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Microscopic Particulate Analysis Consensus Method

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1 Scope and Application

The Microscopic Particulate Analysis (MPA) is a consensus methodology developed for the purpose of identifying those public water systems using groundwater under the direct influence of surface water. Samples are processed by washing the fibers from the MPA filter in a lab stomacher. The resulting sediment is centrifuged into a pellet. Then, depending on the size of the pellet, the sediment is either purified by a gradient floatation procedure or is analyzed directly. Slides are prepared and examined for "bioindicators" such as alga, protozoa, insects and other particulates that are characteristic of surface waters.

The consensus method attempts to equate quantitatively the significant occurrence of primary and secondary indicator organisms to a relative risk score for a particular water supply. It should be emphasized that surface water influence on a groundwater source cannot be determined solely on the basis of MPA. Other pertinent information as described in the USEPA Guidance Manual and elsewhere should be gathered from each individual source in accordance with criteria established by the primary agency. The MPA consensus protocol should be regarded as a tentative method with limited recovery efficiency data available for review. The absence of bio-indicators indicates a negative sample to the extent of the detection limits of the analysis performed; it does not ensure that the source is pathogen-free. Conversely, a positive MPA result does not necessarily signify the presence of pathogens.

2 Definitions

Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions. (SOP Reference 13.3)

3 Interferences

Method interferences may be caused by contaminants in glassware. To abstain from interferences, all glassware must be washed, sterilized and put in the hot air

oven at 180 ºC for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants.

4 Safety

Refer to Laboratory Safety/ Chemical Hygiene Plan & Fire Safety Plan, online revision. (SOP Reference 13.5)

5 Apparatus and Equipment

5.1 Large capacity centrifuge with swing bucket rotor
5.2 250 mL conical centrifuge bottles with screw caps
5.3 500 mL conical centrifuge bottles with screw caps
5.4 Phase contrast or DIC microscope with digital camera system
5.5 Stomacher Lab blender
5.6 Stomacher bags, 3.5 L capacity
5.7 Vortex tube mixer
5.8 Aspiration flask and vacuum source with 0-30 psi gauge
5.9 10 mL pipets and pipet aid
5.10 Micropipette, multivolume
5.11 Whirlpac bags, 5.5X15", sterile
5.12 Polypropylene yarn wound filter tubes (M39R10A)
5.13 Stainless steel pan
5.14 Long tweezers
5.15 Disposable sterile scalpel or utility knife
5.16 4 L and 200 mL beakers
5.17 Pasteur pipettes
5.18 15 mL and 50 mL conical centrifuge tubes
5.19 Microscope slides and coverslips (22X22 mm)
5.20 Immersion oil
5.21 Hydrometer
5.22 Hotplate/Stirrer
5.23 250 mL graduated cylinders

6 Reagents

6.1 Distilled water
6.2 Percoll
6.3 *0.01% Tween 20 (Polysorbate 20)*: Pipette 100uL of Tween into 1L of DI water. Use within one week.
6.4 *2.5 M Sucrose Solution*: Measure 171.16g of sucrose and pre-warm 200mL of DI water to 200˚C. In another beaker, pour in 40mL of the pre-warmed water and begin stirring at a medium speed. While continuing to stir, slowly pour half of sucrose in. After half has been poured, pour in another 40 mL of water. Slowly pour in the remaining half of sucrose. Bring total volume of mixture up to 200mL with remaining water and

loosely cover with foil. Allow mixture to cool before making floatation solution.

6.5 *Percoll – Sucrose Floatation Solution*: Add 124mL of the 2.5 M Sucrose solution and 62ml of Percoll to 100mL of DI water. Mix ingredients thoroughly. Measure specific gravity with hydrometer. Do not use if Sp gravity is less than 1.15. Store at 1-4˚C. Use within one week. (Allow solution to reach room temperature before use.)

6.6 Clear nail polish or Permount (for slides)

7 Sample Collection

Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation and Sample Holding Times. (SOP Reference 13.3)

8 Calibration

There are no calibrations associated with this method. Maintain sterility with equipment, reagents and technique.

9 Quality Control

Refer to Section 14.1 for Quality Control Acceptance Criteria associated with this method.

10 Procedure

10.1 Cutting filter

The filter is cut in half lengthwise to the plastic core using a sterile surgical quality scalpel or utility knife. Cut string filter in the sterile SS pan. Cutting in this manner should result in string fibers, approximately 2 inches in length.

10.2 Cut fibers from the string filter are mechanically agitated using a stomacher lab blender

After the filter is cut into halves, each half of the two inch long fibers are teased apart with tweezers and placed in a 3.5L capacity sterile stomacher bag with 1.5L of .01% polysorbate (Tween) 20/80 water. The filter fibers in each bag are homogenized using the stomacher lab blender for three, three-min intervals over a 15 min. period. In between each three min. interval, the fibers are hand-kneaded to redistribute them within the bag. After homogenization, the liquid contents of each bag is poured into a 4 L collection beaker after which the filter fibers are wrung out into the beaker by cutting a corner of the stomacher bag.

10.3 Centrifuge Washings

After mechanical homogenization, the resulting wash water is poured into six 500ml centrifuge bottles and centrifuged at 1050xg (2144 RPMs) for 10 min. using a large capacity swing bucket rotor. To prevent swilling, make sure the brake control is off allowing the rotor to decelerate slowly.

10.4 Pooling of sediments

Aspirate the supernatant from each centrifuge bottle approximately 5ml above the pellet. Collectively combine the sediments by rinsing the bottles into a small beaker using a minimal amount of Tween water from a squirt bottle. **Note: During aspiration, pressure gauge should not exceed 11.8 in. Hg.**

10.5 Centrifugation of pooled sediment

The unpreserved sediment from section 10.4 is resuspended by mixing (stirbar) for 1-4 min. and transferring into two or more 50 mL or 15 mL conical centrifuge tubes and spun at 1050xg (1100 RCF) for 10 min. As much of the supernatant as possible is aspirated from the tubes and discarded. Observe and record the total packed pellet volume using graduations on the tubes. If volumes are below lowest graduation, mark a "dummy" set of tubes at 150 and 300 uL and visually compare to sample.

10.5.1 If volume of pooled sediments is below a concentration of 20uL/100 gallons, examine directly without Percoll-Sucrose floatation solution. Continue in section 10.7.

10.5.2 If concentration of sediment is >20 uL/100 gallons, weigh the pellet (note: 1 gram of sediment is approximately equal to 0.5 mL). Then resuspend the sediment in each tube with particle free DI water, filling 50 mL tubes to the 40 mark or 15 mL tubes to the 10 mark. Resuspend using a vortex tube mixer. Proceed to section 10.6.

10.6 Percoll/sucrose gradient procedure

10.6.1 Prepare isotonic Percoll /sucrose gradient solution. When overlying, add 75 mL Percoll/sucrose to 250 mL centrifuge bottles or 30 mL to 50 mL centrifuge tubes.

10.6.2 Resuspend the sediments by vortexing for 15-30 seconds. Then carefully layer the suspension onto gradient using a 10 mL pipet. Gently add to sides of centrifuge tube making sure not to disturb gradient interface. (Refer to pellet weight - add no more than 1 g sediment/25 mL Percoll/sucrose.)

10.6.3 After overlaying, place centrifuge tubes on lab bench at room temperature and allow to settle by gravity (static) for 5 min. Do not centrifuge.

10.6.4 If no visible settling occurs after 5 min., centrifuge tubes for 5 min. @ 650xg (700 RCF) if using 50mL tubes or @1687 RPMS if using 250mL centrifuge tubes.

10.6.5 After centrifugation, aspirate down to first cloudy layer and carefully transfer remaining liquid into 5X vol. particle free DI water to dilute Percoll/sucrose (use 250mL or 500 mL conical tube). If the packed sediment in bottom of tube or bottle represents a significant portion of the floated sample (>50 uL/100 gallons), examine at least one slide directly (wet mount) or re-float as in section 10.6.2.

10.6.6 Centrifuge diluted Percoll/sucrose liquid at 650xg (1687 RPMs) for 10 min. Aspirate and retain second pellet.

10.6.7 This second pellet is vortexed for 10-30 sec. with an equal amount (v/v) or 10 mL of sterile DI/Dist water (which ever is greatest) and poured into a new 50mL graduated conical centrifuge tube. Wash sides of tube with squeeze bottle of DI water to insure complete transfer. Vortex (10-30 sec) and centrifuge at 650xg (700 RCF) for 10 min. Aspirate all water down to pellet. This represents the second and final washing.

10.7 Second and final pellet

The vol. of this final pellet is measured in uL, recorded and diluted 1:1 (v/v) or greater using particle-free DI water.

10.7.1 Vortex for 10-30 sec.

10.7.2 Using a micropipette place 20 uL portions onto a standard glass slide and cover with a 22 X 22 mm cover slip.

10.7.3 Drop cover slip in such a manner that an even distribution of particulates occurs on the slide.

10.7.4 Seal with clear nail polish.

10.8 Microscope examination

10.8.1 Analysis can be done by either brightfield, phase-contrast or differential interference contrast (DIC).

10.8.2 Immediately scan entire area of prepared slides and count all bio-indicators @ 100 X.

10.8.3 Counting of other particulates such as amorphous debris, minerals, pollen, etc. is optional but noting their relative concentration per 100 gal is recommended.

10.8.4 Identify all microbiota to at least phyla or class level.

10.8.5 Record and document rare, unusual or unidentifiable microbiota if possible by using a digital camera system.

10.8.6 For questionable microbiota, use a calibrated vertical ocular micrometer to measure the size of the various bio-indicators or other particulates.

10.9 Amount of final pellet to be examined

10.9.1 If final diluted pellet is >200 ul, prepare additional slides (20ul/slide) until the sediment equivalent of 100 gal. of filtered water has been examined.

$$\text{\# of slides to examine} = \frac{(\text{uL of pellet}) \times (\text{dilution factor})}{(\text{\# 100 gal units}) \times (\text{uL per slide})}$$

10.9.2 If the final diluted pellet is 200 uL or less examine entire amount (20 uL/slide = 10 slides).

10.10 Recording of results and procedural parameters

10.10.1 Tally all microbiota and particulates observed.

10.10.2 Record results on bench worksheet.

11 Calculations

Refer to Table 1 and Table 2 below to calculate the Risk factor.

12 Waste Management

12.1 See GA EPD SOP – EPD Laboratory Waste Management Standard Operating Procedures, online revision. (SOP Reference 13.4)

13 References

13.1 EPA Consensus Method for Determining Groundwaters under the Direct Influence of Surface Water Using the Microscopic Particulate Analysis (MPA), October 1992.
13.2 Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources", March, 1991 ed.
13.3 GA EPD Laboratory Quality Assurance Plan, online revision.
13.4 GA EPD Laboratory SOP – EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.5 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Practical Quantitation Limits (PQLs) Precision and Accuracy Criteria, and Quality Control Approach

No PQLs associated with this method.

Table 1. Numerical Range of Each Primary Bio-Indicator Based on Numbers Counted per 100 gallons of Water

	EH	H	M	R	NS
Giardia	> 30	16-30	6-15	1-5	<1
Coccidia	> 30	16-30	6-15	1- 5	<1
Diatoms	> 150	41-149	11-40	1-10	<1
other Algae	> 300	96-299	21-95	1-20	<1
Insects/ Larvae	> 100	31- 99	16-30	1-15	<1
Rotifiers/ Crustacea	> 150	61-149	21-60	1-20	<1
Plant Debris	> 200	71-200	26-70	1-25	<1

1. According to EPA "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources", March, 1991 ed.
2. If Giardia cycts or coccidia are found in any sample, irrespective of volume, score as above.
3. Key= **EH** - Extremely Heavy; **H**- Heavy; **M** - Moderate; **R**- Rare; **NS** - Not Significant
4. Chlorophyll containing.

Table 2. Relative Risk Factors Associated with Primary Bio-Indicators Based on Numbers Counted per 100 gallons of Water.

	EH	H	M	R	NS
Giardia	40	30	25	20	0
Coccidia	35	30	25	20	0
Diatoms	16	13	11	6	0
other Algae	14	12	9	4	0
Insects/ Larvae	9	7	5	3	0
Rotifiers/ Crustacea	4	3	2	1	0
Plant Debris	3	2	1	0	0

1. According to EPA "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources", March, 1991 ed.
2. Refer to Table 1 for range of indicators counted per 100 gallons.
   Key= **EH** - Extremely Heavy; **H**- Heavy; **M** - Moderate; **R**- Rare; **NS** - Not Significant.
3. Risk of surface water contamination:
   ≥20 – High Risk
   10-19 – Moderate Risk
   ≤ 9 - Low Risk

Updates to Previous SOP Version:
Updated for online revision