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Modified Moore Swab Method for Microbiological Surface Waters Sampling

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1

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This protocol describes the use of modified Moore swabs to collect surface water samples for microbiological analysis.

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protocol

Ballesteros-Nova NE, Sánchez S, Steffani JL, Sierra LC, Chen Z, Ruíz-López FA, Bell RL, Reed EA, Balkey M, Rubio-Lozano MS, Soberanis-Ramos O, Barona-Gómez F, Brown EW, Allard MW, Meng J, Delgado-Suárez EJ, Genomic Epidemiology of Circulating in Surface Waters Used in Agriculture and Aquaculture in Central Mexico. Applied and Environmental Microbiology 88(5). doi: [10.1128/aem.02149-21](https://doi.org/10.1128/aem.02149-21)

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Peristaltic pump and rechargeable portable battery



Portable pH meter with temperature probe



Portable turbidimeter



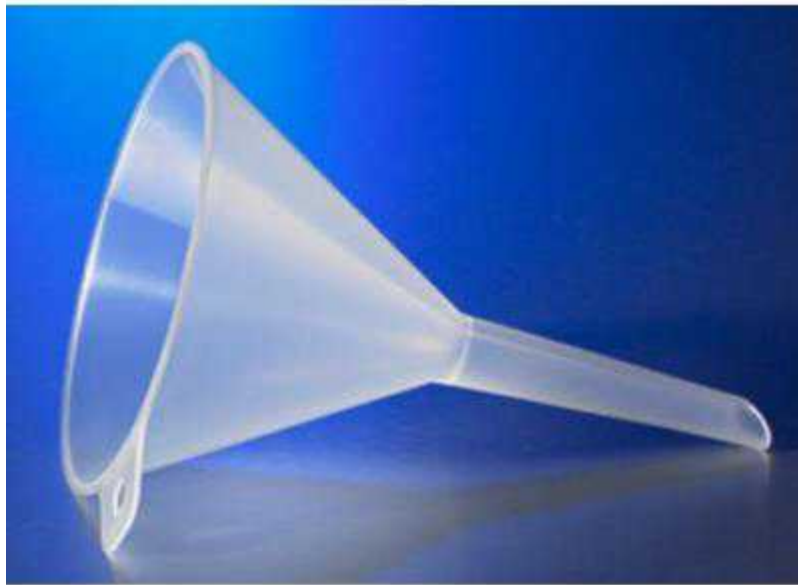
Sterile MMS units



100-mL plastic graduated cylinders



Wash bottle with distilled water



Plastic funnel



14-L bucket



1 to 1.5-m stick



Rope



1-L plastic container

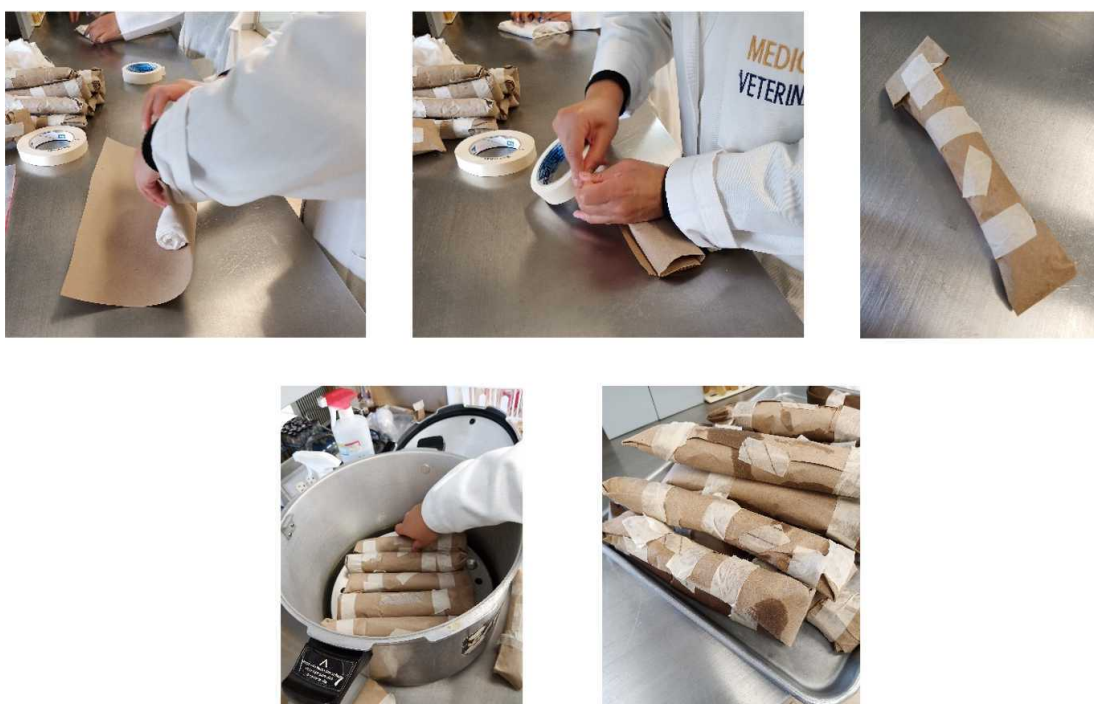
Modified Moore Swab (MMS) Preparation

3d

- 1 Filtration cloth.** Cut a 92 x 95 cm² cheesecloth grade #90 piece and fold it horizontally. Fold it again to its half size and stretch. Fold again each of the sides until they touch each other and press it to get the cloth as flat as possible. Then, tightly roll the cheesecloth to get a cylindrical shape-swab of approximately 12 cm long and 4.4 cm in diameter.



- 2 Wrap the folded cheesecloth in Kraft paper. Use masking tape to seal the paper around the cloth and attach a piece of sterilization indicator tape before autoclaving at 121 °C / 20 min. Let the cheesecloth cool down to room temperature before placing it into the MMS unit.



- 3 **Modified Moor Swabs (MMS).** Assemble a PVC MMS. Collect the required parts and use^{2d} Oatey PVC and CPVC Purple Primer to seal PVC tube joints. Refer to the attached file for a detailed description of the required parts.

 [MMS_parts.xlsx](#)

Example of assembled MMS units. The wide end is used to place the cheesecloth and the narrow one to place the tubing that will be connected to the peristaltic pump.



Place a Phifer BetterVue Insect screen on the wide end of the filtering unit and fix it with a rubber band.



Sterilizing MMS

4h

- 4 Sterilize empty MMS and insect screens by immersion in a 10% chlorine solution for 30 min. For that purpose, use a bucket, making sure the whole MMS is submerged in the chlorine solution. After chlorine immersion, place the MMS in a dry bucket (previously washed and sterilized with chlorine as well) and let them air dry.

Autoclaving MMS is not recommended for it will damage the PVC quickly, causing cracks that will render the units useless.

Use sterile tweezers and gloves to aseptically place the sterilized cheesecloth cylinder into a sterilized MMS. Place the MMS into a sterile bag and close the bag until required for field

sampling.



Assembled MMS unit that is ready to use.

Surface water sampling

40m

- 5 4.1 On site, find a proper location to securely place the peristaltic pump and the battery.
- 4.2 Couple the PVC vinyl tubing to the pump. Put one end of the tubing inside the bucket.



- 4.3 Take a sterile MMS unit and couple it to the other end of the peristaltic pump tubing. Use the rope to tie up the filtering unit to the 1-1.5-m stick, to prevent the MMS unit from being dragged by the stream. Use the stick to submerge the MMS unit in the water.



Always grab the MMS unit by its narrow part to avoid touching the water inlet and contaminating it.

4.4 Turn on the pump and switch the speed knob to maximum flow. Let the water flow for about 1-2 min. This ensures the MMS unit is completely filled with water and it also eliminates air bubbles trapped inside it.

4.5 Switch the speed knob to nearly half of the flow speed. Use the 1-L container and a chronometer to adjust flow to 0.5 L/min.

4.6 Change the water exit to the 14-L bucket, with the 10-L level clearly marked. Continue filtering water until the water level in the bucket reaches the 10-L mark (approximately 20 min).

4.7 Turn off the pump, get the MMS unit out of the water and uncouple it from the tubing.



4.8 Carefully place the MMS unit inside a sterile plastic bag identified with the corresponding sample name. Always wear new gloves for this procedure and avoid touching the inner part of the plastic bag when placing the used MMS unit inside it.



4.9 Close the bag and place it in an insulated container with coolant gels to keep it refrigerated during transport to the laboratory.



4.10 Make sure the pump's tubing is completely emptied. If necessary, hold it vertically to get out any water remaining inside it. Place the pump and the rest of the sampling materials back to the transporting vehicle

RECORDING SAMPLING LOCATIONS AND WATER VARIABLES

4.11 Open the Epicollect5 App in your mobile phone and sign in to the project account as a sample collector. Create a new entry and record the following data:

- Full name of sample collector
- Sample name
- Date and time the sample was taken
- GPS coordinates of the sampling site
- A picture of the location

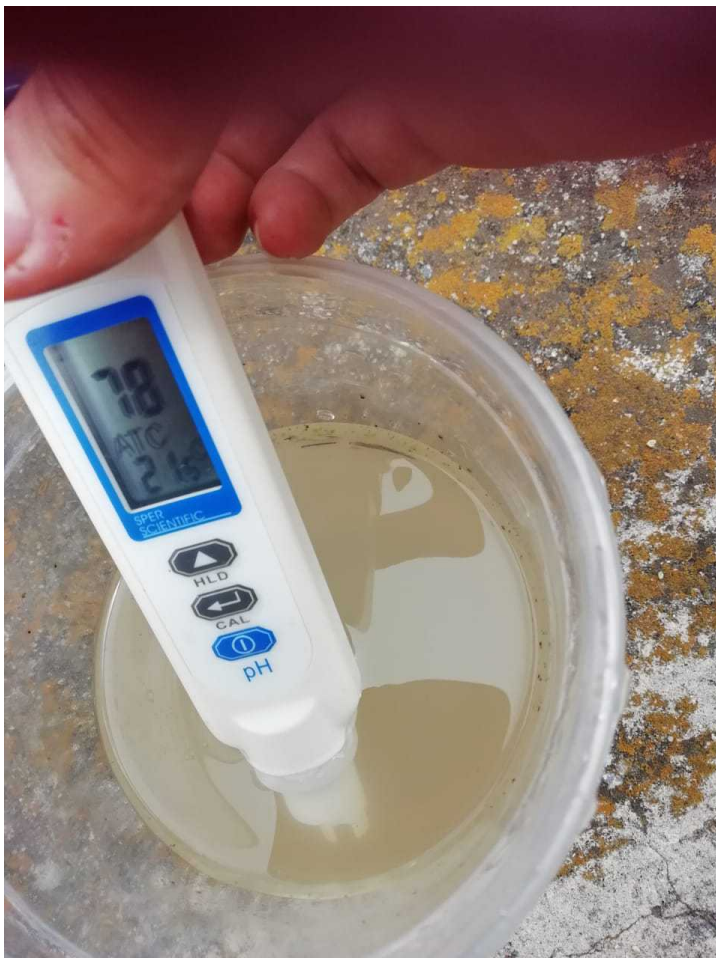
Measuring water pH and temperature

4.12 Take the 1-L container and collect water (≈ 0.5 L) from the same spot where the MMS unit was located. If you have to walk into the water, collect the sample as far away as possible from where you are standing, to make sure no sediment is included in the sample.



4.13 Take a calibrated pH meter and use the wash bottle to rinse its probe with distilled water. Then, use a disposable paper towel to dry it.

4.14 Turn the pH meter on and place it in the water sample. Use the pH meter to slowly shake the water for about 30 s before reading water pH and temperature. Record water pH and temperature in Epicollect5.

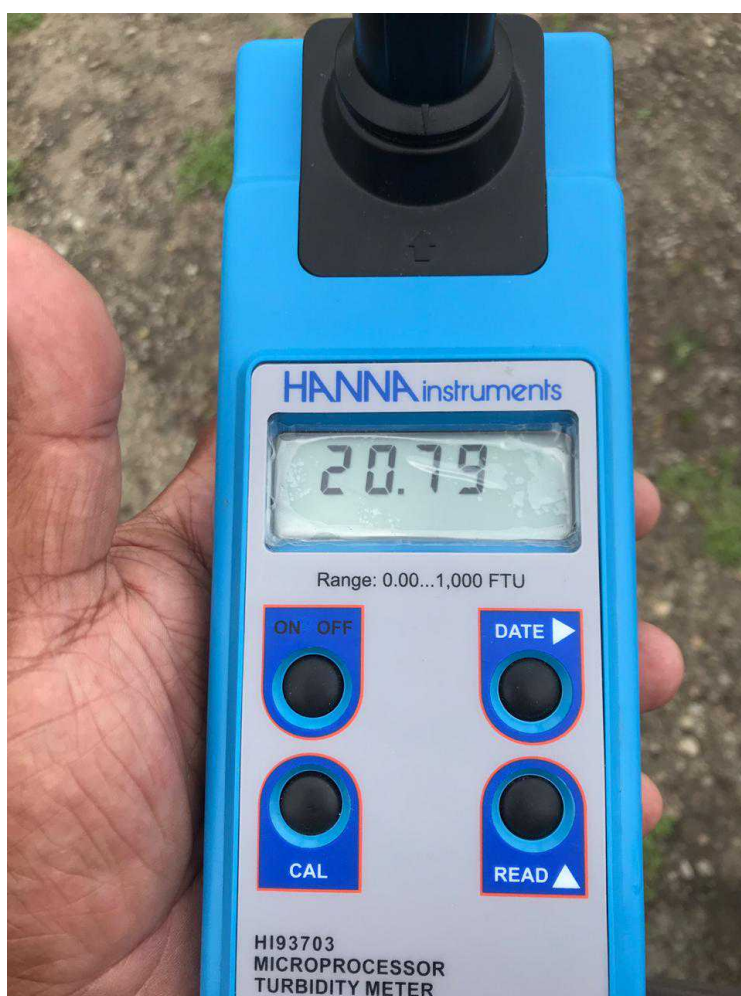


Measuring water turbidity

4.15 Turn on the turbidimeter and fill one cuvette up to the mark with distilled water. To avoid biases in the measurements, the cuvette must be handled from its head. Never touch the part that goes inside the measuring channel. If touched accidentally, rinse the touched part with distilled water and dry it carefully with a disposable paper towel, until the glass looks completely transparent again.

4.16 Measure water turbidity of distilled water and set it as the blank (or zero turbidity).

4.17 Fill a 100-mL graduated cylinder with some of the sampled surface water and use it to fill another cuvette (as described in 4.15). Measure water turbidity and record it in Epicollect5.



4.18 If water turbidity is out of range (too high), prepare 1:10 or 1:100 dilutions, as required:

1:10 dilution. Pour 10 mL of sampled water in a graduated cylinder and fill it up to 100 mL with distilled water.

1:100 dilution. Pour 10 mL of the 1:10 dilution in another graduated cylinder and fill it up again to 100 mL with distilled water. And so on, in case further dilutions are required. Normally, a 1:10 dilution is enough in most surface waters with high turbidity.

