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Moore Swab Methods for Concentrating and Detecting Salmonella Typhi and Salmonella Paratyphi A in Environmental Wastewater Samples

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ABSTRACT

This methods describes a tool to concentrate and testing Salmonella Typhi (ST) and Salmonella Paratyphi A (SPA) from environmental water samples (*e.g.*, wastewater, wastewater-impacted surface waters, etc.), using culture and/or molecular methods. This method should be applied in locations with adequate water flow so that a Moore swab can stay submerged across the sampling period. Results are reported as presence/absence.

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Summary of Moore Swab

1 The Moore swab is placed into the source water for 24-72 hours. When water passes through the Moore swab, bacteria such as *S.* Typhi may be captured by size exclusion, on particulate matter, electrostatic interactions, or by other mechanisms. The sample is enriched in broth to recover the target microorganism, and the enrichment is extracted for molecular testing.

Preparation

2 **2.1. UP broth**

1) Measure 950 mL of reagent-grade water in a beaker
2) Place water into a 1L flask
3) Add the following reagents to the flask

Pancreatic digest of casein	5.0 g
Proteose peptone	5.0 g
Monopotassium phosphate	15.0 g
Disodium phosphate	7.0 g
Sodium chloride	5.0 g
Dextrose	0.5 g
Magnesium sulfate	0.25 g
Ferric ammonium citrate	0.1 g
Sodium pyruvate	0.2 g

4) Mix thoroughly with stir bar
5) Adjust pH to 7.0 ± 0.2 with 10.0 N sodium hydroxide
6) Bring all contents to 1.0 L
7) Autoclave UP broth

2.2. Moore swab

1) Cut Purewip cheese gauze to 20 cm by 150 cm.
2) Starting on the short side of the Purewip cheese gauze, accordion fold to a width of 2-3 cm.
3) Tie the swab in the middle with the fishing line.
4) Fold the swab in half and wrap the string around the folded swab. (as shown below)

5) Autoclave swab 121°C for 15 minutes.

3 Procedures of Moore Swab Sample Collection

Materials

- Monofilament Fishing Line (Zebco Omniflex)
- Purewip cheese cloth (American Fiber & Finishing Inc, cat#53-100)
- Whirlpak bags (Spectrum Chemicals & Lab PROD, cat#972-95886-083)
- Permanent marker
- Cold packs, frozen at -20°C
- Cold chamber 0 °C
- Disinfectant
- Wipes
- Hand sanitizer
- Biohazard bag
- 50 mL UP enrichment broth
- 450 mL UP enrichment broth

1) Put on personal protective equipment (gloves, lab coat, face mask).
    a. *Note: This step is critical to ensure biosafety.*
2) Immerse a swab into a place (pumping station, wastewater treatment plant, manhole, river, drain, etc) with moving water.
3) Leave the swab for at least 24 hours in order to extract S. Typhi.
4) Retrieve the swab from the sewage carefully and transfer the swab to the labeled whirlpak bag with 50 mL of UP broth.
5) Close the Whirlpak bag by carefully and quickly rotating the bag, without spilling the sample, and then twisting the wire tabs together. Make sure that the bag is completely closed and not leaking.
6) Wipe outside of the whirlpak bag with a disinfectant.
7) Place the whirlpak bag in a cooler with cold packs and transport the sample to the laboratory.
8) Place any disposable items (*e.g.,* gloves, lab coat, face mask, etc.) in a biohazard bag for disinfection and disposal.
9) Change gloves or sanitize hands with alcohol-contained sanitizer or 70% ethanol.

4 Moore Swab Enrichment Procedures

1) Upon sample receipt at the laboratory, carefully transfer the Moore swab into a 1 L flask containing 450 ml of UP enrichment broth.
2) Shake the sample at 37°C for 24 hours.

3) Take out the swab from the flask and placed it in a bio-hazard bag. Leave the UP broth only.
4) Proceed the UP broth to the membrane filtration step.

5 Membrane Filtration

Equipment and Supplies:

- Capped pre-sterilized plastic bottles
- Vacuum manifold or vacuum flask and tubing
- Membrane Filter Holder & Funnel
- Vacuum Pump
- Filter apparatus with vacuum source
- Filter membranes, 47-mm diameter, 0.45-µm pore size (MF-Millipore™, cat# HAWP04700)
- Forceps
- Enrichment flask (sterile 150-ml Erlenmeyer flasks containing 25-ml APW)
- Phosphate buffered solution (PBS), pH 7.4
- 70% ethanol
- 10% bleach
- Sterilized water / deionized water
- 2 mL Microcentrifuge tubes

Preparation:

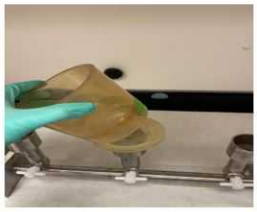
a. Wipe down bench or hood with 10% bleach followed by 70% ethanol.

b. Assemble the filtration funnel and the base.

c. Prepare alcohol burner with lighter.
d. Prepare a small beaker with 100% ethanol for sterilizing forceps. The ethanol should be 2-3 cm deep, just enough to cover the tips of the forceps when they are resting in the beaker.

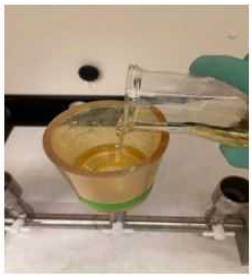
Procedures:

1) Attach the filtration funnel to the holder and rinse the funnel with distilled water.



2) Flame forceps for approximately 5 seconds horizontally in case burning to your hand.
3) Use the sterile forceps to remove the filter and place it onto the filter base into the funnel assembly.

4) Pour 10 mL of PBS on the filter.



5) Turn on vacuum, open the valve and close the manifold valves.
6) Pour another 10 mL of PBS on the filter and vacuum it through.

7) Flame the forceps again to sterilize. Carefully, remove membrane filter from filter base with sterile forceps, avoiding contact with the center of the membrane.
8) Place the filter, gridded side up, labeled "Negative Control" and placed the filter paper into a 2 mL microcentrifuge tube.
9) Repeat steps 2 to 5.
10) Filter 10 to 20 ml UP-enriched sample through a 47-mm, 0.22-µm pore size polycarbonate filter. Note: the amount of sample chosen is determined by the concentration of *S. Typhi* in the sample.
11) Add the membranes with attached bacteria to a 5 ml PowerWater DNA bead tube and label the tube with the sample name.
12) Clean up the bench or hood using 10% bleach followed by 70% ethanol.
13) Carefully process the labeled tubes to the next step. If there's not enough time, store the samples at -70 to -80°C for further use to follow with DNA extraction.
14) If you have more sample, start from the step 2. Note: One negative processing control for 1-10 samples. For 11-20 samples, two negative processing controls are necessary.

6 DNA Extraction

Materials:

- Qiagen DNeasy PowerWater kit (Cat# 14900-100-NF)
- Flat blade forceps
- 10-20 ml 70% ethanol
- Vortexer
- 1 ml and 200 ul pipettes
- 1 ml and 200 ul tips
- Discarding beaker
- Marker
- Microcentrifuge with speed up to 13,000 rpm

Procedure: DNeasy PowerWater Kit product page at https://mobio.com/powerwater-filter.

1) Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube.
2) Secure the tube horizontally to a Vortex Adapter (cat. no. 13000-V1-5/13000-V1-15).
3) Vortex at maximum speed for 5 min.
4) Transfer the supernatant to a clean 2 ml Collection Tube (provided in the kit). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.
   Note: *Placing the pipette tip down into the beads is required. Pipette until you have removed all the supernatant. Expect to recover 600–650 µl of supernatant.*
5) Centrifuge at 13,000 x *g* for 1 min at room temperature.
6) Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube.
7) Add 200 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
8) Centrifuge the tubes at 13,000 x *g* for 1 min.
9) Avoiding the pellet, transfer all of the supernatant to a clean 2 ml Collection Tube (provided in the kit).
10) Add 650 µl of Solution PW3 and vortex briefly to mix.
11) Load 650 µl of supernatant onto an MB Spin Column. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through. Repeat until all the supernatant has been processed.
12) Place the MB Spin Column Filter into a clean 2 ml Collection Tube (provided in the kit).
13) Add 650 µl of Solution PW4 (shake before use). Centrifuge at 13,000 x *g* for 1 min.
14) Discard the flow-through and add 650 µl of ethanol and centrifuge at 13,000 x *g* for 1 min.
15) Discard the flow-through and centrifuge again at 13,000 x *g* for 2 min to remove all traces of ethanol.
16) Place the MB Spin Column into a clean 2 ml Collection Tube (provided in the kit).
17) Add 100 µl of Solution EB to the center of the white filter membrane. Wait for 2-3 min to allow the DNA to dissolve in EB buffer.
18) Centrifuge at 13,000 x *g* for 1 min.
19) Discard the MB Spin Column. Label the collection tube with sample ID and Date, and store the DNA at -20^{O}C.

7 Real-Time PCR detection of *Salmonella* Typhi and *Salmonella* Paratyphi A

Equipment and Materials:

- iQ powermix (Bio Rad, cat#1725849)
- S. typhi primer Forward (10 µM)
- S. typhi primer Reverse (10 µM)
- S. typhi Probe (10 µM)
- S. Paratyphi A primer Forward (10 µM)
- S. Paratyphi A primer Reverse (10 µM)
- S. Paratyphi A Probe (10 µM)
- 96-well plates (Bio-rad, cat# HSP9601)
- Microseal B Adhesive seals for PCR plates (Bio-rad, cat# MSB1001)
- Bio-rad Real-Time PCR (qPCR) machine

Procedures:

1) Spray surface of hood and pipettes with 70% ethanol, then wipe off
2) Thaw the reagents from the Bio-Rad iQ powermix, primers, and probes at room temperature

3) Place all the reagents on ice.

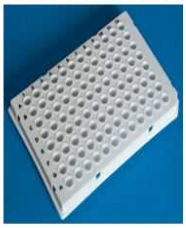
4) Prepare the following mixture under a clean hood:

Reagent	S. Typhi Volume (ul)	S.Paratyphi (ul)
Bio-Rad iQ Multiplex Powermix	12.5	12.5
S. Typhi forward primer (10 µM)	1.0	
S. Typhi reverse primer (10 µM)	1.0	
S. Typhi probe (10 µM)	0.5	
S. Paratyphi forward primer (10 µM)	-	1.0
S. Paratyphi reverse primer (10 µM)	-	1.0
S. Paratyphi probe (10 µM)	-	0.5
Molecular Water	5.0	5.0
Total	20.0	20.0

5) Calculate how much the reagents needed in total (according to the number of the wells needed for RT-PCR).
6) Aliquot 20 µl of the mixture created to each well of the PCR plate
a. Dispose of used pipet tips and plastic cup into trash
7) Move the PCR plate into a new area
8) Spray surface of the new area and pipettes with 10% bleach, then wipe off

9) Spray surface of the new area and pipettes with 70% bleach, then wipe off
10) Add 5 µl of DNA into the PCR plate

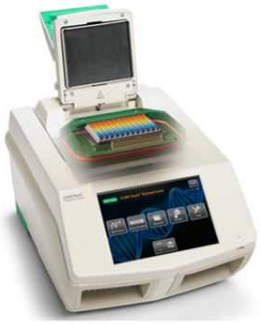
- For the PCR negative control, add 5 µl of molecular grade water
- For the Extraction negative control, add 5 µl of extraction material
- For the DNA extraction positive control, add 5 µl of DNA control



11) Dispose of used pipet tips and plastic cup into trash
12) Place samples in Bio-Rad Detection System with the following program:

95°C	15 mins	
95°C	30 secs	} 40 cycles
60°C	30 secs	
72°C	30 secs	

13) Place the 96-well plate into the Bio-rad PCR machine. Remember to correctly select the probe/fluorescence to FAM and start the cycles.



14) Results are interpreted using computer software available with Real-Time PCR machines by monitoring increase in fluorescence throughout the amplification cycles and reported as Ct value. The Ct value is the number of amplification cycles required to detect a fluorescent signal above a given threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample. In general, Ct values < 29 are considered strong positive reactions and are indicative of abundant target nucleic acid in the sample, while Ct values of 30 to35 are positive reactions indicative of moderate amounts of the target, and Ct values of 38 to 40 are considered weak reactions with little or no target nucleic acid in the sample.