

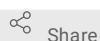


Aug 23, 2021

Protocol for detection of *Salmonella* Typhi and *Salmonella* Paratyphi A in Surface water (and other types of water samples)

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dx.doi.org/10.17504/protocols.io.bv72n9qe

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ABSTRACT

The protocol describes method for qualitative detection (presence/ absence) of *Salmonella* Typhi and *Salmonella* Paratyphi A in surface water by enrichment culture followed by real-time PCR.

The same protocol can be used for other types of water samples such as drinking water, drain water or flood water.

DOI

dx.doi.org/10.17504/protocols.io.bv72n9qe

PROTOCOL CITATION

Renuka Kapoor, Ashutosh Wadhwa, Christine Moe 2021. Protocol for detection of *Salmonella* Typhi and *Salmonella* Paratyphi A in Surface water (and other types of water samples). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bv72n9qe>

FUNDERS ACKNOWLEDGEMENT

Bill & Melinda Gates Foundation
Grant ID: OPP1150697

KEYWORDS

Surface water, water, *Salmonella* Typhi, *Salmonella* Paratyphi A, enrichment, detection, environment

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CREATED

Jun 30, 2021

LAST MODIFIED

Aug 23, 2021

PROTOCOL INTEGER ID

51162

MATERIALS TEXT

- Gloves
- 70% ethanol
- Felt tip pen
- Biosafety hood
- 37°C incubator
- Sterile 1 L flask (1/sample)
- UP (Universal pre-enrichment) Broth (450 mL/sample)

Preparation of UP broth

Measure 950 mL of reagent-grade water in a beaker

Place water into a 1 L flask

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

Mix thoroughly with stir bar

Adjust pH to 7.0 ± 0.2 with 10.0 N sodium hydroxide

Bring all contents to 1.0 L

Autoclave UP broth

NOTE: The UP broth will form a precipitate at the bottom after autoclaving. Shake the bottle well before use.

- 1 Sterile 500 mL graduated cylinders
- Serological pipettes and pipette controller
- Sterile 1 L flask (1 per sample)
- 0.45 µm membrane filter and filtration unit
- Sterile forceps
- PowerWater DNA extraction kit (Qiagen, Cat. No.: 14900-100-NF)

1. Processing of Surface water sample

- 1 The surface water does not require any processing and can be used directly with or without enrichment culture. The following steps describe bench preparation up to enrichment stage.

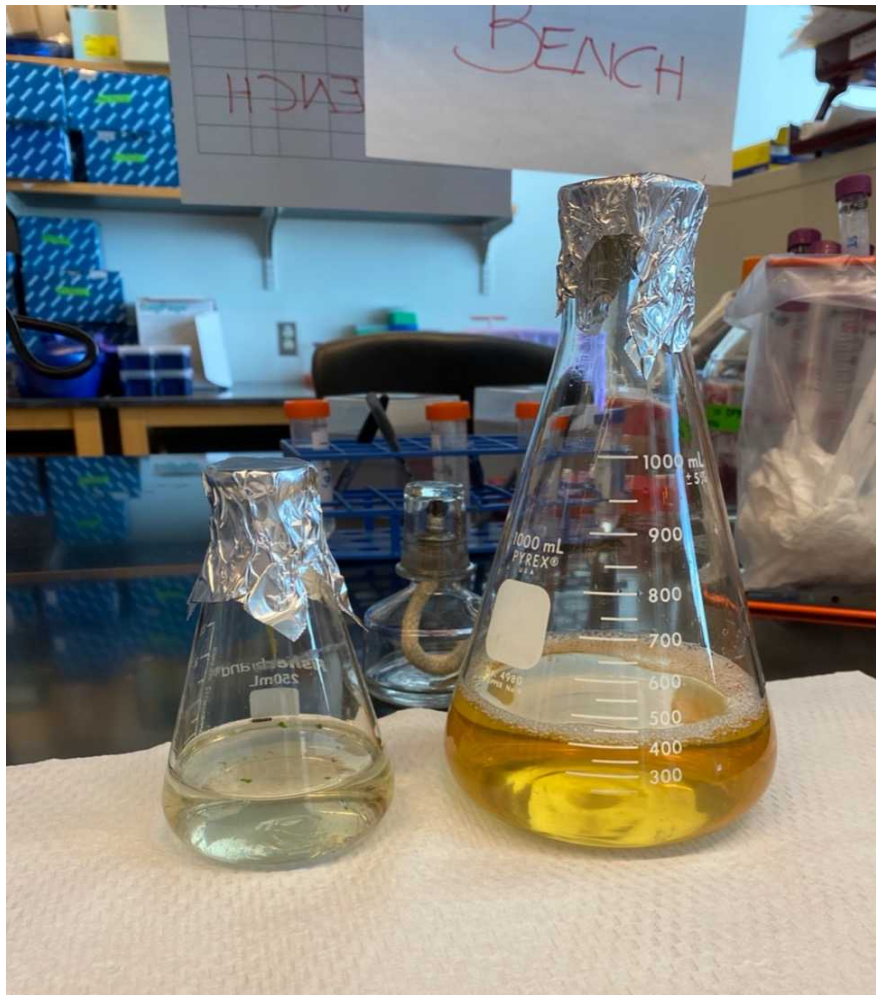
- 1.1 Put on gloves and spray hands with 70% ethanol. Rub hands together to sanitize all surfaces of the gloves.

- 1.2 Prepare your work surface by cleaning it with 70% ethanol.

- 1.3 Samples should arrive at the lab in a Whirl-Pak bag or glass bottles. Spray the outside of the bag/bottles with 70% ethanol and rub it well.
- 1.4 Rotate the Whirl-Pak bag or bottle with the sample five times to mix the sample.

2. Enrichment culture

- 2 The following steps are for optional enrichment of the sample. If enrichment is not performed, the sample can be used directly for membrane filtration and DNA extraction (Section 3).
 - 2.1 In a biosafety hood, add **450 mL** of Universal Pre-enrichment (UP) Broth ([USEPA Standard Analytical Protocol for Salmonella Typhi in Drinking Water](#)) to a sterile 1 L flask.
 - 2.2 Transfer **50 mL** of sample to the flask containing the UP Broth.



- 2.3 Incubate the flask in shaking incubator **37 °C** overnight (16-18 hr).



3. DNA Extraction

5m

- 3 The following steps are for membrane filtration and DNA extraction. They can be performed following step 1.4 or step 2.3.

3.1 Clean your workspace and set up your filter units.

If enrichment was performed, remove the flask from the incubator.

3.2 Using sterile forceps, place a clean membrane filter on the base of the filter unit.



3.3 Place the cup on top of the filter.

Make sure the cup is placed flush against the base. If there are any gaps the sample will spill out.



3.4 Add mL of enriched sample to the cup and turn on the vacuum.

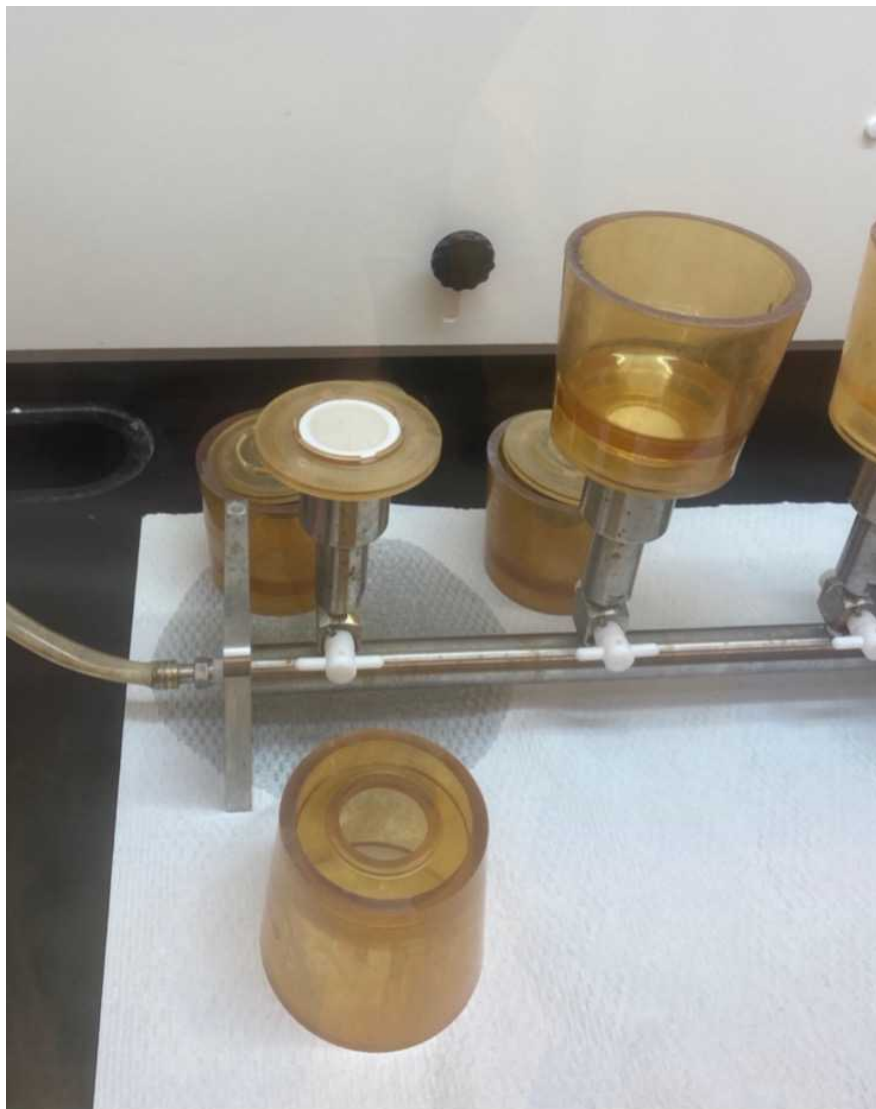
For samples processed without enrichment, use 100ml of sample for membrane filtration.



3.5 Allow the sample to filter until liquid is no longer visible on the filter.

The time needed for this step varies depending on the sample type and dirtiness or turbidity. Another way to tell if it is finished is when the ridges of the filter unit base are visible on the filter.

3.6 Turn off the vacuum and remove the cup from the base.



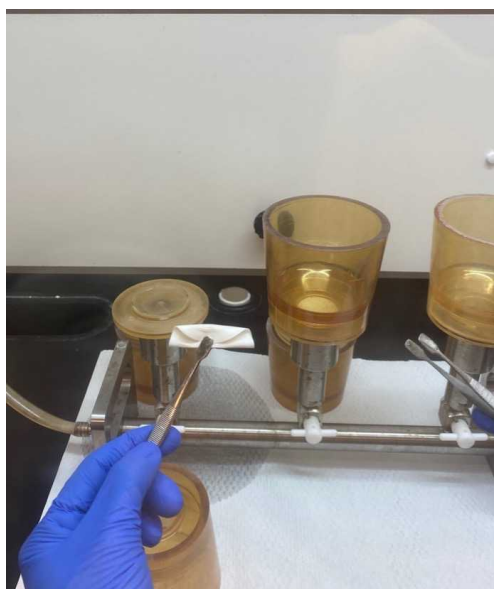
3.7 Using a sterile forcep, remove the filter from the base.



- 3.8 Using two sterile forceps, fold the filter in half inward, so the cells are now contained inside the folded filter.



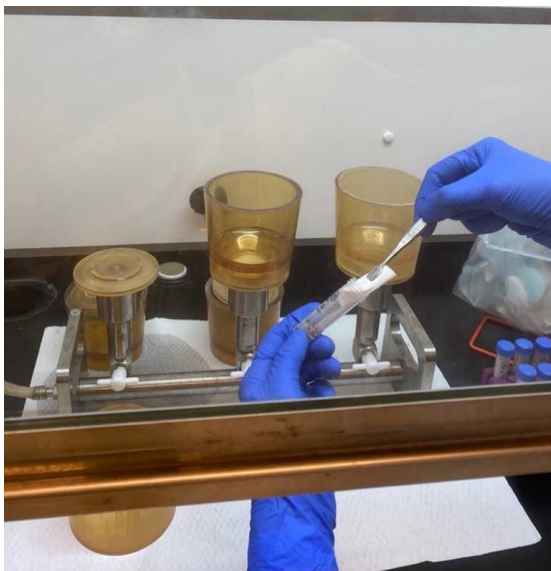
3.9 Fold the filter in half again.





3.10 Transfer the folded filter to a bead tube (from Qiagen DNeasy PowerWater kit).

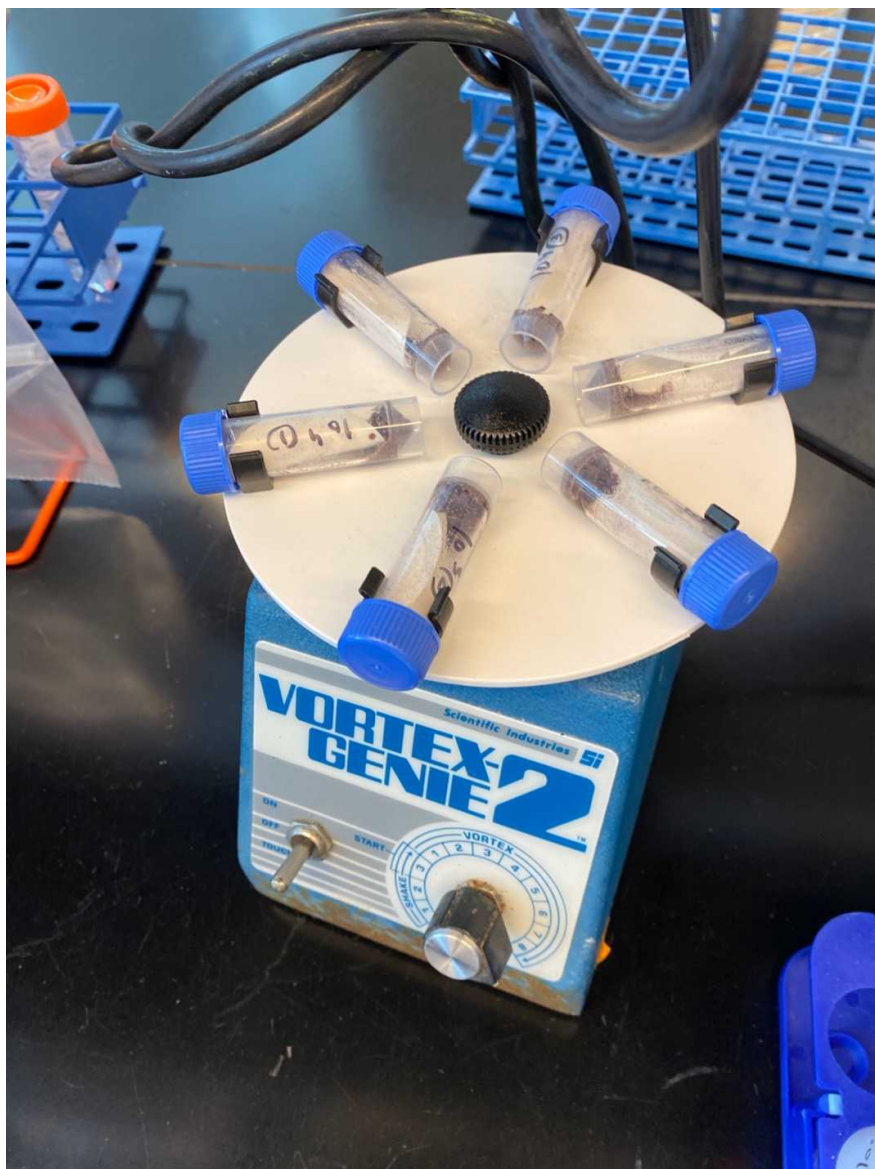


Bead tube (Qiagen DNeasy PowerWater kit)



3.11 Add  1 mL of Buffer PW1 (Qiagen DNeasy PowerWater kit) to the bead tube and vortex for  00:05:00 .

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- 3.12 Proceed with the DNA Extraction according to manufacturer's protocol (Qiagen DNeasy PowerWater kit).

4. Real-time PCR

- 4 Test DNA extracts for *S. Typhi* and *S. Paratyphi A* using Taqman-based quantitative real-time PCR (qPCR) platform.

4.1 Detection of *S. Typhi*

S. Typhi is detected using duplex PCR protocol developed by researchers at the University of Washington (Scott Meschke and team) using primers and probes targeting the *tviB* and *staG* genes ([Nair et al., 2019](https://doi.org/10.1186/s13059-019-1750-4)).

*tviB*_F 5'TGTGGTAAAGGAACTCGGTAAA-3';
*tviB*_R 5'-GACTTCCGATACCGGGATAATG-3';

tvB_P HEX-TGGATGCCGAAGAGGTAAGACGAGA-BHQ1;
staG_F 5'-CGCGAAGTCAGAGTCGACATAG-3';
staG_R 5'-AAGACCTCAACGCCGATCAC-3';
staG_P FAM-5'-CATTTGTTCTGGAGCAGGCTGACGG-3'-BHQ1

The reaction mixture contain 0.65 µl of *tvB_F* (20µM), 0.75 µl each of *tvB_R* (20µM), *staG_F*(20µM), and *staG_R* (20µM), 0.5 µl each of the probe *tvB_P* (10µM) and *staG_P* (10 µM), 12.5 µl of SsoAdvanced Universal Probes Supermix (Bio-rad), and 5 µl of DNA in a final volume of 25 µl. The PCR reaction conditions include initial denaturation at 95°C for 5 min, followed by 45 cycles of 95°C 30 sec, 64°C 30 sec, 72°C 10 sec, and final extension at 72°C for 5 min.

4.2 Detection of *S. Paratyphi A*

S. Paratyphi A Is detected using primers and probe targeting *SPA2308* ([Nga et al., 2010](#))

SPA2308_F 5'-ACGATGATGACTGATTTATCGAAC-3';
SPA2308_R5'-TGAAAAGATATCTCTCAGAGCTGG-3';
SPA2308_PCY5-CCCATACAATTCATTCTTATTGAGAATGCGC-BHQ2

The reaction mixture containing 1 µl of each primer (10µM), 0.4 µl of probe (10µM), 200µM of dNTPs, 5mM of MgCl₂, 5U of HotStar Taq DNA polymerase (Qiagen), and 5 µl of DNA in a final reaction volume of 25 µl. The PCR reaction conditions include initial denaturation at 95°C for 5 min, followed by 45 cycles of 95°C 30 sec, 60°C 30 sec, 72°C 30 sec, and final extension at 72°C for 10 min.