fibers to express as much of the liquid as possible before discarding.

9.6 The 2 aliquots of wash water, bag rinse water and residual filter water obtained from the filter housing are combined in one 4 liter beaker.

9.7 Record the volume of the total particulate solution.

Note: The use of Immunofluorescent Assay for *Giardia & Cryptosporidium* is optional at this time, but is required if ***Giardia & Cryptosporidium*** reporting is included in the analysis. If IFA is chosen, follow the latest method publicized in the federal register. The fibers from those samples for which IFA is being done in addition to MPA will be washed a second time in the IFA prescribed eluting solution (Federal Register 1994). An FA eluting solution was developed because more *Giardia* cysts and *Cryptosporidium* oocysts could be recovered

during the washing process than by using particle-free water alone. However, many non-encysted organisms subjected to the FA eluent deteriorate and cannot be recognized or identified for MPA. Therefore, a particulate extraction scheme will need to be followed so that both MPA and FA can be done from the same sample. The particle-free water/particulate solution from the initial wash is halved for MPA and FA. The half retained for IFA is combined with half of the secondary wash solution to provide a known quantity of IFA/particulate solution.

Using Palmer-Maloney Cell

The Sedgwick Rafter (S-R) may be used in addition to the PMCC for enumeration of larger zooplankton if long working distance 20x or 40x objectives are available and if written records of calculation and methodology following Standard Methods are kept.

10.0 Subsample Examination: The total particulate solution represents a solution of the particulates recovered from the number of gallons sampled; therefore, an accurate record of the number of gallons sampled is a prerequisite for the calculations needed to do any further processing. Convert the number of gallons to liters.

10.1 Thoroughly mix the total particulate solution by pouring it back and forth in glass containers of sufficient size to hold the whole sample, or alternatively, use a sterile stir bar and magnetic plate and mix for 10 minutes. After mixing, immediately remove a 200 ml aliquot and place into a 250 ml conical centrifuge bottle. Vortex for 15 seconds.

10.2 Immediately after mixing, withdraw a 0.1 mL subsample with an calibrated Eppendorf, or equivalent, pipette.

10.3 Inject the subsample into the Palmer-Maloney counting chamber by introducing the sample with the pipette into one of the two 5-mm channels on the sides of the chamber with the cover slip in place.

10.3.1 Calculate and record the liter equivalent of the withdrawn subsample using this proportion ratio:

$$\frac{\text{Total Volume in 250 ml conical centrifuge bottle}}{\text{Liter equivalent in centrifuge bottle}} = \frac{0.1\text{mL}}{X}$$

where: X = liters equivalent of 0.1 ml subsample
Liter equivalent in centrifuge bottle is calculated from this proportion ratio:

$$\frac{\text{Total volume of particulate solution (in ml)}}{\text{Total \# of Liters sampled}} = \frac{\text{200 ml aliquot}}{\text{Liter equivalent in centrifuge bottle}}$$

Note: If returning to this step for a second time, skip the above calculation; double the previously calculated Liter equivalent in centrifuge bottle (triple if a third 200 ml aliquot is added), then subtract the liter equivalent of previously withdrawn 0.1 ml subsample (10.7.1).

Note: If previous history of this treatment plant warrants it, centrifugation of the entire wash water may be performed.

10.4 View the subsample at 100x magnification. If $\geq$10 plankters (plural for individual plankton organisms) are present per field of view, proceed to section 11.0.

10.5 If <10 plankters per field of view are present, centrifuge the bottle at 1050 x g for 10 minutes. Alternatively, if the turbidity of the particulate solution examined is comparable to a 1.0 McFarland standard, this will provide an acceptable density of particulates in most instances. *(See McFarland Standards in Appendix 4.)*

10.6 Aspirate the supernatant down to 4cm above the bottom sediment or the bottom of the bottle, this should provide approximately 50 ml. Measure and record the volume of combined centrifugate and supernatant.

10.7 Thoroughly mix the solution by vortexing for 15 seconds and immediately withdraw a 0.1 ml subsample for charging the Palmer -Maloney counting chamber.

10.7.1 Calculate and record the liter equivalency of the 0.1 mL aliquot using this proportion ratio: *(For example of calculations see Appendix 6.)*

$$\frac{\text{Volume of Remaining Particulate Solution (in mL)}}{\text{Remaining Liter Equivalency}} = \frac{0.1\ \text{mL}}{X}$$

where:
X = liter equivalent of 0.1 ml subsample

Vol. of Remaining Particulate Solution = Vol. recorded in 10.6.

Remaining Liter Equivalency = Liter equivalent in centrifuge bottle calculated in 10.3.1 less the liter equivalency of previously withdrawn 0.1 mL subsample(s).

10.8 If ≥10 plankters are present per 100x magnification field of view, proceed to section 11.0.

10.9 If <10 plankters per field of view are present, add an additional 200 ml aliquot of the total particulate solution by repeating steps 10.1 to 10.9 until the plankter density is correct. Alternatively, if the turbidity of the particulate solution examined is comparable to a 1.0 McFarland standard, this will provide an acceptable density of particulates in most instances. *(See McFarland Standards in Appendix 4)*

NOTE: Frequently, raw surface water may be examined without centrifugation after filter washing; whereas, finished water often requires several centrifugation steps. Sometimes samples are over-concentrated (too dense for microscopic visualization due to overlapping) and need to be diluted. If further dilution is necessary, remember to include this in the calculations in 10.3.1 or 10.7.1. Occasionally, interfering amorphous debris (ex: detritus or flocculent from a conventional treatment plant) prohibits a density of 10 plankters per field and these samples must be examined as described in section 11.0 at a particle density as dense as possible but without overlapping particulates that could obscure visualization of the plankters.

11.0 Microscopic Analysis

11.1 After application of sample, allow a 10 minute settling period before counting. The entire Palmer-Maloney counting chamber is systematically examined at a minimum of 100x magnification using phase optics, brightfield, DIC or HMO. Begin scanning the chamber at one edge and use an up-and-down or a side-to-side scanning pattern (see Figure 5). If the distribution of organisms is random and the population fits a Poisson distribution, the counting error may be estimated. If 100 units are counted, the 95 % confidence limits approximate ± 20 %. (3,4).

11.1.1 Separate counts are made for each of the following size ranges: < 10 um, 10 -25 um, 25 - 100 um, 100 - 200 um, > 200 um and total number and should be recorded on the data sheet. An ocular micrometer calibrated against a stage micrometer should be used when performing particle sizing. All categories from 11.1.4, as well as amorphous debris and unclassified biological material will be included in this count. Using a calibrated whipple grid (Appendix 1), count all particles, at a minimum of 100X magnification, present in a total of 20 - 30 whipple grid fields chosen randomly from the PMCC. Averaging methods are acceptable with densities too high for accurate counts. Record the number of particles found in each size range in each of the grid fields. Formula for calculation of # per 100 l is in section 11.2.3 and 11.2.5.

11.1.2 Any microbiota seen that are not too numerous to count at a minimum magnification of 100x are identified following the Standards of Identity section.

11.1.3 Identification to the lowest level of taxonomic resolution known by the analyst is recorded.

11.1.4 Separate counts are made for each of the following categories: nondiatomaceous algae, diatoms, plant debris, rotifers, nematodes, pollen, ameba, ciliates, colorless flagellates, crustaceans, other arthropods and "other" (see Standards of Identity section). Counts are made by the natural unit (clump count enumeration) method defined as follows: any unicellular organism or natural colony is counted as one organism. Alternatively, total count method can be used, defined as follows: each cell is counted as 1 organism. Making a total cell count is time-consuming and tedious, especially when colonies consist of thousands of individual cells. The natural unit is the most easily used system, however it is not necessarily the most accurate because sample handling, collection or water treatment may result in breakdown of organisms leading to inaccurate removal rates between raw and finished water samples. Analyst must report the enumeration method used. Counts are made for those categories of organisms that are not too numerous to count.

11.1.5 The counts for each category are extrapolated from the 0.1 mL aliquot used in the Palmer Cell to numbers per 100 Liters as follows:

11.1.5.1 Calculate X using this proportion ratio: *(For calculation example see Appendix 6.)*

$$\frac{\text{\# of organisms}}{0.1\text{mL}} = \frac{X}{V}$$

where: # of organisms = the count from one category
V = volume from which 0.1 ml aliquot was withdrawn
(derived from 10.3.1 or 10.7)

11.1.5.2 Calculate the number of organisms per 100 liters for each category using this proportion ratio:

$$\frac{X}{\text{liters equiv. of soln from which 0.1 ml aliquot was withdrawn}} = \frac{\text{number of organisms}}{100 \text{ liters}}$$

where:
X = calculation from 11.1.5.1

11.2 The common organisms, or particles that are too numerous to count at 100x are counted in calibrated Whipple grid fields at 400x. Specific calibration of each microscope used is essential *(see 12.0)*.

11.2.1 As many Whipple grid fields as necessary to obtain a minimum of a 100 organism count are observed. (95 % CL of approximately ± 20 %.) Use a single place hand held counter to count the number of fields observed. Use a manual differential counter (10 gang) or 10 place electronic tabulator to count the number of organisms observed in each of the categories listed in 11.1.4. Count only those categories that were not counted in section 11.1.4.

11.2.2 Record the number of organisms or particles found in each category as well as the identity of the organisms observed at the lowest level of taxonomic resolution known by the analyst.

11.2.3 Calculate the number of organisms or particles per mL in each category using the following formula:

$$\text{No./mL} = \frac{C \times 1000mm}{A \times D \times F}$$

where:

C = number of organisms counted for each category from 11.2.2
A = area of a field (Whipple grid image), mm^2 ($width^2$ - see 12.0)
D = depth of a field (P-M chamber depth = 0.4mm)
F = number of fields counted

11.2.4 Calculate the number of organisms in the remaining particulate solution from which 0.1 mL of solution with ≥10 plankters per field was withdrawn as follows:

$$\frac{N}{1mL} = \frac{X}{\text{mLs of remaining particulate solution}}$$

where:

N = number of organisms per mL (calculated in section 11.2.3)
X = number of organisms in the remaining particulate solution

11.2.5 Calculate the number of organisms per 100 liters using this proportion ratio:

$$\frac{X}{L} = \frac{\text{No. of organisms}}{\text{100 liters}}$$

where:

X = the number of organisms in the remaining particulate solution calculated in section 11.2.4.
L = liters equivalency of the remaining particulate solution (Total number of liters sampled less the liter equivalence of withdrawn subsamples).

12.0 Whipple Grid and Ocular Micrometer Calibration: Place a Whipple grid in an eyepiece of the microscope and a stage micrometer that has a standardized, accurately ruled scale on a glass slide. The whipple disk has an accurately ruled grid subdivided into 100 squares. One square near the center is subdivided further into 25 smaller squares. The outer dimensions of the grid are such that with a 10x objective and a 10x ocular, it delimits an area of approximately 1 mm^2 on the microscope stage. At 40x and with the ocular and stage micrometers parallel and in part superimposed, match the line at the left edge of the Whipple grid with the zero mark on the state micrometer scale. Determine the width of the Whipple grid image to the nearest 0.001 mm from the stage micrometer scale. *(APHA 1995)*

13.0 Centrifugate Pellet measurement. *(Figure 4)* Centrifugate pellet measurement may provide information about the overall plant performance. However, it is not intended to be used as a sole method for determining filtration efficiency.

13.1 If the liter equivalent in the 250 ml centrifuge bottle (10.3.1) is > 100 liters proceed to 13.1.1. If not, proceed to 13.2.1.

13.1.1. Centrifuge the 250 ml bottle at 1050 x g for 10 minutes. Measure the centrifugate pellet volume. If volumes are below lowest graduation, mark a "dummy" set of tubes using water injected from calibrated pipettes and compare to sample.

13.1.1.1. Calculate the volume of centrifugate pellet per 100 liters using the following proportion ratio:

$$\frac{\text{Total Centrifugate Pellet}}{\text{Liter equivalent in 250 ml centrifuge bottle}} = \frac{X}{100 \text{ liters}}$$

where:

Total Centrifugate pellet = volume of centrifugate from 13.1.1
X = The volume of centrifugate pellet per 100 liters

13.2.1 Remove a subsample from the remaining particulate solution equivalent to 100 liters. For example: if the total particulate solution is 4,000 mL representing 400 liters sampled, 1,000 mL would be removed.

13.2.1.1 Pour the subsample into 50 mL tubes. Centrifuge at 1050 x g for 10 minutes. Aspirate the supernatant down to 5 mL above the bottom sediment. The remaining subsample may be poured on top of the centrifugate pellet and remaining supernatant in the 50 mL tubes. Centrifuge again at 1050 x g for 10 minutes. This may be repeated until all of the 100 liter equivalent subsample has been centrifuged.

13.2.1.2 Aspirate the supernatant down to 5 mL above the centrifugate pellet. Combine the pellets and the remaining 5 mL of supernatant into one 50 mL tube. Centrifuge at 1050 x g for 10 minutes.

13.2.1.3 Record the centrifugate pellet volume.

14.0 Recording of Results and Procedural Parameters

14.1 Field data should include the following:

14.1.1 Total water volume filtered in gallons

14.1.2 Water source identified as to type and location

14.1.3 Record the type of filtration, any pretreatment and kind of disinfection

14.1.4 Record both address and exact location of water source being evaluated

14.1.5 Date and time of sample device installation and removal

14.1.6 Name, address and phone number(s) of sampler(s)

14.1.7 Field measurements, such as turbidity, pH, conductivity, chlorine residual

14.1.8 Record use of sodium thiosulfate if applicable

14.2 Laboratory data should include the following:

14.2.1 Total volume of packed pellet (centrifugate volume)

14.2.2 Number of each bioindicator from 0.1 ml sample aliquot. Proper significant figures should be used in calculations. Refer to ***Standard Methods for Water and Wastewater, 19th ed*** for details.

14.2.3 Number of particulates from each slide for the size ranges described in 11.1.1

14.2.4 Type of microscopy employed
- brightfield
- phase contrast
- other

14.2.5 Magnification of objective(s) used

14.2.6 Number of whipple fields per PMCC at 400 X or other magnification

14.2.7 Wash Solution constituents (e.g. Tween 20, Sodium citrate)

14.2.8 Sedgewick Rafter or Palmer Maloney Counting Cell used

14.2.9 Kind of count used; total or natural unit

15.0 Interpretation of Results: MPA for filtration plant optimization identifies and enumerates a subsample of the organisms/particles eluted from a HVF waterborne particulate sample collected through a 1 um nominal porosity filter cartridge. Other particulate measurements and observations are also reported. If raw and finished samples are analyzed, an estimate of filtration plant efficiency can be determined. In addition, since biological organisms are identified, potential filtration plant problems may be identified and can lead to optimization of plant operation.

15.1 Filter Color. The one micrometer filter cartridge changes color during sampling depending on the water's particulate composition and color as well as the amount of water sampled. The cartridge color can provide useful information about the general quality of water and can be used to make some process control decisions. For example: an efficient water treatment plant will often have a brown raw water sampling cartridge and a white finished water sampling cartridge. The presence of a green tinge on only the finished filter cartridge may indicate the presence of algae growth within the filter beds.

15.2 Centrifugate pellet volume. The centrifugate pellet volume in ml per 100 liters is a direct measurement of the final pellet of particulate matter recovered from the sampling cartridge after particulate elution and centrifugation. The percent reduction or log removal between raw and finished centrifugate pellet volume can be useful in interpretation of overall filtration plant efficiency. However, it is important to realize that the volume of pellet can be strongly influenced by sampling technique and other factors and therefore should not be used as the sole factor in determining filtration efficiency. Treatment problems may be identified when finished sediment is greater in volume than the raw sediment. Situations such as this may occur when excess treatment chemicals are used. Percent reduction of centrifugate volume through the treatment system is calculated as follows:

15.2.1 $$\% \text{ reduction} = \frac{(\text{raw centrifugate} - \text{finished centrifugate})}{\text{Raw centrifugate}} \times 100$$

$$\text{Log removal} = \text{Log (Raw Centrifugate)} - \text{Log (Fin. Centrifugate)}$$

Example:

Raw Water = 3.0 mL per 100 liters
Finished Water = 0.3 mL per 100 liters

$$\textbf{\% Reduction} = \frac{\textbf{3.0 mL (in) - 0.3 mL (out)}}{\textbf{3.0mL (in)}} \times 100 = \textbf{90\%}$$

The above example would equal 1.0 log removal.

15.3 Amorphous Debris. This category lists the types of inorganic matter and non-living organic matter (detritus) in order of predominance. Also included is the size range of these particulates observed under the microscope. Particulates are reported in numbers per 100 liters, using proper significant numbers (scientific notation is optional) as outlined in Standard Methods for the Examination of Water and Wastewater (19 ed, 1-17)

15.4 Nondiatomaceous Algae through Other (refer to data sheet). These categories signify the different types of organisms found and their respective numbers. Organisms are reported in numbers per 100 liters, using proper significant numbers. A qualitative approach can be taken for each category of organisms or a more objective approach can be taken by examining the total organisms percent reduction or log removal by the treatment system.

15.5 The percent reduction and log removal of organisms is calculated as follows:

15.5.1 Calculate the total number of organisms found in the raw water and in the finished water.

15.5.2 $$\% \text{ Reduction} = \frac{(\text{Raw total} - \text{Finished total})}{\text{Raw total}} \times 100$$

$$\text{Log Removal} = \text{Log}_{10}\,(\text{Raw total}) - \text{Log}_{10}\,(\text{Finished total})$$

15.5.3 Example:

Raw Water Total Organisms = 40,000,000/100 liters
Finished Water Total Organisms = 400,000/100 liters
% Reduction = 99 %, or 2 log removal. 99.9 % reduction is equivalent to 3 log removal.

15.5.4 Percent reduction and log removal can be approximated for plants with more than one raw water source if MGD or percent use records are kept for each source. Separate counts from each source are weighted by the appropriate percentage to calculate a total influent count.

For example: A water utility filters 21 MGD; it is composed of 14 MGD (67 % of total) of reservoir water and 7 MGD (33 % of total) from a river. Analysis of both influent sources provides the following information on algal content:

1) Reservoir contains 50,000 per 100 liter
2) River contains 100,000 per 100 liter

The total influent count to be used in percent log removal (river and reservoir combined) would be calculated as follows:

(50,000 x 0.67) + (100,000 x 0.33) = 60,000 algae per 100 liter.

15.5.5 Occasionally organism numbers may increase in the finished water, and it is suspected that either reproduction is occurring in the filter beds or another source of raw water is being introduced that has not been accounted for such as backwash return water or some other in-plant recycle operation. In such instances, information about the kind of organisms present in the finished water may assist the plant operator in improving plant operation. Likewise, the total centrifugate volume may increase in the finished water, or may demonstrate less log removal than the organism removal. For example, flocculent may be passing through the system. Identification of this substance microscopically, may provide useful information to the plant operator.

16.0 Analyst Qualifications

Interpretation of results derived from the consensus method will depend upon numerous factors, the most important of which will be the level of training and experience of the analyst(s) employing this technique.

16.1 Analyst should have a strong background in limnology and freshwater biology as well as an academic background and/or training in parasitology, protozoology, phycology, invertebrate zoology and bacteriology.

16.2 Analyst should have extensive experience with a light microscope with skills in brightfield, phase contrast and DIC or HMC microscopy.

16.3 Analyst should have experience in examining a sufficiently large number of Surface Water MPA samples.

16.4 A working knowledge of conventional treatment plants, slow and rapid sand filters, and alternative filtration methods is essential to providing adequate interpretation of the results and recommendations for controlling treatment plant conditions.

17.0 Standards of Identity

Diatoms: Diatoms are a group of algae which are distinctive because their cell wall is composed of silica. This contributes to their ability to resist environmental, mechanical and chemical insults. There are numerous species found in surface waters. They contain chlorophyll and need sunlight to live and reproduce. The size of these organisms is dependent on the nutritional quality of the water. Some species are known to be nuisance organisms because they clog filtration systems. A preponderance of 1 or 2 species in the finished water indicates possible reproduction or retention in the filter beds rather than the actual passage through the filtration plant. It is important to categorize the diatoms presence as living (containing internal structures) or dead (empty silica skeletal remains). The number of empty or dead diatoms may be of interest in certain types of filtration plants (e.g. effluent from DE filters frequently contains large numbers of empty diatoms).

Other Algae This category is comprised of a large number of chlorophyll containing filamentous, colonial and unicellular divisions of algae. Like diatoms, these genera of chlorophyll-bearing algae require sunlight for their metabolism. Surface water contains more than 10,000 known species with about 100 different species being commonly found. Diversity, abundance and organism size are dependent on available nutrients, water temperature, time of year, and other environmental and biological factors. Some species are known to be nuisance organisms causing taste and odor problems. Some cause filters to clog and add color to the water. Some species will reproduce in the filtration system and be present in the effluent. This can usually be detected because there is an overall decrease in the variety of species and an increase of only a few species between the raw and finished water.

Rotifers: A major taxonomic group with over 2500 species, of which more than 2375 species are of fresh water origin. They are associated with a variety of habitats including small puddles, damp soils and vegetable debris. They are also found associated with mosses, which can often be found in or around ground water sources. The vast majority of rotifers encountered are females ranging in size from 70-500 um. Rotifer growth in filtration beds has been suggested.

Plant debris: This group may be defined as either unidentifiable plant material containing chlorophyll or undigested fecal detritus from herbivorous animals, usually muskrat and beaver. Plant debris is very light weight material which is large in size (50 - 100 um). If the plant material is fecal detritus, it can suggest that animals are present in the watershed and may shed cysts or oocysts.

Nematodes: These include some 2,000 known free-living species found in fresh water. Some species show an amazing ability to survive and thrive in aquatic habitats under a wide range of ecological conditions. Benthic sediments of lakes and rivers can contain high numbers of nematodes, as can sewage effluent. The top layer of soil can contain over 1 million nematodes per square meter. Soil runoff is a major source of nematodes in source waters for treatment plants. Nematodes and/or their eggs are common in healthy water sources. Nematodes found in finished potable water do not portray a quality product to the public and may also compromise the microbiological integrity of the drinking water. These organisms seem to grow or reproduce in filter beds and distribution systems, so proper backwashing and super chlorination of the filter beds, as well as, proper maintenance of the distribution system should be conducted routinely.

Pollen: This includes all microspores produced by plants. In the spring and fall, pollen is everywhere, both airborne and waterborne. Because pollen can become trapped in the filter

cartridge during insertion of the filter or in the laboratory while the filter is being processed for examination, it is only rarely useful for assessing filtration efficiency, by itself.

Ameba: These include the ameboid, flagellated and cyst stages ranging in size from 10 to 600 um. This group is characterized by the formation of pseudopodia of one type or another. The external surfaces of these ameba are usually very thin compared to the cell coverings of ciliates and most flagellates. Most species are free-living and feed on bacteria, algae, other protozoa and debris. Ameba are common in surface waters and proper filtration removes them, but reproduction may occur in filter beds.

Ciliates: These free-living protozoa are very common. Ciliates are distinguished from other protozoa by the presence of a macronucleus. Like amoeba, they feed on bacteria, algae, small metazoa, other protozoa and debris. Proper filtration removes ciliates, but a few species may reproduce in filter beds.

Colorless flagellates:
Although many flagellates are phototrophic, there are many colorless species that grow in the absence of light if sufficient dissolved nutrients are available. They are common in surface water and can be removed by filtration; however, some species may reproduce in filter beds. Flagellates possessing chlorophyll are included in the algae category.

Crustaceans: These include all aquatic arthropods which have two pairs of antennae and are fundamentally biramous. The vast majority of known species (>35,000) are marine, but approximately 1,200 are found in freshwater. Adults range in size from 250 to 500 um, with eggs from 50 to 150 um. Several species occur in healthy surface and ground water. Daphnia and Bosmina species have been known to reproduce in very high numbers under the right environmental conditions and cause filter clogging problems for water treatment plants. Finished and distribution waters can contain large numbers of crustaceans. It is suspected that eggs will hatch in the filter beds or pass through the filters and hatch in the distribution system. Identification of crustaceans is often difficult because of fragmentation and observation of only small portions of the organism.

Other Arthropods:
There are a large number of organisms, all with jointed appendages, in the phylum Arthropoda. This category includes only the arthropods that are not classified as crustaceans or those which are identifiable only to the phylum level due to the decomposed and fragmented condition of the organism. Chironomid (insect) larvae and eggs are commonly reported in surface waters as are arthropod pieces. Seen less frequently are other insects, water mites and seed ticks.

Other: This category includes any organism seen that does not fit into the above categories. Examples include, iron bacteria, fungal spores, gastrotichs and/or tardigardes.

18.0 Quality Assurance. Listed below is the minimum recommended QC to be followed to under a laboratory QA/QC program. Documentation of testing is extremely important and careful records need to be maintained at all stages of analysis. Additionally, users of this method should develop their own internal QA/QC, and attempt to determine precision and bias at least at the analyst level for particulate counts.

18.1. QC on equipment and supplies.

18.1.1 Large capacity high/low speed centrifuge (preferably refrigerated).

18.1.1.1 Equipped with swing bucket rotors. Records maintained on rotor(s) usage at designated RPM. Post Manufacturer recommendation with regard to life time hours on rotor.

18.1.1.2 Rotor speed checked with tachometer on a yearly basis.

18.1.1.3 Determine and record RPM necessary for each rotor to attain desired g force. Post near centrifuge.

18.1.1.4 Annual PM agreement in force or internal maintenance protocol/records in place.

18.1.2 Brightfield/phase-contrast/DIC/HMO microscope (Appendix 1)

18.1.2.1 Phase rings checked for each objective before each use period. Kohler illumination adjusted for each objective for DIC/HMO.

18.1.2.2 Ocular micrometer (reticle) in place and calibrated against a stage micrometer for each objective in use. Calibration data posted near microscope. Re-check on an annual basis.

18.1.2.3 Microscope must be cleaned and optics realigned and adjusted on a frequent schedule.

18.1.2.4 Annual PM agreement in force or internal maintenance protocol/records in place.

18.1.2.5 Whipple grid used must be designed for installation into the laboratory's microscope ocular and must be calibrated against a stage micrometer.

18.1.3 Stomacher brand (model 3500) laboratory blender.

18.1.3.1 Operated according to manufacturers recommendations. The use of the blender is carefully timed to insure consistent washing of filter fibers. Stomacher is properly adjusted, with set screws, to accept entire filter.

18.1.3.2 Stomacher unit is maintained and internal paddle cover is cleaned/disinfected after each use with dilute detergent/bleach solution.

18.1.4 MPA sampling apparatus

18.1.4.1 Apparatus is cleaned with dilute detergent and bleach solution, rinsed thoroughly with hot tap water, followed by a particle-free water rinse in the lab. Apparatus is flushed with water in the field prior to inserting the filter.

18.1.4.2 Water meter is periodically checked for accuracy by timing the rate of flow into a measured gallon container.

18.1.5 Sample processing

18.1.5.1 All laboratory supplies used during sample processing are autoclaved or chemically sanitized.

18.1.5.2 Particle-free water has been tested and shown to contain less than 100 particles per 100 ml.

18.2 Analytical QC

18.2.1 Analyst has available identification keys and pictorial atlases to assist in classification of microbiota. *(see reference list)*.

18.2.2 Strict adherence to the Consensus Method and the definition of Standards of Identity will aid in maintaining intralaboratory and interlaboratory consistency.

References for Internal Document

1. Vasconcelos, G.J. and S.I. Harris. EPA in-house document. Microscopic Particulate Analysis and Particle Size Analysis for Determination of Filtration Efficiencies. EPA Manchester Environmental Laboratory.

2. Hancock, C.M., J.V. Ward, K.W. Hancock, P.T. Klonicki and G.D. Sturbaum. Assessing Water Treatment Plant Performance Using Microscopic Analysis (MPA). Proc AWWA WQTC Nov. 1994, San Francisco.

3. Palmer, C.M. and T.E. Maloney. 1954. **A New Counting Slide for Nannoplankton.** Amer. Soc. of Limn. and Oceano. special pub. no. 21, Environ. Health Center, Public Health Service, Cincinnati, Ohio.

4. Rashash, D. and D.L. Gallagher. 1995. An Evaluation of Algal Enumeration. Journal AWWA, April 1995. 127 - 132.

References for Microscopic Identification

American Public Health Association, American Water Works Association, Water Environment Federation. 1995. Standard Methods for the Examination of Water and Wastewater. Section 10200 F. Phytoplankton Counting Techniques & color plates. 19th Edition, A.P.H.A., Washington, D.C.

AWWA (1995) Selected Problem Organisms in Water Treatment. M7 Operator's Identification Guide.

A.P.H.A., A.W.W.A., W.E.F. 1994. Section 9711 B. Immunofluorescence Method for *Giardia* and *Cryptosporidium* spp. Standard Methods for the Examination of Water and Wastewater (Proposed). 19th Edition, A.P.H.A., Washington, DC, pp. 9-111.

Belehery, Hilary, 1979. An Illustrated Guide to Phytoplankton. H.M. Stationery Office, London.

Boutros, S.N. 1993. Microscopic Particulate Analysis (MPA) in Studies of Groundwater. Proc. AWWA WQTC, Nov. 15-19, 1992, Toronto, Ontario.

Brady, Nyle C. 1974. The Nature and Property of Soils. MacMillan Publishing, New York, 637 pp.

Federal Register, **Monitoring Requirements for Public Drinking Water Supplies**; Proposed Rule, February 10, 1994. 40 CFR Part 141. Vol. 59, No. 28 Proposed Rules.

Foged, Niels, 1981. Diatoms in Alaska, Bibliotecha phycoligica. J.Cramer Inder A.R. Gantner Verlag Kommaniditgesellschaft.

Garnett, W.J., 1965. Freshwater Microscopy in U.S. Dover Publishing, Inc., N.Y., N.Y.

Lee, J.J., Hunter, S.H. and E.C. Bovee, editors. 1985. An Illustrated Guide to the Protozoa. Society of Protozoologists, Lawrence, KS.

Lund, JWG and H. Canter-Lund, 1995. Freshwater Algae: Their Microscopic World Explored. Biopress Ltd., Bristol, England.

Palmer, C. Mervin, 1962. **Algae in Water Supplies**. U.S. Dept. of Health, Education and Welfare, Public Health Publication No. 657, Washington, D.C.

Pennak, R.W. 1989. **Freshwater Invertebrates of the United States - Protozoa to Mollusca**. 3rd edition. John Wiley and Sons, Inc., New York.

Pentecost, A. 1984. **Introduction to Freshwater Algae**. Richmond Publishing Co. Ltd., Richmond, England.

Pesez, Gaston, 1977. **Atlas de Microscopie des Eaux Douces**. 19 rue Augereau, Paris.

Prescott, G.W., 1954. **How to Know the Freshwater Algae**. In: Pictured Key Nature series, University of Montana Press, Montana.

Smith, G.M., 1950. 2nd ed. **Freshwater Algae of the United States**. McGraw Hill Book Co., Inc., N.Y.

United States Environmental Protection Agency. 1992. **Consensus Method for Determining Groundwaters Under the Direct Influence of Surface Water Using Microscopic Particulate Analysis (MPA)**. Manchester Environmental Laboratory Report #910/9-92-029. 53 p.

Vinyard, W.C. 1979. **Diatoms of North America**. Mad River Press, Inc., Eureka, CA.

Appendix 1.

The microscope portion of this procedure depends upon very sophisticated optics. Without proper alignment and adjustment of the microscope the instrument will not function at maximal efficiency and the probability of obtaining the desired image will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.

While microscopes from various vendors are configured somewhat differently, they all operate on the same general principles. Therefore, slight deviations or adjustments may be required to make these guidelines work for the particular instrument at hand.

1) **Transmitted Light Adjustment.** This section assumes that you have successfully replaced the transmitted bulb in your particular lamp socket and reconnect the lamp socket to the lamp house. Make sure that you have not touched any glass portion of the transmitted light bulb with your bare fingers while installing it. These instructions also assume the condenser has been adjusted to produce Kohler illumination.

   Step 1 Usually there is a diffuser lens between the lamp and the microscope which either must be removed or swung out of the light path. Reattach the lamp house to the microscope.

   Step 2 Using a prepared microscope slide and a 40 X objective, adjust the focus so the image in the oculars is sharply defined.

   Step 3 Without the ocular or Bertrand optics in place the pupil and filament image inside can be seen at the bottom of the tube.

   Step 4 Focus the lamp filament image with the appropriate adjustment on your lamp house.

   Step 5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on your lamp house.

   Step 6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

2) **Adjustment of Interpupillary Distance and Oculars for Each Eye.** These adjustments are necessary, so eye strain is reduced to a minimum. These adjustments must be made for each individual using the microscope. this section assumes the use of a binocular microscope.

   A) **Interpupillary Distance.** The spacing between the eyes varies from person to person and must be adjusted for each individual using the microscope.

      Step 1. Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

      Step 2. Using both hands, adjust the oculars in and out until a single circle of light is observed while looking through the two oculars with both eyes.

   B) **Ocular Adjustment for Each Eye.** This section assumes a focusing ocular(s). This adjustment can be made two ways, depending upon whether or not the microscope is capable of photomicrography and whether it is equipped with a photographic frame which can be seen through the binoculars. Precaution: Persons with astigmatic eyes should always wear their contact lenses or glasses when using the microscope.

1) **For microscopes not capable of photomicrography.** This section assumes only the right ocular is capable of adjustment.

Step 1. Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

Step 2. Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.

Step 3. Now transfer the card to between the left eye and ocular. Without touching the coarse or fine adjustment and with keeping both eyes open, bring the image for the left eye into sharp focus by adjusting the ocular collar at the top of the ocular.

2) **For microscopes capable of viewing a photographic frame through the viewing binoculars.** This section assumes both oculars are adjustable.

Step 1. Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

Step 2. After activating the photographic frame, place a card between the right ocular and eye keeping both eyes open. Using the correction (focusing) collar on the left ocular focus the left ocular until the double lines in the center of the frame are as sharply focused as possible.

Step 3. Now transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the image of the double lines in the center of the photographic frame into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

3) **Calibration of an Ocular Micrometer** (for Whipple grid also) This section assumes that an ocular reticle has been installed in one of the ocular by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed the ocular reticle should be left in place. The more an ocular is manipulated, the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is an optivar[3] on the microscope, then the calibration procedure must be done for the respective objective at each optivar setting.

Step 1. Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.

Step 2. Adjust the stage and ocular with the micrometer so the 0 line on the ocular is exactly superimposed on the 0 line on the stage micrometer.

Step 3. Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.

Registered trademark product of the Zeiss company. A device between the objectives and the oculars that is capable of adjusting the total magnification.

Step 4. Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition.

For example: Suppose 48 ocular micrometer space equal 0.6 mm.

Step 5. Calculate the number of mm/ocular micrometer space.

For example:

$$\frac{0.6\text{mm}}{48 \text{ ocular micrometer spaces}} = \frac{0.0125 \text{ mm}}{\text{ocular micrometer space}}$$

Step 6. Since most measurements of microorganisms are given in um rather than mm, the value calculated above must be converted to um by multiplying it by 1000 um/mm.

For example:

$$\frac{0.0125 \text{ mm}}{\text{Ocular Micrometer Space}} \times \frac{1{,}000 \text{ um}}{\text{mm}} = \frac{12.5 \text{ um}}{\text{Ocular Micrometer Space}}$$

Step 7. Follow steps 1 through 6 for each objective. It is helpful to record this information in a tabular format, like the example, which can be kept near the microscope.

Item #	Obj. Power	Description	No of Ocular Microm. Spaces	No. of Stage Microm. mm [a]	um/Ocular Micrometer Space [b]
1	10 X	N.A.[c] =			
2	20 X	N.A. =			
3	40 X	N.A. =			
4	100 X	N.A. =			

[a] 1000 um/mm

[b] (Stage Micrometer length in mm x (1000 um/mm) ÷ No. ocular micrometer spaces

[c] N.A. = Numerical aperture. The numerical aperture value is engraved on the barrel of the objective.

4) **Kohler Illumination.** This section assumes that Kohler illumination will be established for each DIC or HMO objective. Each time the objective is changed, Kohler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Kohler illumination is not properly established, then DIC or HMO woptics will not work to their maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore.

Step 1. Place a prepared slide on the microscope stage, move the required objective into place, turn on the transmitted light, focus the specimen image using the coarse and fine adjustment knobs.

Step 2. At this point, both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.

Step 3. Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the filed to the center of the visual field.

Step 4. Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up or down while you are looking through the binoculars. Once you have accomplished the precise focusing of the iris field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

Step 5. The aperture diaphragm of the condenser is adjusted now to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

Step 6. After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish either DIC or HMO optics.

Appendix 2.

Use of Electronic Particle Counter

Electronic Particle Counting for Filtration Plant Optimization

Introduction

Alternative methods for determining filtration efficiency, such as particle size analysis, have been recommended in the "Guidance Manual for Compliance with Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources", USEPA, March 91. The data accumulated from particle size analysis should be used in conjunction with microscopic particulate analysis data to determine over-all filtration efficiency. The Federal Register, Vol. 59 number 28, Feb. 10, 1994, states that particle counting data could be used as a tool for treatment process efficiencies and could possibly be used as a surrogate for *Giardia* and *Cryptosporidium* monitoring. Either electronic particle counters or treatment plant in-line installations that measure continuously can be used. These instruments give particle size ranges and the number of particles per size range. A comparison of raw water particle counts verses the finished water particle counts can be used to calculate percent removal or log reduction and an estimate of filtration efficiency established.

Sample Collection

1.0 The samples collected for electronic particle counting can be grab samples or composite samples. If a composite sample is collected, then the procedure used to collect and process the sample is described in the MPA for Filtration Plant Optimization procedure. A subsample from this composite sample will need to be diluted with particle free water before analysis. However, there are potential problems with composite sampling that need research and depend greatly on the type of water being analyzed.

2.0 Grab samples require little processing and may be useful for this reason. The main concern with the grab sample is that collection occurs over a very discrete amount of time and therefore may not be representative of the water supply. This problem can be resolved by either showing repeatability or by comparing the grab sample results to a composite sample which is more representative.

3.0 **Grab sampling:** "dedicated" glass containers are to be used. A dedicated container is one which is used exclusively for a certain type of water (one set for raw waters and another set for finished waters). These containers should be cylindrical glass bottles that have been scrupulously cleaned with a mild laboratory detergent and then rinsed a minimum of three times in particle-free water. Prior to sampling, the container should be rinsed a minimum of three times with the water being sampled. Plastic bottles should be avoided because they may shed particles into the sample.

4.0 **Collection**: Collect grab samples as close to the source as possible. A continuously flowing tap is recommended, if not available, flush the sample tap for 5 minutes prior to sampling. Run the water down the inside of the bottle to lessen air entrapment. Make sure to label the bottle: sample identification, date, time, and sampler name.

5.0 **Holding time:** Analyze as soon as possible after collection. Raw samples are especially prone to organic growth, adsorption of particles to the bottle walls and decay of original sample, all resulting in alteration of the particles. Filtered, or otherwise treated samples, are not quite as critical, but should be analyzed assoon as possible. If analysis can not happen immediately samples should be stored at 4°C and sealed with a teflon screw bottle cap. Do not expose the sample to sunlight or let it freeze.

Particle Analysis

6.0 Analysis by electronic counter for grab and composite samples: Electronic counting can be performed by one of two types of counters; a light blockage device (HIAC, Met One, Hach or Particle Monitoring System to name a few) or an electrical sensing zone device (Coulter Counter or Elzone).

7.0 Prior to analysis, verify the instrument's sizing capabilities according to manufacturer's instructions using latex beads representing the particle sizes of interest. If a light blockage device is used, the calibration is done during installation and on a routine basis recommended by the manufacturer. The most widely accepted dimensions of *Giardia* cysts are 7- 12 um. *Cryptosporidium* oocysts are in the dimensional range of 3 - 7 um. Individual electronic particle counters measure these organisms differently. Testing of individual instruments will be needed to determine the actual size measured by a specific particle counter.

8.0 If sample has been refrigerated bring it to room temperature very slowly. Mix samples gently, by swirling just prior to analysis. Minimize bubble formation, do not shake.

9.0 Run at least 3 rinse samples with particle free water to stabilize the instrument.

10.0 After running the sample in triplicate, run a rinse with particle free water or electrolyte to re-stabilize the instrument.

11.0 Always run the cleanest sample first and proceed to the most concentrated.

12.0 Between analyses, keep particle-free solution in the instrument chamber. The sample is run in triplicate to assess the instrument precision. The individual results should not vary more than 10 % from the average of the three runs, except in low particle count water (less than 10 particles per mL).

13.0 To avoid coincidence errors, in concentrated samples (raw samples), dilution of the final sample will undoubtedly be required. Coincidence occurs when more than one particle passes through the detector at a time, causing inaccurate counting and diameter measurements. This dilution should be done with the particle-free solution recommended by the manufacturer. Generally speaking, a dilution between 1:5 to 1:20 with particle-free solution will suffice. It is best to use a dilution as close to tolerance for coincidence error as possible to decrease the number of background counts. It may also be necessary to screen filter the sample if large debris repeatedly block the orifice tube. For composite samples 1:1000 dilutions are not uncommon. Any pipettes or glassware used to make dilutions should be calibrated.

14.0 The average of the three values obtained are recorded in the corresponding box on the data sheet.

15.0 Calculation of the percent removal and log reductions can be done for both the total number of particles or for each size range. **See example data sheet.**

References for Particle Counting:

1. Vasconcelos, G.J. and S.I. Harris. Procedure draft. **Microscopic Particulate Analysis and Particle Size Analysis for Determination of Filtration Efficiencies**. EPA Manchester Environmental Laboratory.

2. A.P.H.A., A.W.W.A, W.E.F. 1995. Section 2560 Particle Counting and Size Distribution (Proposed) in: **19th Edition Standard Methods**, A.P.H.A., Washington, DC, pp 2-60.

3. Federal Register. 1994. **Proposed Monitoring Requirements for Public Drinking Water Supplies: *Cryptosporidium*, *Giardia*, Viruses, Disinfection Byproducts, Water Treatment Plant Data and Other Information Requirements**; Proposed Rule, February 10, 1994. 40 CFR Part 141. Vol. 59, No. 28 Proposed Rules. pp 6336.

4. Hargesheimer, E.E., C.M. Lewis, and C.M Yentsch 1992. **Evaluation of Particle Counting as a Measure of Treatment Plant Performance.** A.W.W.A. Research Foundation Denver . pp 319.

5. USEPA. Science and Technology Branch. 1991. **Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems using Surface Water Sources. Criteria and Standards Division, Office of Drinking Water, Washington, D.C.**

Appendix 3
Sample Data Forms and Report Forms

Microscopic Particulate and Particle Size Analysis
For Water Treatment Evaluations

Lab # Raw_____ Sampler's name _____
Lab # Finished _____ Agency _____
Water Treatment Plant _____
Sample sites: Raw _____ Finished_____
Raw water source: *(circle one)*

River Creek Spring well
Infiltration gallery Horizontal Collector Other _____

Field Measurements:

	T. Cl	F. Cl	Turb (NTU)	pH	Temp (C)	TC/ 100 ml	FC/ 100ml
Raw							
Finished							

Operational Parameters: circle appropriate choice

Pre-treatment	alum	lime	Polymer	carbon	other
Filtration	R. sand	S.sand	Press. Filter	Cartr	other
Disinfection	Cl	Cl-A	Ozone	Other	

Processing Information:

	Raw	Finished
Total volume filtered (Liters)		
Total filter sediment collected (Packed pellet) (g)		
Centrifugate volume/ 100 liters		

Particle Size Distribution and Percent Removal Using Microscope.

Average of 20 - 30 fields @ 100 X magnification

Particle Size category	Number in Raw Water	Number in Finished Water	Percent Removal
< 10 um			
10 - 25 um			
25 - 100 um			
100 - 200 um			
> 200 um			
Total			

Particle Size Distribution and Percent Removal Using Electronic Particle Counter.

Channels	Number in Raw Water	Number in Finished Water	Percent Removal

Particle Counting Example

Filtration Efficiency

Using an Electronic Particle Counter*

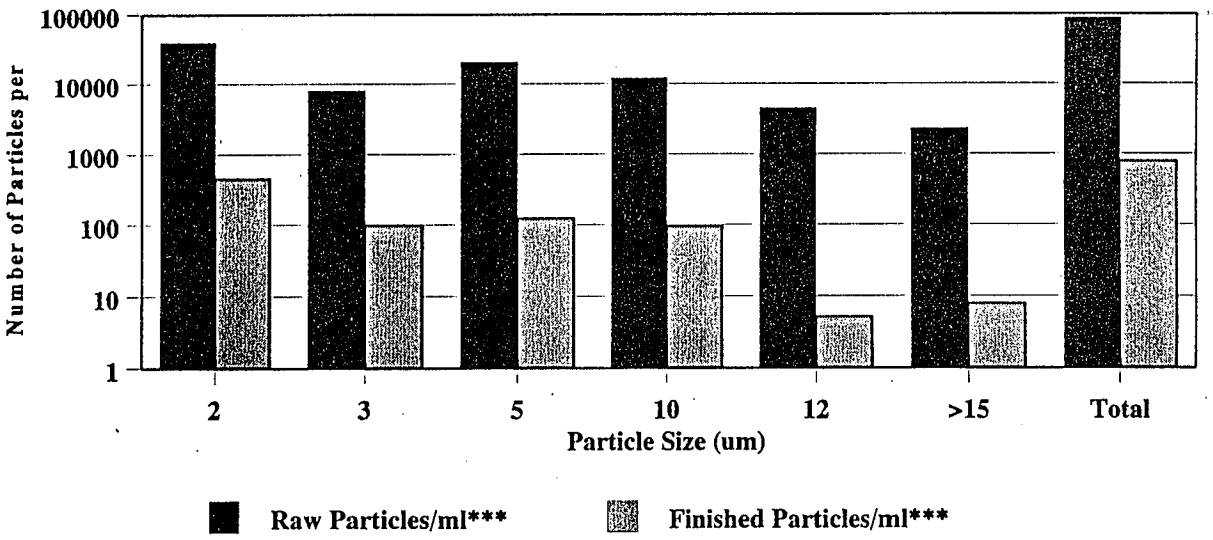
Date:	**May 20, 1994**
Raw Water:	**Raw River Water**
Finished Water:	**Finished Water**

Samples:	**Composite or Grab**
Code number:	**2**
Code number:	**1**

*Particle size (um)***	*Raw Particles/mL ****	*Finished Particles/ml ****	*% Reduction*	*Log Reduction*
2	**40,556.3**	**456.3**	**98.8749 %**	**1.9488**
3	**8,225.6**	**100**	**98.7843**	**1.9152**
5	**20,985.5**	**125**	**99.4044**	**2.225**
10	**12,358.8**	**96.6**	**99.2184**	**2.107**
12	**4,569.8**	**5.2**	**99.8862**	**2.9439**
> 15	**2345.6**	**8**	**99.6589**	**2.4672**
Total	**89,041.6**	**791**	**99.1115**	**2.0514**

*All limitations of the analytical methods, laboratory dilutions and instrument apply.
**Measured in equivalent spherical diameter.
***Numbers represent the average from 3 subsample counts.

Raw vs. Finished



Analysis for Waterborne Particulates

Customer <CUSTOMER_NO>
<CUSTOMER>
<ADDRESS>
<CITY_STATE_ZIP>

PWSID# <PWSID>

Sample Information: <SAMPLE_ID> <SAMPLE_INFO>
Date/Start: <DATE_START> Hrs **Date/Stop:** <DATE_ST> Hrs **Sampler:** <SAMPLER>
Gallons: <GALS> **Filter Color:** <FILTER_COLOR> **Centrifugate:** <CENTRIF> mL/100 gals

Results of Microscopic Particulate Analysis[1]:

Amorphous Debris:	<AMORPHOUS_DEBRIS> μM diameter <RANGE>
Nondiatomaceous Algae:	<ALGAE_VALUE> <ALGAE_SPECIES>
Diatoms:	<DIATOMS_VALUE> <DIATOMS_SPECIES>
Plant Debris:	<PLANT_DEBRIS>
Rotifers:	<ROTIFERS>
Nematodes:	<NEMATODES>
Pollen:	<POLLEN>
Ameba:	<AMEBA>
Ciliates:	<CILIATES>
Colorless Flagellates:	<COLORLESS_FLAG>
Crustaceans:	<CRUSTACEANS>
Other Arthropods:	<OTHER_ARTHROPODS>
Other:	<OTHER>

Comments: <COMMENTS> <COMMENTS2>
Type of Wash water used; total or natural count used, type of counting chamber used

Laboratory Information:
<DELIVERY>; <DEL_DATE>; <DEL_TIME> Hrs; <FILTER_TYPE>; <FILTER_COND>;
Results submitted by:

Appendix 4
Formulation of McFarland Standards

McFarland standards provide laboratory guidnace for the standardization of numbers of bacteria for susceptibility testing or other procedures requiring a standardization of inoculum. They are devised to replace the counting of individual cells are are designed to correspond to appropriximate cell densities.

1) Make solution 1 1 % H_2SO_4
Make solution 2 1.175 % $BaCl_2$

Dispense in the following amounts of desired standard totaling 10 ml in 16 X 125 mm tubes. Cap and label. Should be replaced every six months.

Standard Concentration	$BaCl_2$ Volume in ml	H_2SO_4 volume in ml
0.5	0.05 ml	9.95 ml
1	0.1 ml	9.9 ml
2	0.2 ml	9.8 ml
3	0.3 ml	9.7 ml
4	0.4 ml	9.6 ml
5	0.5 ml	9.5 ml

Appendix 5
Figures for Document

Figure 1 Raw Water Sampling Apparatus

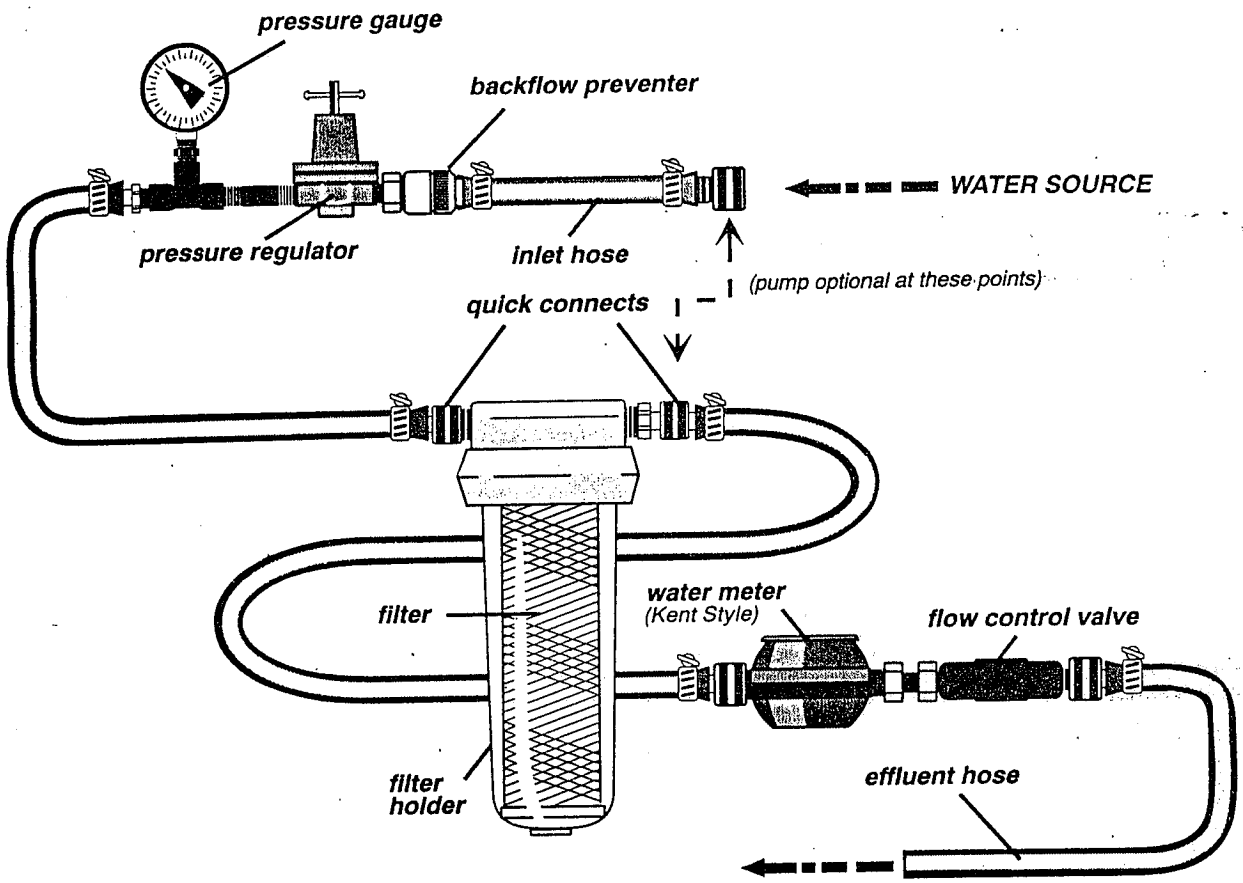


Figure 2 **Finished Water Sampling Apparatus**
(For chlorinated water only)

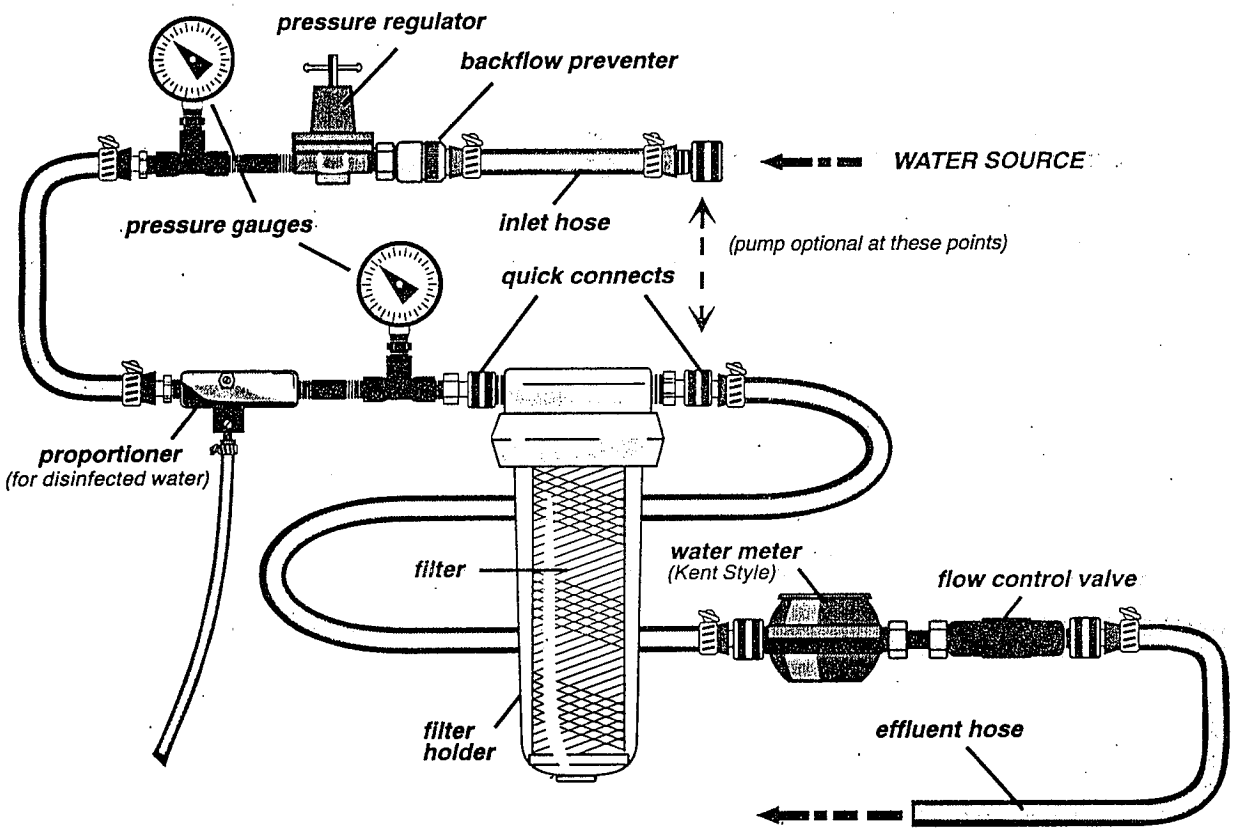


Figure 3 **Processing Flow Chart for Palmer Maloney Counting Chamber**

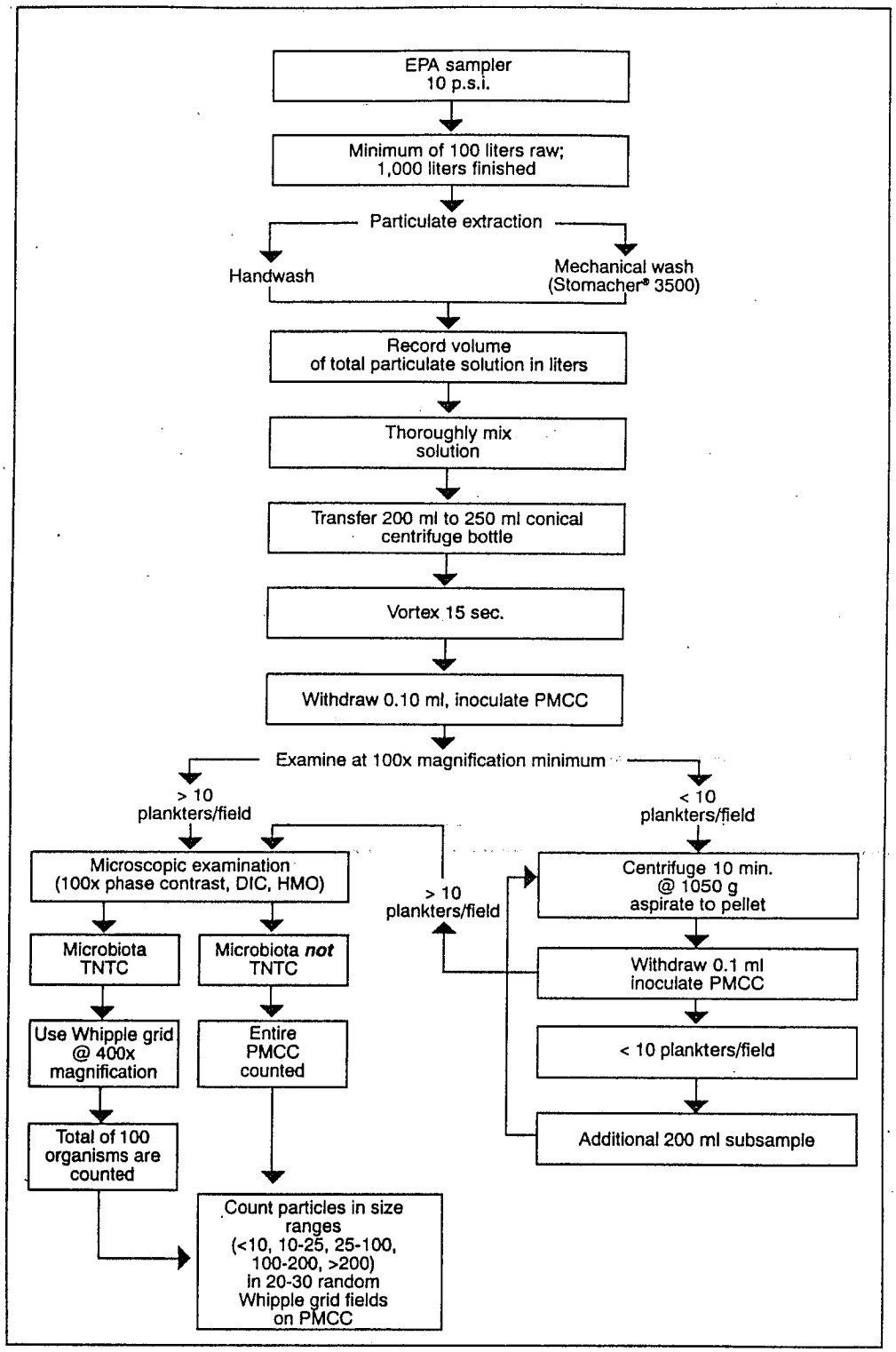


Figure 4 **Centrifugate Pellet Volume Determination**

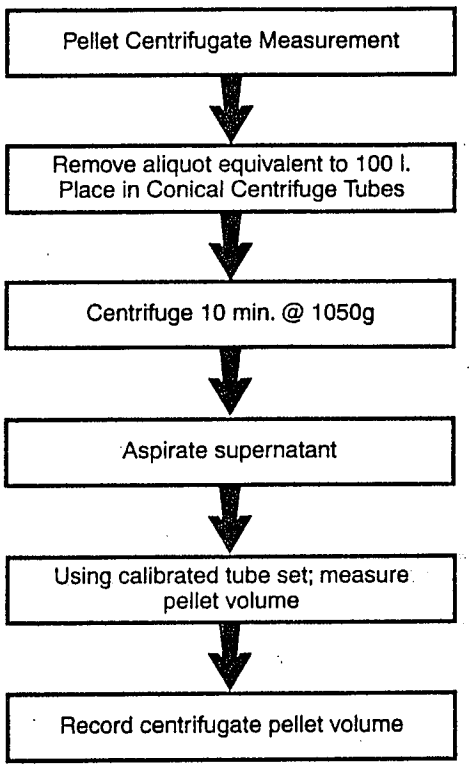
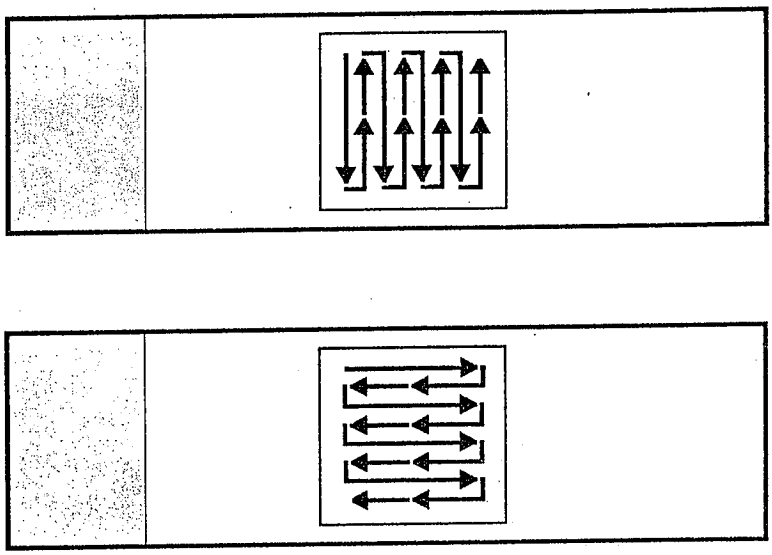


Figure 5 **Scanning Cover Slip Method**



Appendix 6
Example of Sample Calculation

Assume that a 1000 Liter (380 gal) water sample was collected. The sample was eluted resulting in 3 L of particulate solution. The solution was thoroughly mixed and a 200 mL aliquot was placed in a centrifuge bottle. A 0.1mL aliquot from the centrifuge bottle was examined in a Palmer-Maloney Chamber but only 5 plankters per field were observed, so the 200 mL subsample was centrifuged and 150 mL of the supernatant was aspirated. The remaining 50 mL of supernatant and pellet was thoroughly mixed before 0.1 mL was examined in a Palmer-Maloney Chamber. Because 20 plankters per field were observed, the plankters were identified and counted.

The liter equivalent in the centrifuge bottle was calculated based upon these facts:

$$\frac{\text{3000 mL of particulate solution}}{\text{1000 L sampled}} = \frac{\text{200 mL aliquot in centrifuge bottle}}{\text{66.667 Liter equivalent in centrifuge bottle}}$$

The liter equivalent in the first 0.1 mL subsample (x), where 5 plankters per field were observed, is calculated using the proportion ratio in 10.3.1:

$$\frac{\text{200 mL in centrifuge bottle}}{\text{66.667 Liter equivalent in centrifuge bottle}} = \frac{\text{0.1 mL [observed in Palmer-Maloney Chamber]}}{\text{x = 0.033 Liter}}$$

The liter equivalent of the remaining 50 mL of supernatant and pellet is 66.634 which is the 66.667 Liter equivalent in the centrifuge bottle prior to centrifugation minus the 0.033 L equivalent withdrawn in the first 0.1 mL subsample (calculated in 10.3.1).

The following organisms were identified and counted in the second 0.1mL subsample when the entire Palmer-Maloney Cell is scanned at 100x:

10 *Keratella* (rotifers)
3 *Vorticella* (ciliates)
1 Nematode

The numbers per 100 Liter are calculated using the proportion ratios in 11.1.5.1 and 11.1.5.2:

$$\frac{\text{10 } \textit{Keratella}}{\text{0.1 mL}} = \frac{\text{x = 5,000}}{\text{50 mL}}$$

Because the 50 mL of particulate solution in the centrifuge bottle is equivalent to 66.634 L:

$$\frac{\text{5,000}}{\text{66.634 L equivalent}} = \frac{\text{7503 } \textit{Keratella}}{\text{100 L}}$$

Similarly,

$$\frac{\text{3 } \textit{Vorticella}}{\text{0.1mL}} = \frac{\text{1500}}{\text{50 mL}}$$

$$\frac{\text{1500}}{\text{66.634 L}} = \frac{\text{2254 } \textit{Vorticella}}{\text{100 L}}$$

and

$$\frac{1 \text{ Nematode}}{0.1 \text{ mL}} = \frac{500}{50 \text{ mL}}$$

$$\frac{500}{66.634 \text{ L}} = \frac{750 \text{ Nematodes}}{100 \text{ L}}$$

The algae were too numerous to count at 100x so 100 algal cells were counted in 5 whipple grid fields at 400x. The number of algae is calculated as in 11.2.3, 11.2.4, and 11.2.5:

$$\frac{100 \text{ alga counted} \quad x \quad 1000mm}{A \times 0.4 \times 5 \text{ fields}} = \text{No. algae/mL}$$

A = the area of the whipple grid field, which must be calculated for the microscope used for the counts (section 12.0). For example A = .074. (0.4 is the depth of the Palmer-Maloney chamber.)

then,

$$\frac{100 \quad x \quad 1000}{.074 \times .4 \times 5} = 675{,}675.67 \text{algal cells/mL}$$

then,

$$\frac{675{,}675.67}{\text{mL}50} = \frac{33{,}783{,}783}{\text{mLs}}$$

and because 50 mL is equivalent to 66.634 L:

$$\frac{33{,}387{,}783}{66.634\text{L}} = \frac{50{,}700{,}500 \text{ Algae}}{100 \text{ Liters}}$$

Report the organism values in significant figures using the guidelines in Standard Methods. For this example the values would be reported in #'s/100 L as follows:

8,000 *Keratella*
2,000 *Vorticella*
800 Nematodes
50,000,000 Algae