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Environmental sampling using Moore swabs

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Typhoid Environmental Surveillance

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A protocol for the collection of environmental samples (sewage and wastewater) using Moore swabs and subsequent laboratory processing up to DNA extraction. This is part of a set of protocols for environmental surveillance of *Salmonella* Typhi.

Bacteria in the wastewater are trapped in the Moore swab. Trapped bacteria are enriched during incubation in Universal Pre-Enrichment broth. The broth is then filtered under a vacuum to capture the bacteria on filter discs ready for DNA extraction.

This protocol is adapted from the Moore swab protocol described in Liu *et al* (2021).

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protocol

Liu P, Ibaraki M, Kapoor R, Amin N, Das A, Miah R, Mukhopadhyay A, Rahman M, Dutta S, Moe C (2021), Development of Moore Swab and Ultrafiltration Concentration and Detection Methods for *Salmonella* Typhi and *Salmonella* Paratyphi A in Wastewater and Application in Kolkata, India and Dhaka, Bangladesh. Frontiers in Microbiology, doi: 10.3389/fmicb.2021.684094

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It is recommended that sample collection be done in the morning.

Universal Pre-Enrichment Broth **Fischer**

Scientific Catalog #DF0235-17-8

Moore swabs
Wide-mouth jars (500ml capacity)
Cold packs and ice box
20ml pipet
Membrane filtration apparatus
45mm 0.45um filter discs
Empty Powerbead tubes or equivalent (2ml screw cap microcentrifuge tubes)

Appropriate PPE (Disposable lab coats, nitrile gloves, face mask) and disinfectant

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Ensure that you wear appropriate PPE during sampling (e.g. gloves, disposable lab coat, appropriate footwear, facemask).

Sample Collection

1 Sterilise Moore swabs by autoclaving prior to taking them to the field site.

2 Immerse the swab in the sewage or wastewater channel and tie it to a stable support using the tailing thread of the swab.

3 Leave in place for 24-72 hours

4 Prepare 450ml of Universal Pre-Enrichment broth per sample, following the manufacturer's instructions

5 Cut the thread of the Moore swab and transfer the swab to an appropriately labelled container with 450ml of UPE broth

6 Place the container on ice and transport back to the lab within 4 hours of sample collection

Processing the Moore Swab

7 Incubate the container with the Moore swab in UPE broth at **37 °C** for 21 - 24 hours

8 After incubation, swirl the mixture and pipet two 20ml aliquots from the enrichment broth into 50ml conical flasks

9 Assemble the membrane filtration unit according the manufacturer's instructions and attach to the vacuum pump

10 Using sterile tweezers, place a 47mm 0.45µm filter disc onto the filtration unit head and attach the filtration unit cup

11 Pipet the first 20ml sample into the filtration cup and turn on the vacuum to filter the sample through the disc

12 Once complete, cut the filter into several strips using sterile forceps then place the strips into an empty PowerBead tube. If possible, place the strips so that the "dirty" side faces the inside of the tube.

13 Repeat steps 10 to 12 with the second 20ml sample.

14 Store samples at **-20 °C** until extraction.

15 Thoroughly disinfect filtration cups, containers and conical flasks after use to avoid cross contamination of samples.