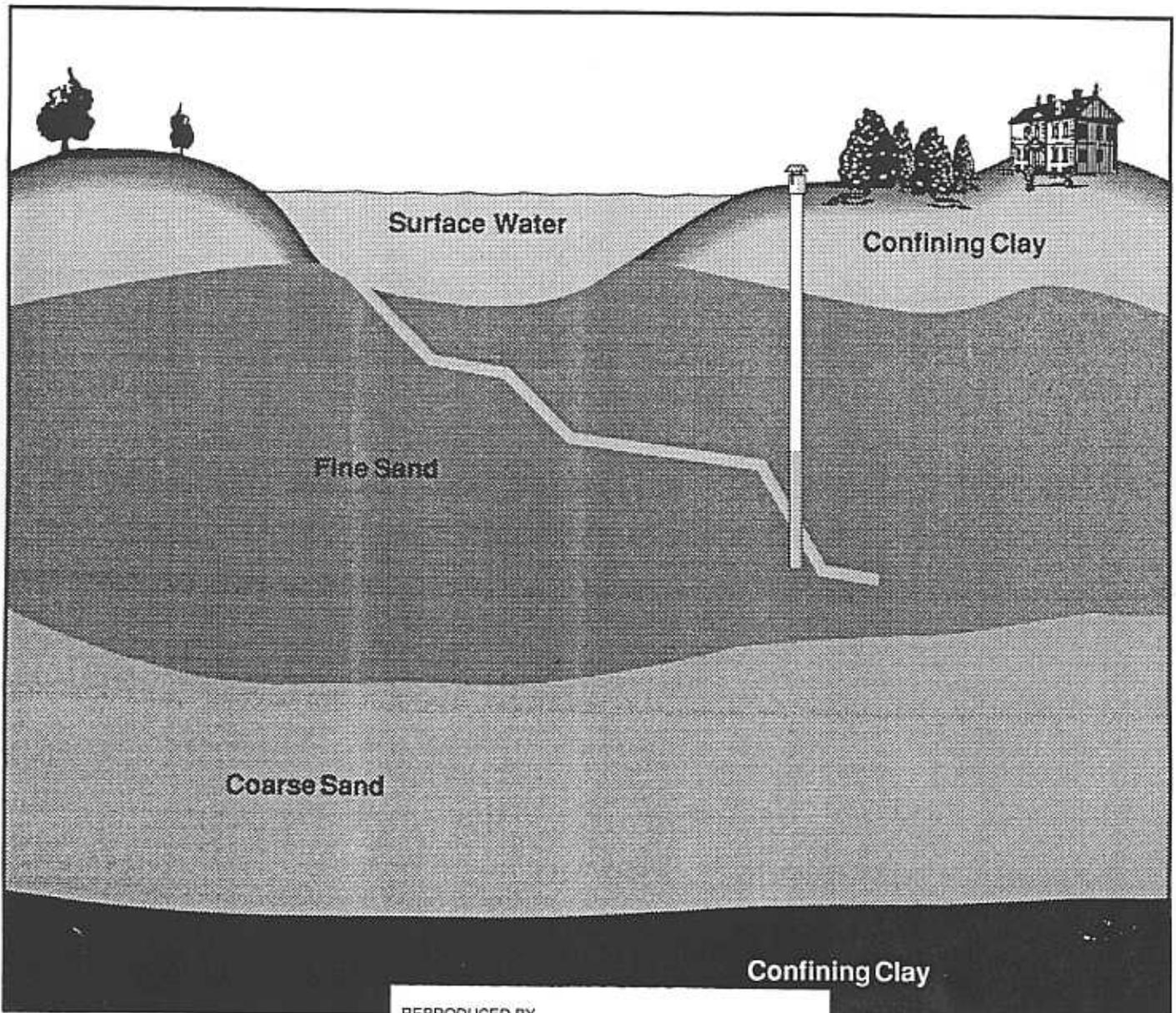




Consensus Method for Determining Groundwaters Under the Direct Influence of Surface Water Using Microscopic Particulate Analysis (MPA)



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CONSENSUS METHOD FOR DETERMINING GROUNDWATERS UNDER THE DIRECT
INFLUENCE OF SURFACE WATER USING MICROSCOPIC PARTICULATE ANALYSIS
(MPA)

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INTRODUCTION

With enactment of the 1986 Amendment to the Safe Drinking Water Act, 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 (GWDI). These requirements are often referred to as the Surface Water Treatment Rule (SWTR). As part of the SWTR, states will have primary responsibility for identifying those ground waters directly influenced by surface water and consequently at risk to waterborne diseases such as giardiasis. Traditionally, many states have defined surface sources as all waters located above ground such as lakes, ponds, rivers, creeks, etc. Similarly, subsurface sources such as shallow wells and springs have been defined as ground waters.

The microscopic particulate analysis (MPA) which evolved from the analysis of Giardia and filtration efficiency determinations is a useful laboratory tool in the identification of ground water supplies suspected of being under the direct influence of surface water. This may include, but is not limited to, water sources with open channel contamination (eg. cracked well casing), systems receiving recharge from a nearby surface source and obvious surface sources such as creeks and rivers.

This document is a collaborative effort combining the experiences and knowledge from contributors throughout the country into an acceptable consensus method. The procedures employed during MPA are too new and the analyst's experience too diverse for a standard method to be proposed at this time. 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. The range of values for each bio-indicator was taken from data submitted from laboratories throughout the country.

It should be emphasized that surface water influence on a groundwater source cannot be determined solely on the basis of one or two MPA's. Other pertinent information as described in the USEPA Guidance Manual(1) and elsewhere(2) should be gathered from each individual source in accordance with criteria established by the primary agency.

Finally, the MPA consensus protocol should be regarded as a tentative method with limited recovery efficiency data available for review. The absence of Giardia cysts, coccidia or other 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 Giardia or pathogen-free. Conversely, a positive MPA result does not necessarily signify the presence of Giardia or other related pathogens.

SAMPLE COLLECTION FOR MPA

1.0 Sample Equipment and materials (see Appendix 1 for parts and supplies).

1.1 A MPA Sampling device consists of the following parts (refer to Fig. 1):

1.1.1 Inlet hose with backflow preventor (Watts #8)

1.1.2 Pressure regulator (Watts IR56) plus pressure gauge (Baxter GS 202-2), 0-100 psi

1.1.3 Ten inch cartridge filter housing, preferably Commercial Filter model LT-10 (3)- (part #9499-5015)

1.1.4 Water meter readable in gallons, suggest a Kent C700 with plastic housing

1.1.5 Flow control valve (limiting flow orifice) rated at 1.0 gal/min (3.8 L/min)

1.1.6 Discharge hose

1.2 MPA Sampling Materials

1.2.1 Ten inch, 1 um polypropylene, yarn wound (string), nominal porosity cartridge filter, preferably commercial Honeycomb Filter Tubes (M39R10A) (3).

1.2.2 Whirl pak plastic bags (5.5"X14") or ziploc freezer bags

2.0 Sample Collection parameters

2.1 Minimum sample volume of 500 gallons, recommend 1000 gallons over a 8-24 hour period

2.2 Pressure over filter face controlled at 10 psi using an

- in line pressure regulator and gauge (0-50 psi)
- 2.3 Flow through the filter unit should be controlled at 1 gpm (3.8 L)/min using a limiting flow orifice rated at 1 gpm.
 - 2.4 Filter samples are collected at the groundwater source
 - 2.4.1 Avoid sample sites within the distribution system.
 - 2.4.2 Use of electric or gasoline powered pumps are recommended if no positive pressure is available at the groundwater source. All tubing or hose should be flushed with particle free water prior to use.
 - 2.4.3 If collection at the source not possible, final report must "qualify" sample
 - 2.4.4 Spring boxes should be cleaned prior to sampling by scrubbing the walls and removing all visible debris. Following cleaning the spring should be flushed for a day or more before samples are collected.
 - 2.5 Samples are collected prior to any blending, disinfection or other treatment
 - 2.6 A minimum of two samples should be collected
 - 2.6.1 One sample collected following a heavy rain fall (i.e. minimum of 2 inches within a weeks prior) or snow melt or other critical period (ie irrigation season).
 - 2.6.2 One sample collected during the late summer or

- 2.6.2 One sample collected during the late summer or following an extended period of little or no rainfall
- 2.6.3 If only one sample can be collected, it should be taken during worst case period, i.e. after a rainfall or during a spring snowmelt.
- 2.7 Samples must be shipped iced (3°C) in insulated, water-tight containers. Blue ice is acceptable but filters must not be in direct contact with the blue ice during transit.
- 2.8 The maximum transit/holding time should not exceed 48 hours
- 2.9 Multiple samples should be clearly labeled preferably marked in or on the tube filter transport bag using a waterproof lab marker pen.
- 3.0 Sample collection procedure
 - 3.1 The sampling unit should be cleaned and flushed with hot tap water prior to use. A mild detergent and soft brush can be used if the unit is soiled. A final rinse with particle free water (as defined in section 2.19) is required.
 - 3.2 Connect sampling unit to pressure source or pump in the direction of flow indicated on filter housing. Flush the unit without a filter for 3-5 min with the source water

to be sampled.

- 3.3 Record the date, time or day and gallon reading from water meter before and after sampling. Document the name, address and location of each sample site in addition to the exact sample point. Identify the water source as a spring, dug well, drilled well, artesian well or other. Document the distance to the nearest rivers, stream, irrigation canal, lake or pond.
- 3.4 Insert filter in the housing and tighten it with the plastic wrench provided. Make sure rubber washer or "O" ring is in place between filter housing bowl and base.
- 3.5 After installing filter, turn water on slowly with the unit in an upright position. Invert unit to make sure all the air within the housing has been expelled. When housing is full of water, return unit to upright position and turn volume on completely.
- 3.6 Check reading on pressure gauge. If not reading 10 psi, loosen lock nut and adjust regulator. Retighten lock nut.
- 3.7 The sampling unit should be allowed to run for a 8-24 hour period. Volumes sampled over this protracted time may vary from 500 to over 1000 gallons. Sample volumes filtered will usually be dictated by the time available for sampling, turbidity and particulate content of the source water being tested.
- 3.8 After filtering sample turn off the faucet or pump and

disconnect hose from incoming water source. Unscrew housing bowl from the top and pour off all but 100-200 mL. Do not touch filter with bare hands, use sanitary rubber gloves or plastic bag to remove filter, and place in a plastic whirl-pak /ziploc bag. Each filter must be placed in its own individual bag. Pour the water remaining in the filter bowl (100-200 ml) into the whirlpak/ziploc bag with the filter. Seal the bag securely.

- 3.9 Pack the filter(s) in a small insulated container or ice chest with a bag of ice and/or blue ice packs. Do not place blue ice in direct contact with filters because this can cause the filters to freeze. Frozen fibers cannot be analyzed for MPA. If possible place the filter bags in an upright position with the seal at the top.
- 4.0 If provided, fill out the sampler data sheet providing all information requested. Place data sheet(s) in plastic bag and send with filters.

4.1 Send filters and data sheets via 24-hour delivery services (Federal Express, etc.) to the address below:

4.2 If there are any further questions regarding the operation of the sampling unit contact:

EQUIPMENT AND SUPPLIES

1.0 Equipment

- 1.1 Large capacity refrigerated centrifuge (non-refrigerated if samples preserved).
- 1.2 Large capacity swing-bucket rotor (90°) 1-6 L/run
- 1.3 250 ml flat bottom or conical, autoclear bottles with screw caps (polycarbonate or glass).
- 1.4 1L flat bottom or conical, autoclear bottles with screw caps (polycarbonate or glass).
- 1.5 Combination brightfield/phase contrast and/or DIC microscope with Köhler-type illumination and 10-16X, 20-40X and 100X objectives.
- 1.6 35 mm, polaroid camera system or video image printer.
- 1.7 Five degree refrigerator.
- 1.8 Stomacher Lab blender - mode 3500 (optional).
- 1.9 Vortex tube mixer.
- 1.10 Aspiration flask and vacuum source with 0-30 psi gauge.
- 1.11 Pipet aid or bulb or 30 ml syringe with large bore canula.
- 1.12 Motorized multivolume microliter pipette (Rainin edp plus) or manual equivalent.
- 1.13 Hollow glass tubes (ca 1/4" bore).
- 1.14 Giardia filtration device (see Fig. 1).
- 1.15 70°C Steam bath for melted vaspar (if vaspar is used).

- 1.16 Sonicator (optional).
- 1.17 Manual differential counter (10 gang) or 10 place electronic tabulator.

2.0 Supplies

- 2.1 Whirlpac® bags, 5.5 X 15", sterile. For filter transportation.
- 2.2 Polypropylene yarn wound (string) filter tubes (M39R10A).
- 2.3 Sterile surgical gloves.
- 2.4 Stainless steel pan.
- 2.5 Ziploc bags, 7 X 8"
- 2.6 Bandage scissors, autoclavable.
- 2.7 Scalpel handle, autoclavable.
- 2.8 utility knife, autoclavable.
- 2.9 Scalpel blades, sterile.
- 2.10 4 liter beakers.
- 2.11 Pasteur pipettes, sterile.
- 2.12 10% buffered formaldehyde, pH 7.0.
- 2.13 15 ml or 50 ml conical centrifuge tubes, polystyrene, sterile, or borosilicate glass.
- 2.14 Microscope slides, coverslips (22 X 22 mm).
- 2.15 Membrane filter (optional) 25mm, 0.45 mm porosity.
- 2.16 Polysorbate 20 (Tween 20).
- 2.17 Percoll® (Pharmacia Fine Chemicals Inc Uppsala, Sweden)
- 2.18 2.5 M Sucrose (855.75 gram sucrose/liter)

- 2.19 Particle-free water (deionized distilled water passed through a series of three 0.22 um disc filters-Millipak 40, Millipore MPGLO4SK2 or equivalent). Particle free water should contain less than 100 particles/ml.
- 2.20 0.01% Polysorbate 20 in particle-free water.
- 2.21 Vaseline.
- 2.22 Paraffin.
- 2.23 Sodium Citrate - GR($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$).
- 2.24 Clear nail polish
- 2.25 Cotton-tipped applicator sticks.
- 2.26 Lugol's Iodine (Iodine (Powder Crystals - 5g, potassium iodine 10g, distilled water-100 mL)
- 2.27 3.5 L capacity stomacher bags (Seward Medical, Tekmar Co.)
- 2.28 Non-drying immersion oil (Cargille formula: code 1243, type A at 20°C or equivalent).
- 3.0 Processing Reagents
- 3.1 Percoll-Sucrose floatation solution (sp gravity 1.15):
- 3.1.1 62 ml Percoll
- 3.1.2 100 ml particle-free water
- 3.1.3 124 ml 2.5 M Sucrose solution
- 3.1.4 Mix ingredients thoroughly, measure sp gravity with hydrometer. Sp gravity should be between 1.15 and 1.16, do not use if less than 1.15.

Store at 1-4 C, use within 24 hours.

3.2 Non-sterile stock wash water(dilute 1:10)

3.2.1 sterile erylenmeyer flask (1,2 or 4L)

3.2.2 Particle-free water

3.2.3 Sodium citrate

3.2.4 0.01% polysorbate 20

3.2.5 mix these chemicals in the following

proportions for stock solution:

	1L	2L	4L
3.3 Sodium citrate			
(optional if Iron present)	5.0 g	10.0 g	20.0 g.
0.01% polysorbate	10 ml	20 ml	40 ml
Particle free water			
(QS to)	1L	2L	4L
Final pH to 7.0 +/- .2			

4.0 Vaspar

1 part vaseline to 1 part paraffin (w/w), melt and mix ingredients in beaker. To remelt, heat in 70°C water bath, apply to slide with cotton-tipped applicator.

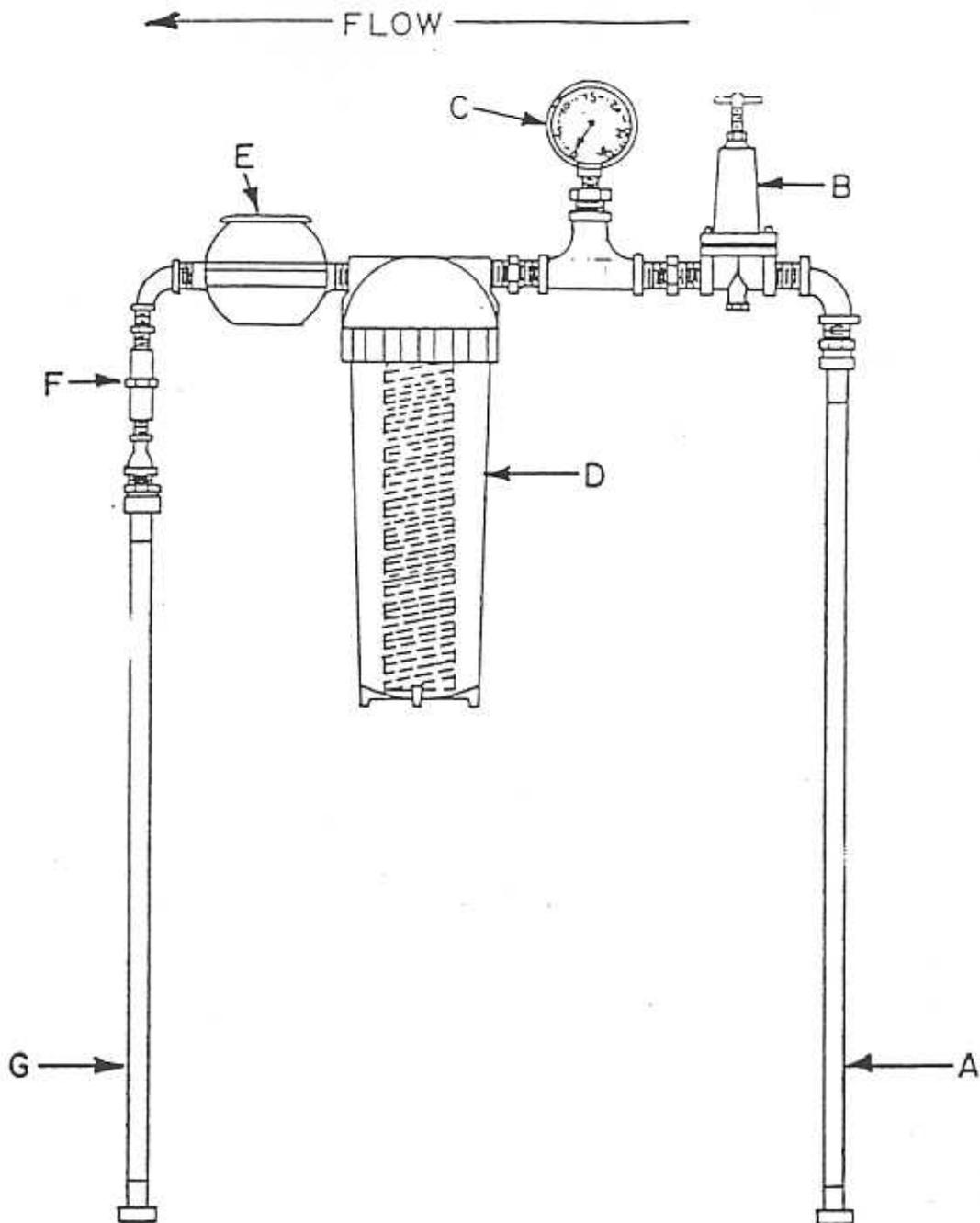


Figure 1. Water Sampling Device

- A. Six-foot inlet hose with backflow preventer. (HG-80 female fittings)
- B. Pressure regulator, adjustable, pre-set at 10 psi (Watts Regulator Co., Lawrence, MA, U.S.A., # 3-26A, model 3-50)
- C. Pressure gage, 0-100 psi
- D. Filter holder housing (Fulflo (Lebanon, IN, U.S.A.), model F15-10), containing polypropylene yarn filter (Carborundum Co., Lebanon, IN., U.S.A., model M39R10A, 2.5 inches diam x 9.75 inches long.)
- E. Gallon meter (Kent C700)
- F. Limiting flow orifice (faucet control), 1.0 gal/min (3.79 L/min) (Dole FM-C, Carol Stream IL, U.S.A.)
- G. Six-foot discharge hose.

FILTER PROCESSING AND ANALYSIS

- 1.0 Filter processing can be accomplished by one of two quasiaxenic techniques.
 - 1.1 Unwinding Filters - The filter is unwound on a sterile rod over a sterile SS pan. The end of the string filter is found and unwound by hand dividing it into roughly fourths, which are hand washed in successive 1 L deionized/dist. (pH 6.5-7.5) aliquots of particle free water containing 0.0001% polysorbate (Tween) 20/80 (4).
 - 1.2 Cutting filter - The filter is cut in half lengthwise to the plastic core using a sterile surgical quality scalpel or utility knife. Cutting in this manner should result in string fibers, approximately 2 inches in length which are washed in particle free water containing 0.0001% polysorbate (Tween) 20/80 water (4).
- 2.0 Cut or unwound string filter are either hand washed or mechanically agitated using a Stomacher lab blender, model 3500 (Tekmar Co., Cincinnati, OH)
 - 2.1 Handwashing of string filter - The four-six portions of the unwound filter are individually hand rinsed in successive 1 L aliquots of Tween water in 4 L beakers until the filter fibers appear clean. After several

rinsing, the fibers are wrung out into the final collection beaker by placing them in individual 8"X 8" interlocking polyethylene bags which have one corner snipped off to allow for drainage. Express all fluid from the four bags into one 4 L beaker. Alternatively, the portions of cut filter are washed separately in successive 1 L aliquots of Tween water. Wash filters until clean, place in polyethylene bags and proceed as described above.

- 2.2 Mechanical washing(5) - If filter is cut into halves, each half of the two inch long fibers are teased apart and placed in a 3.5 L capacity sterile stomacher bag (Seward Medical, Tekmar Co.) with 1.5-1.75 L Tween 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 the 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. Alternatively, each quarter portion of unwound filter skeins are placed in Stomacher bags containing 1.50-1.75 L Tween water and processed in the same manner as the cut filter described above.

- 3.0 Centrifuge Washings - After mechanical homogenization or handwashing the resulting wash water is poured into sterile 1 L centrifuge bottles and centrifuged at 1050xg for 10 min. using a large capacity swing bucket rotor. Use of a refrigerated centrifuge is recommended but optional at this time. To prevent swilling, make sure the brake control is off allowing the rotor to decelerate slowly.
- 4.0 Pooling of sediments - Aspirate the supernatant from each centrifuge bottle and collectively combine the sediments by rinsing the bottles into a 2-4 L beaker using a minimal amount of Tween water from a squirt bottle. Add sufficient 10% buffered (pH 7.0) formaldehyde to the combined sediments to make a 1% solution. The resulting fluid is mixed (stirbar) for 1-4 min with the beaker covered with foil. At this point the sample can be stored at 5°C for analysis the next day. If pooled sediment is processed the same day, addition of buffered formaldehyde is not required (optional).
- 5.0 Centrifugation of pooled sediment - The formaldehyde preserved or unpreserved sediment from section 4.0 is resuspended by mixing (stirbar) and transferring into two or more 50 mL or 15 mL conical centrifuge tubes and spun at 1050xg 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.

5.1 If volume of pooled sediment is below a concentration of 20 uL/100 gallons, examine directly without floatation.

5.1.1 Dilute 1:1 (v/v) and continue as in section 7.0 or

5.1.2 Dilute as needed and filter thru one or more 25mm, 0.45 um, cellulose acetate membrane filters (MF) and clear MF with type A immersion oil. Cover with a round coverslip and continue as in section 8.0.

5.2 If concentration of sediment is >20 uL/100 gallons continue by resuspending the sediment in each tube with particle free DI/Dist water, filling 50 mL tubes to the 40 mark or 15 mL tubes to the 10 mark. Resuspended using a vortex tube mixer.

6.0 Percoll/sucrose gradient procedure (6,7)

6.1 Prepare isotonic Percoll/sucrose gradient solution (1.15 sp. gr.). When overlying, add 75 mLs Percoll/sucrose to 250 mL cent. bottles or 30 mL to 50 mL cent. tubes.

6.2 Resuspend the sediment by vortexing for 15-30 sec. then

layer over 70-90, or 10-30, mL tube of resuspended sediment onto a 250 mL cent. bottle or 50 mL cent. tube, respectively. Add no more than 1 g sediment/25 mL Percoll/sucrose. Layer onto gradient carefully using a large bore glass tube, pipet-aid or 30 mL syringe with large bore canula. Gently add to sides of cent. bottle/tube making sure not to disturb gradient interface.

- 6.3 After overlying, place cent. bottles/tubes on lab bench at RT and allow to settle by gravity (static) for five min. Do not centrifuge.
- 6.4 If, after five min. on the lab bench (static) no visible settling occurs, centrifuge bottles/tubes for five min. @ 650xg.
- 6.5 After centrifugation, aspirate down to first cloudy layer and carefully transfer remaining liquid into 5X vol. particle free DI/Dist water to dilute Percoll/sucrose. If the packed sediment in bottom of tube or bottle represents a significance portion of the floated sample (>50 uL/100 gallons), examine at least one slide directly (wet mount) or re-float as in section 6.2.
- 6.6 Centrifuge diluted Percoll/sucrose liquid at 650xg for 10 min. Aspirate and retain second pellet.
- 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 50 mL

graduated conical cent. tube. Wash sides of bottle/tube with squeeze bottle to insure complete transfer. Vortex (10-30 sec) and centrifuge at 650xg for 10 min. Aspirate all water down to pellet. This represents the second and final washing.

7.0 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/Dist water.

7.1 Vortex for 10-30 sec.

7.2 Using a micropipet place 20 uL portions onto a standard glass slide and cover with a 22 X 22 mm coverslip.

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

7.4 Seal with vaspar or clear nail polish.

8.0 Microscope examination - Analysis can be done by either brightfield, phase-contrast or differential interference contrast (DIC). If using phase-contrast/DIC microscopy do not stain with iodine solution. If using brightfield add 2-3 μ L of Lugol's iodine per 50 μ L of diluted sample.

8.1 Immediately scan entire area of prepared slides (approximately 79 fields/slide @ 100 X) and count all bio-indicators using a manual differential counter or

electronic tabulator. Refer to Standards of Identity section for definitions.

- 8.2 Counting of other particulates such as amorphous debris, minerals, pollen, etc. is optional but noting their relative concentration per 100 gal is recommended.
- 8.3 Identify all microbiota to at least class or phyla level.
- 8.4 Record and document rare, unusual or unidentifiable microbiota using a 35 mm/Polaroid camera or video image printer.
- 8.5 Use a calibrated vertical ocular micrometer (reticle) calibrated against a stage micrometer (for each objective) to measure the size of various bio-indicators and other particulates.

9.0 Amount of final pellet to be examined

- 9.1 If final diluted pellet is $>200 \mu\text{L}$, prepare additional slides ($20 \mu\text{L}/\text{slide}$) until the sediment equivalent of 100 gal. of filtered water has been examined, i.e. 300 uL of pellet from 500 gal filtered water would be determined as follows:

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

$$\begin{array}{r} 300 \times 2 \\ \# \text{ of slides to examine} = \frac{\quad}{5 \times 20} = 6 \end{array}$$

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

10.0 Recording of results and procedural parameters.

10.1 Using a manual differential counter or electronic tabulator tally all microbiota and particulates observed.

10.2 Record results using data sheet similar to the one below.

10.3 Field data should include the following:

10.3.1 Total water volume filtered in gallons

10.3.2 Water source identified as to type and location

10.3.3 If dug or drilled well request depth and distance from nearest body of water (river, canal, stream, lake, pond, etc.)

10.3.4 Record both address and exact location of ground water source being evaluated.

10.3.5 Date and time of sample device installation and removal.

10.3.6 Name, address and phone numbers of sampler(s).

10.3.7 Field measurements such as turbidity, pH, temperature, conductivity, chlorine residual,

etc.

10.3.8 Other field parameters are described in the section on Sample Collection For MPA.

10.4 Laboratory data should include the following:

- 10.4.1 Total volume of pooled sediment from filter washings
- 10.4.2 Volume of suspended floated material recovered from Percoll/sucrose float after washing.
- 10.4.3 Volume of packed pellet at bottom of Percoll/sucrose gradient tube(s) or bottle(s).
- 10.4.4 Number of each bio-indicator and other particulates from each slide containing 20 uL of floated material.
- 10.4.5 Type of microscopy employed.
 - Brightfield
 - Phase contrast
 - DIC
 - Other
- 10.4.6 Type of material examined
 - direct examination of unfloated sediment by wet mount or filtered thru MF
 - Floated (suspended) pellet
 - Floated packed pellet

- 10.4.7 Dilution of material examined before placing on slide
- 10.4.8 Magnification of objective in use
- 10.4.9 Number of fields/coverslip at 100X or other magnification

11.0 Qualification of Analyst

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.

11.1 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.

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

11.3 A working knowledge of ground water hydrogeology and soils.

11.4 Analyst should have experience in examining a sufficiently large number of groundwater samples.

11.5 Familiarity with the construction development and maintenance of wells (horizontal and vertical), spring boxes, artesian wells and infiltration galleries.

12.0 Interpretation of results

Until further research is completed, several guidelines should be followed when interpreting laboratory findings and what they mean relative to ground waters under the direct influence (GWDI).

12.1 Identification of Giardia cysts, coccidia and helminths in any concentration should be considered conclusive evidence of GWDI.

12.2 The repeated occurrence of a significant number of pigment bearing diatoms (not diatomal frustules) and other chlorophyll containing algae should be considered strong evidence of GWDI. Blue green, green and brown algae require sunlight for their metabolism which is unavailable in a true, protected ground water source.

12.2.1 Algae in question must be chlorophyll-bearing.

12.2.2 Certain types of algae may be present in unenclosed spring box walls open to direct sunlight or in springs that come to surface as pools or ponds.

12.2.3 If possible compare algae found to those in nearby surface water.

12.2.4 The morphology and sizing of algal cells is important. Do not confuse smaller filamentous iron and sulfur bacteria

(Crenothirix, Beggiatoa, Thiospirillum,
Phaecrotilus) as algae.

12.3 Some insect, insect parts and larvae are indicative of surface water. Obviously, some forms are more important than others. Until further information is acquired, insects, and insect larvae should be considered evidence of GWDI.

12.3.1 The strength of this evidence increases if source is not protected and within 200 ft. of surface water.

12.3.2 When dealing with adult forms or flying insects (stoneflies, damselflies, mayflies, dragonflies, etc) consider they may be airborne in unprotected sources.

12.3.3 If possible identify all insect/larvae to genus level - common names are acceptable.

12.3.4 Certain types of insect larvae are important while others are not.

12.4 Arthropods such as soil and water mites (Hydracarina) are of little significance.

12.5 The occurrence of sessile or free-swimming rotifers can indicate a source is either influenced by surface water or that the supply contains sufficient organic debris, fungi, bacteria, etc to provide a food supply, and therefore is not influenced.

- 12.5.1 Some free-living rotifers have highly specialized food habits not always associated with surface water.
 - 12.5.2 Some genera, mainly Notommatides, feed on the fluid contents of filamentous algae.
 - 12.5.3 Rotifers are usually seldom found in cold springs habitats (<8°C).
 - 12.5.4 At present, the presence of rotifers should be supported by other bio-indicators or physical evidence such as nearness to surface water or significant fluctuations in temperature, turbidity, etc.
- 12.6 The presence of "plant debris" is a broad category that is open to interpretation. Some microscopists have been defining it differently than originally proposed. Refer to Standards of Identity for definition.
- 12.6.1 Original definition applied to the undigested fecal detritus of herbivorous animals, usually muskrat and beaver.
 - 12.6.2 Others have expanded the definition to include unidentifiable plant materials that are chlorophyll-bearing. Intact plant material lacking chlorophyll is indicative of breakdown time of conductive tissue cell walls.
 - 12.6.3 Large infestations of certain insects

such as the Gypsy Moth (larval form) produce large amount of fecal pellets which may be confused with the fecal detritus of herbivorous animals. The larval form of this insect feeds on over 300 varieties of trees and shrubs native to many watersheds.

- 12.7 Some microscopists consider the occurrence of secondary bio-indicators such as crustacea and free-living protozoa (ciliates) as indicative of surface water. However, both crustacea and ciliates can be found living in the soil interstitium. Others consider the presence of large numbers of free-living amoebae and amoebic cysts as a significant parameter when investigating GWDI.
- 12.8 Although many green flagellates are obligate phototrophs, many species live and grow in the absence of sunlight, assuming sufficient nutrients are present. Consequently, if they are colorless, and translucent their occurrence in groundwaters may be of questionable value.
- 12.9 Sub-terranean caves, underground limestone areas and artesian wells sometime contain primitive or highly specialized amphipods, isopods, decapods, copepods and turbellarians.
- 12.9.1 Most sub-terranean species are colorless, translucent or "whitish."
- 12.9.2 Eyes are absent or non-functional.
- 12.9.3 Antennae and other tactile structures are

longer, more developed.

12.10 Pollen - Seasonal plant pollen is everywhere; both airborne and in the water. Therefore, the observance of pollen grains in a ground water is of little significance.

12.11 Relative risk factors - To help clarify the relative importance of each bio-indicator or group a relative risk factors has been assigned to each indicator based upon their importance as a health risk indicator, their significance as an indicator of surface water contamination and their concentration per 100 gallons of water.

12.11.1 Based on our present knowledge a relative "weight" is assigned to each bio-indicator or particulate based on the above factors (Table 2).

12.11.2 Since the SWTR (54 FR 27486-27541) defines GWDI as "any water beneath the surface of the ground with: (i) significant occurrence of insects or other macroorganisms, algae or large-diameter pathogens....", we can no longer approach the use of MPA on a presence or absence basis.

12.11.3 Application of the definition of GWDI as it exists in the

SWTR mandates a quantitative approach, i.e. "significant occurrence." Therefore, using existing data from several laboratories a concentration range for each bio-indicator or group is constructed (Table 1).

12.11.4 Using a quantitative approach per unit volume linked to a relative risk factor would place a ground water source at either a low, moderate or high risk of surface water contamination. The occasional spurious occurrence of flying insects, pupae, rotifers, crustacea, ciliates, colorless flagellates or plant debris without diatoms/algae, Giardia or coccidia would place the source at low to moderate risk. On the other hand, the present of Giardia or coccidia in any amount would place the system in the high risk category.

TABLE 1. Numerical range of each primary bio-indicator (particulate) counted per 100 gallons water.

Indicators of surface water ¹	EH ³	H	M	R	NS
Giardia ²	>30	16-30	6-15	1-5	<1
Coccidia ²	>30	16-30	6-15	1-5	<1
Diatoms ⁴	>150	41-149	11-40	1-10	<1
Other Algae ⁴	>300	96-299	21-95	1-20	<1
Insects/Larvae	>100	31-99	16-30	1-15	<1
Rotifers	>150	61-149	21-60	1-20	<1
Plant Debris ⁴	>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 cysts or coccidia are found in any sample, irrespective of volume, score as above.
3. Key= EH -extremely heavy M -moderate NS -not significant
H -heavy R -rare
4. Chlorophyll containing

TABLE 2. Relative surface water risk factors associated with scoring of primary bio-indicators (particulate) present during MPA of subsurface water sources.

Indicators of surface water ¹	Relative Risk Factor ³				
	EH ²	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
Rotifers	4	3	2	1	0
Plant Debris	3	2	1	0	0

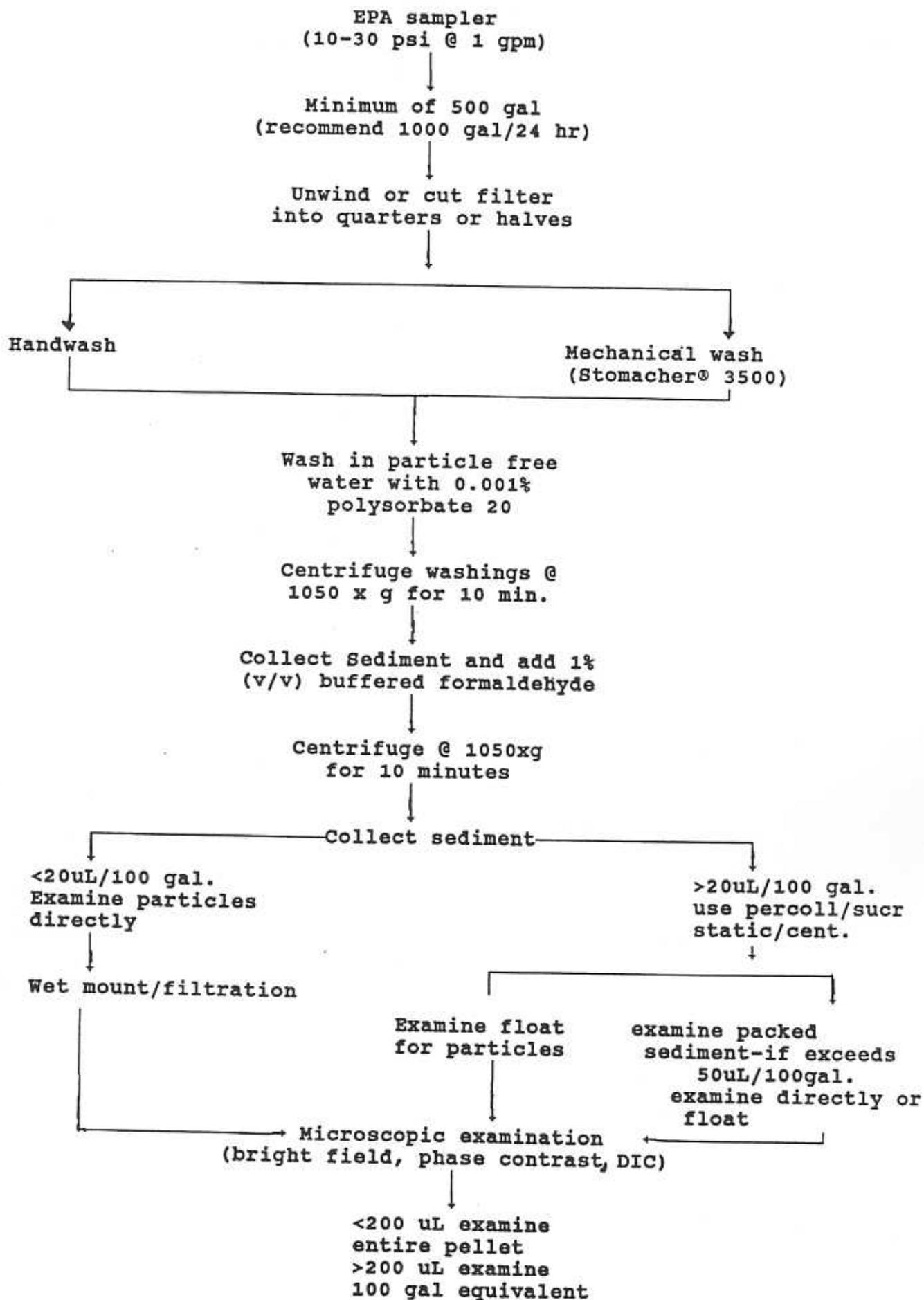
1. According to EPA "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources", March 1991 ed.

2. Refer to Table 1 for range of indicators counted per 100 gallons.

Key= EH -extremely heavy M -moderate NS -not significant
H -heavy R -rare

3. Risk of surface water contamination:
≥20 - high risk
10-19 - moderate risk
≤9 - low risk

PROPOSED EPA CONSENSUS METHOD FOR MPA



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STANDARDS OF IDENTITY

Giardia: The appearance of Giardia cysts under brightfield (Iodine), phase-contrast or DIC should be confirmed by internal morphology. Examine cysts under 800-1000X for proper shape and size. Record the length and width of the cysts with a calibrated ocular micrometer. If two or more morphological characteristics (2-4 nuclei, axonemes, median bodies) are observed, record as confirmed identification.

Coccidia: Coccidia are a subclass of intracellular parasites which occur primarily in vertebrates. This category covers mammalian, avian and fish coccidia which infect various tissues and organs, including the intestinal tract (eg. Cryptosporidium). Though not frequently identified by low power magnification (100X) using transmitted light microscopy, coccidia are good indicators of direct surface water contamination because they usually require a vertebrate host. Cryptosporidium oocysts are commonly found in surface water, but require extensive experience to detect using light microscopy. Because of its small size (4-6 um), less experienced microscopists should use specific IFA techniques for Cryptosporidium identification.

Diatoms: For purposes of this test, diatoms have been separated from other algae (green, blue-green) because they are the most resistant group of algae and are able to withstand a large amount of environmental, mechanical and chemical insult. Several species are present in surface water and are indicative of a healthy source. However, it is important that this determination be based on the presence of living diatoms and not their empty silica skeletal remains.

Other algae: Comprise a large number of chlorophyll containing filamentous, colonial and unicellular divisions of algae. Chlorophyll-bearing algae require sunlight for their metabolism (as do diatoms). For this reason their repeated presence in a ground water source is indicative of direct surface influences. Although surface water contains a great diversity in algal forms, only a few types have been found in groundwater. Their abundance and number is dependent upon available nutrients, water temperature and time of year.

Insect: This category includes insects, insect parts, larvae, eggs and another group of Arthropods, the Arachnids. Healthy surface waters should have insect larvae, nymphs and/or eggs of species that inhabit surface waters. Likewise, insects or their parts may originate

from the surrounding soil or may be airborne. At certain times of the year, Arachnids such as seed ticks (larval ticks) and soil mites are often present in a surface water.

Rotifers: A major taxonomic group that is often characteristic of fresh water. There are over 2500 species, of which > 2375 species are restricted to fresh waters. 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 a ground water sources. The vast majority of rotifers encountered are females ranging in size from 70-500 um. They generally are only good indicators of surface water influences when supported by presence of other bio-indicators. A few species have nutritional requirements which may be satisfied by food sources not necessarily associated with surface indicators. These latter species may not be good indicators of GWDI.

Plant debris: This is subtle term for the undigested fecal detritus from herbivorous animals, usually muskrat and beaver. Plant debris is very light weight (low density) material and is large in size (50-100 um). All experienced microscopists can usually recognize the

differences between beaver and muskrat feces because of the difference in diet most times of the year. If well trained in diagnostic parasitology one can often recognize the detritus (cellulose) from ruminant animals. Since it may take years for undigested plant tissue (cellulose) to breakdown in water, fecal detritus of this type may be present in the water long after Giardia cysts have died off. While plant debris does not always indicate the presence of Giardia and/or Cryptosporidium, it does suggest that animals are present and if not shedding cysts today, they may at some future date. To other microscopists, plant debris may be defined differently to include all unidentifiable plant material containing chlorophyll.

Large These are large particles, > 5 um in diameter, of amorphous material, usually organic detritus including biofilms, debris: fixed growth slimes and on occasion, large grains of sand. Large conglomerates of mixed debris are also included in this category. Since this material is non-specific and ubiquitous in all water sources it is not a good indicator.

Fine Generally this is a combination of silica and organic amorphous detritus, ranging in size from 1 - 5 um in debris: diameter, depending on the sources and times of year.

Small unrecognizable matter from decaying vegetation may also fall within this category.

Minerals: These are solid, homogeneous crystalline chemical compounds that result from the inorganic processes of nature. Microscopically most of these crystalline materials have a fractured or "broken glass" appearance. Some mineral material, such as apatite, have a very high birefringence; others, such as bentonite and lignite do not. One of the more common minerals is quartz which appears as colorless, transparent to translucent, sharply angular chips.

Plant This includes all microspores produced by seed plants.

Pollen: In the spring and fall, pollen is everywhere, both airborne and water borne. Pollen can become trapped in the filter cartridge during insertion of the filter or even in the laboratory while the filter is being processed for examination.

Nema- These include some 2000 known free-living species found
todes: in fresh water. Some species show an amazing ability to survive and thrive in aquatic habitats under a wide range of ecological conditions. Nematodes and/or their

eggs are common in healthy water sources and in spring boxes containing plant material or other detritus. They occur in widely differing habitats. Their appearance in groundwater is of little assistance in determining GWDI.

Crusta- These include all aquatic Arthropods which have two
ceans: pairs of antennae and are fundamentally biramous. The vast majority of known species (>35,000) are marine but approximately 1200 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 water and frequently are found in eastern lakes during the summer months. The significance of these larger organisms in ground waters is unknown at this time.

Free- These include the amoeboid, flagellated and cyst stages
living of such Sarcodina as Naeqleria, Amoeba, Acanthamoeba,
amoeba: and Difflugia, ranging in size from 10 to 600 um. The external surfaces of these amoeba are usually very thin as opposed to ciliates and most flagellates which are thicker (protective pellicle). These amoeba are very common in healthy surfaces waters, especially eastern lakes during the summer months. In western waters they may be present in lower numbers. One investigator has

reported seeing testate amoebae (order: Testacida) in a number of ground waters.

Ciliates: These free-living protozoa are extremely common in, and around healthy surface sources. Like amoeba, they feed on bacteria, algae, small metazoa, other protozoa and extraneous debris.

Flagellates: Many flagellates are plant-like, possessing chlorophyll and chromatophores. Although many flagellates are phototrophic, there are numerous species that grow in the absence of light providing sufficient dissolved nutrients are available. Since these protozoa have broad feeding and nutritional abilities (mixotrophic), their usefulness as indicators of GWDI may be limited.

Other: This category is important for listing any other biota found in a sample. The significance of "other" organisms may increase as further research is completed on ground waters and/ or surface water populations.

QUALITY ASSURANCE FOR MPA

Due to the subjective nature of the MPA, the adoption of a comprehensive QA/QC program at this time is somewhat limited. Listed below are just a few areas to be considered under a QA/QC program.

1.0 QC on equipment and supplies.

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

1.11 Equipped with swing bucket rotors. Records maintained on rotor(s) usage (time at designated RPM).

1.12 Rotor speed checked with tachometer on a quarterly/yearly basis.

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

1.14 Annual PM agreement in force or internal maintenance protocols/records in place.

1.2 Brightfield/phase-contrast/DIC microscopes.

1.21 Phase-rings checked for each objective before each use period.

1.22 Ocular micrometer (reticle) in place and calibrated against a stage micrometer for each objective in use. Re-check on an annual basis.

1.23 Records maintained on use hours of all tungsten

microscope bulbs.

- 1.24 Microscopes must be cleaned and optics realigned and adjusted on a frequent schedule.
- 1.25 Annual PM agreement in force or internal maintenance protocol/records in place.
- 1.3 Stomacher brand (model #3500) laboratory blender.
 - 1.31 Operated according to manufacturers recommendations. The use of the blender is carefully timed to insure consistent washing of filter fibers.
 - 1.32 Stomacher unit is maintained and cleaned after each use.
- 1.4 MPA sampling apparatus
 - 1.41 Apparatus is detergent cleaned in the lab and flushed with water in the field prior to filtering (without filter in housing).
 - 1.42 Kent water meter is periodically checked for accuracy by timing the rate of flow into gallon container.
- 1.5 Set of calibrated hydrometers
 - 1.51 Set ranges from 0.700 to 1.800 spec. gravity.
 - 1.52 Reading are temperature compensated.
- 1.6 Sucrose or Percoll/Sucrose solutions.
 - 1.61 Solutions checked for sterility and for spec. gravity with use of calibrated hydrometers.
 - 1.62 Solutions are not used beyond their expiration

date.

- 1.7 Sterile gloves are worn when handling any potentially contaminated objects and sterile technique used during processing.
- 1.8 PBS and DI water used during the sample preparation are checked for sterility and for Ph after autoclaving.
- 2.0 Analytical QC
 - 2.1 Analysts should be well founded in the fields of limnology, freshwater biology, parasitology, protozoology, phycology, invertebrate zoology, bacteriology, as well as, hydrogeology and soils.
 - 2.2 Extensive experience with microscope and skills in phase contrast, differential interference contrast and fluorescence microscopy.
 - 2.3 Documentation of unusual or unidentifiable or unusual microbiota should be by photomicrograph.
 - 2.4 Availability of identification keys and pictorial atlases to assist in classification of microbiota.
 - 2.5 Down the road, perhaps a round robin study involving identification of microbiota specific to each region.
 - 2.6 Strict adherence to the Consensus Method and the definitions of Standards of Identity will aid in maintaining intralaboratory and interlaboratory QA.

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APPENDIX 1

(EPA water sampling device parts)

WATER SAMPLING DEVICE PARTS

- A) Backflow preventor, Watt no 8 (for hose bib application)
Familian N.W. Inc.
1305 Marine Drive
Bremerton, WA 98310
(206) 479-9713
- B) Watts regulator, adjustable. No. 3-26A, Model M. 3-50 psi.
Female and male connections = 3/8".
Familian N.W. Inc.
above
- B) Watts hose connection vacuum breaker No 8.
Familian N.W. Inc.
above
- C) Pressure gauge, 2 1/2" stem. No shock model 25.300.30. 0-50
psi, adjustable.
Branon Instruments
PO Box 80308
Seattle, WA 98108
(206) 762-6050
- D) Filter housing- commercial filter LT -10 part # 9499-5015
Montgomery Bros, Inc Gaskets no 2620.5045 and 4154-6000
14844 NE 31st Circle
Redmond, WA 98052
(206) 881-9393
- E) Filters M39R10A 10" polypropylene
Montgomery Bros, Inc.
above
- F) Kent Water Meter
C-700 Kent Polymer 1/4 to 50 gpm
5/8" X 1/2" water meter
American Power, Inc
808 South Fidalgo
Seattle, WA 98108
(206) 362-2321
- F) Limiting Flow Orifice. Dole flow control valve. model FMC 1.0
gpm
George Scott and Associates
2700 NW St. Helens Rd
Portland, OR 97210
(503) 228-8643
- G) Misc galvanized nipples and bushings
Coast to Coast hardware
3/4" X 1/2" Hex bushing galvanize
1/2" Tyy galvanize
3/4" X 1/2" reducing 90 galvanize

1/2" X 3/8" Hex bushing galvanize
1/2" close nipple galvanize
1/2" X 2" nipple galvanize
1/2" X 1/4" Hex bushing galvanize
3/8" close nipple galvanize
3/8" X 1/2" bell galvanize
1/2" FIP X 3/4" MIP hose galvanize
3/4" T galvanize
3/4" close nipple galvanize
3/4" X 1/4" Hex bushing galvanize
6' Washing machine hose F to F

APPENDIX 2

(Field data sampling and Analytical Forms)

MPA SOURCE WATER IDENTIFICATION

Lab# _____ City/utility _____ Sampler(s) _____
 Project Code _____ Address _____ Agency _____
 Account# _____ Phone _____ Address _____
 Date(s) sampled _____ System _____ public _____ comm _____ Phone _____
 Date recieved _____ System _____ public _____ comm _____ non-comm _____ other _____

Water source location _____ Meter reading: before _____ after _____
 Sample taken from _____ Total volume filtered _____

Water Source ID as: _____ spring _____ infil galley _____ artesian well
 _____ dug well _____ drilled well _____ horizontal well _____ other _____
 If well: depth _____ ft Distance from river/stream/lake _____ ft

Field Measurements:	(Date)	Turb (NTU)	pH	Cond.	T. Chlo.	F. Chlo.
visit one	_____	_____	_____	_____	_____	_____
visit two	_____	_____	_____	_____	_____	_____

Other MB Analysis:	(Date)	TC/100 mL	FC/100 mL	HPC/mL
visit one	_____	_____	_____	_____
visit two	_____	_____	_____	_____

Processing Information:

Total volume filtered _____ Time required _____
 Total filter sediment collected _____
 uL sediment/100 gal _____
 Percoll®/surcose floatation pellet volume _____ uL
 Percoll®/surcose floatation packed sediment _____ uL
 uL floatation pellet volume/100 gallons filtered _____ uL

Floataion Parameters:

_____ Percoll®/surcose gradient _____ ZNSO₄
 _____ surcose gradient _____ other _____
 _____ potassium citrate _____

MPA CLASSIFICATION AND QUANTITATION OF PARTICULATES

Date _____ Analyst _____ Dilution _____ Magnification _____ Microscopy _____ Vol final pellet _____ uL

	slide 1	slide 2	slide 3	slide 4	slide 5	slide 6	slide 7	slide 8	slide 9	slide 10	Total	#/100 gallon	Risk Factor
Primary Particulates													
Giardia													
Coccidia													
Diatoms													
Other Algae													
Insect/larvae													
Rotifers													
Plant Debris													
Secondary Particulates													
Large amorphous debris													
Fine amorphous debris													
Minerals													
Plant pollen													
Nematodes													
Crustacia													
Amoeba													
Ciliate/Flagellates													
Other													

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