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Environmental Grab Sampling and Membrane Filtration

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Typhoid Environmental Surveillance

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A protocol for the collection of environmental samples (sewage and wastewater) using grab sampling and membrane filtration. This is part of a set of protocols for environmental surveillance of *Salmonella* Typhi.

1L of sewage or wastewater is collected then filtered under a vacuum to capture the bacteria on filter discs ready for DNA extraction.

This protocol is adapted from the method described in Rigby *et al* (2021).

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protocol

Rigby J, Elmerhebi E, Diness Y, Mkwanda C, Tonthola K, Galloway H, MIles R, Henrion M, Edwards T, Gauld J, Msefula C, Johnston R, Nair S, Feasey N, Elviss N (2021), Optimized methods for detecting *Salmonella* Typhi in the environment using validate field sampling, culture and confimratory molecular approaches. Journal of Applied Microbiology, doi: 10.1111/jam.15237

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It is recommended that sampling be done in the morning.

Ringer's lactate solution

Bucket/container with 1L capacity
Membrane filtration apparatus
47mm 0.45um filter discs
Coffee filters (if necessary)
Whirl-Pak bags

Appropriate PPE and disinfenctants

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

Sample Collection

1 Using a bucket or another method, fill a 1L collection container with sewage or wastewater and securely close the container.

2 Wipe the outside of the collection container with disinfectant.

3 Place the container in a cold container with ice packs for transport back to the laboratory

4 Place any disposable items in a biohazard bag for disinfection and disposal. Place any reusable items into a biohazard bag for return to the library and disinfection. Sanitise hands with hand sanitiser.

Sample Processing

5 Upon receipt in the lab the sample should be stored at 4-8ºC until filtration

6 Assemble the membrane filtration apparatus according to the manufacturer's instructions

7 Measure the sample into a large, sterile graduated cylinder

8 Place a 47mm 0.45µM filter disc on the filtration unit head and attach the filtration unit cup.

If necessary, place a coffee filter on top of the cup as a pre-filter to remove any larger solid matter.

9 Pour sample into the filtration cup and turn on the vacuum to allow sample to slowly filter through the disc for 1 hour.

If the filter becomes clogged, pipette the remaining sample back into the measuring cylinder, and replace the filter disc and coffee filter with fresh filters. Repeat as necessary with up to 5 filters.

10 Using sterile forceps, transfer the filter discs to a small WhirlPak bag, add 10ml of Ringer's lactate and then seal the bag shut

11 Elute the filter discs by massaging the bag until the filters are clean or have fallen apart

12 Pipette 1ml of the eluate into a 1.5ml microcentrifuge tube and centrifuge at

10000 x g, 00:10:00

Pipette off the supernatant and store the pellet at -20ºC until DNA extraction.

13 Thoroughly disinfect membrane filtration cups and measuring cylinders after use to avoid cross contamination of samples.