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Protocol for detection of *Salmonella* Typhi and *Salmonella* Paratyphi A in Surface Swabs

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ABSTRACT

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

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PROTOCOL CITATION

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KEYWORDS

surface, tile, wood, metal, swab, Salmonella Typhi, Salmonella Paratyphi A, enrichment, detection, environment

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MATERIALS TEXT

- Gloves
- 70% ethanol
- Biosafety hood
- Vortex
- 37°C incubator
- 4°C refrigerator
- Serological pipettes and pipette controller
- Sterile 100 mL graduated cylinder
- PBST (1X PBS with 0.05% Tween 80) (40 mL/sample)
- UP Broth (180 mL/sample)

Preparation of UP broth
Measure 950 mL of reagent-grade water in a beaker
Place water into a 1L flask
Add the following reagents to the flask
Pancreatic digest of casein5.0 g
Proteose peptone5.0 g
Monopotassium phosphate15.0 g
Disodium phosphate7.0 g
Sodium chloride5.0 g
Dextrose0.5 g
Magnesium sulfate0.25 g
Ferric ammonium citrate0.1 g
Sodium pyruvate0.2 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.

- 50 mL sterile conical tube (1 per sample)
- Sterile 500 mL flask (1 per sample)
- 0.45 mM membrane filter and filtration unit
- Sterile forceps
- Qiagen PowerWater DNA extraction kit (Cat. No.: 14900-100-NF)

1. Processing of surface swab samples

1 The following steps describe processing of surface swabs up to enrichment stage.

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

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

1.3 Label a sterile 50 mL conical tube each with the Sample ID, the date, and your initials.

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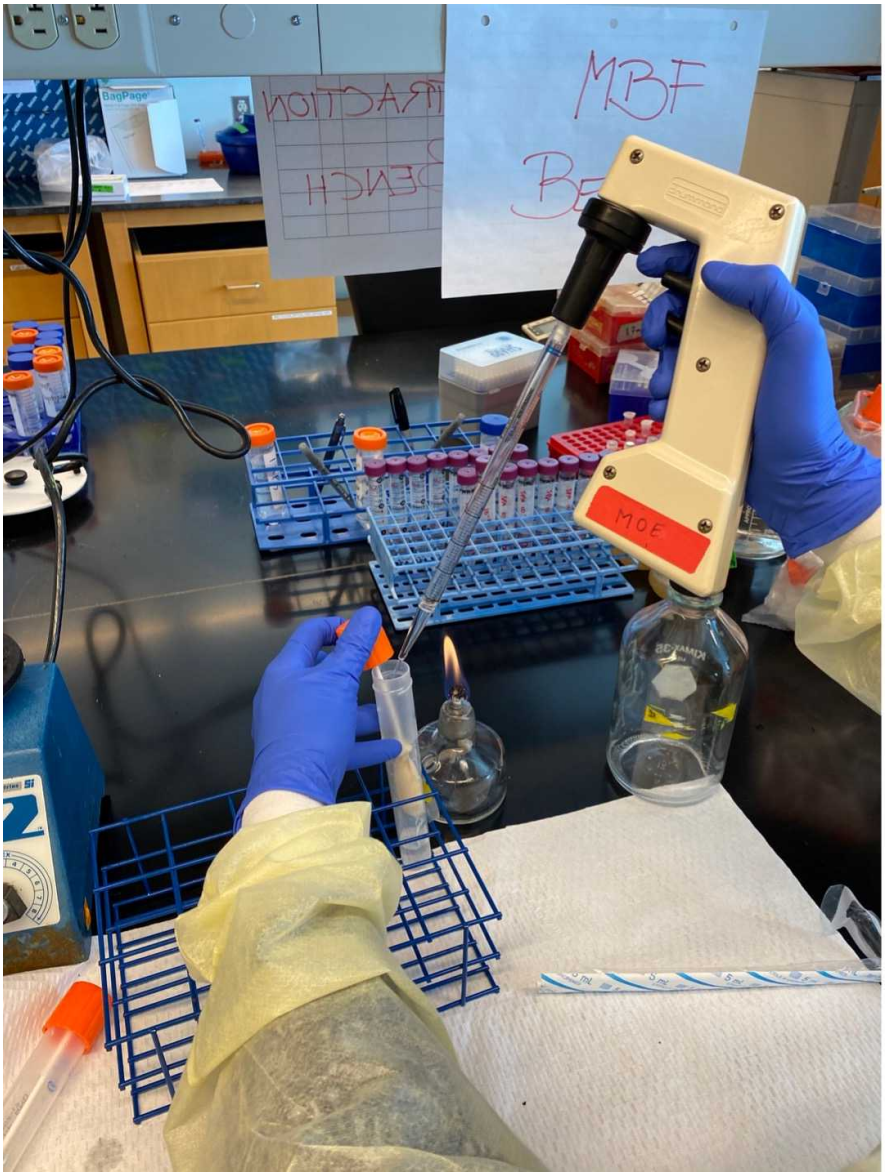
1.4 Carefully unscrew the cap of the tube with the swab.

Take care not to touch the swab itself, nor any other part of the swab aside from the lid.

1.5 Use a sterile 10 mL pipet to carefully add **10 mL** of PBST to the swab containers.



1.6 Place the swab back into the tube, screwing the lid down well.

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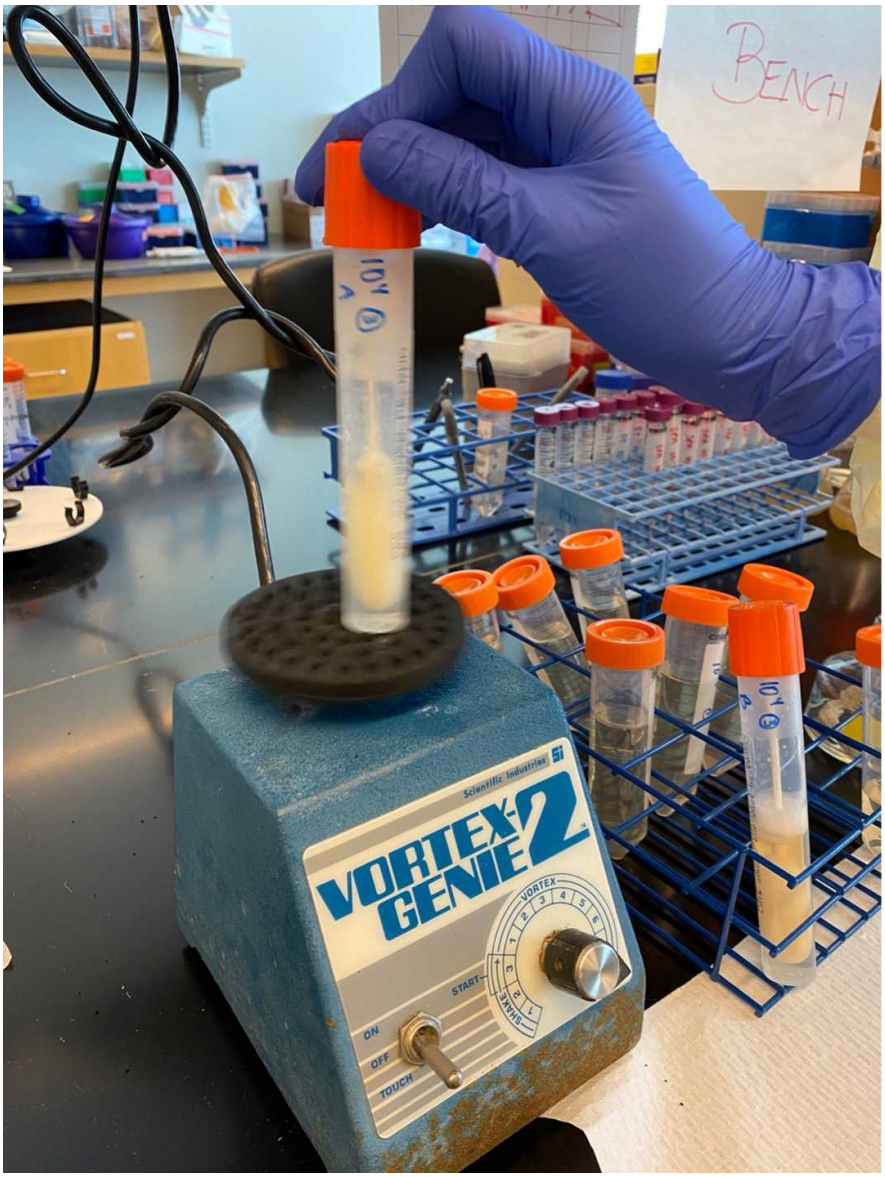
30s

1.7 Vortex the tubes containing the swabs for **00:00:30** .



5m

1.8 Incubate for **00:05:00** at **Room temperature** .

30s

1.9 Vortex for another **00:00:30** .

1.10 Reopen swab container and pour the swab elute into the empty labeled 50 mL conical tube.

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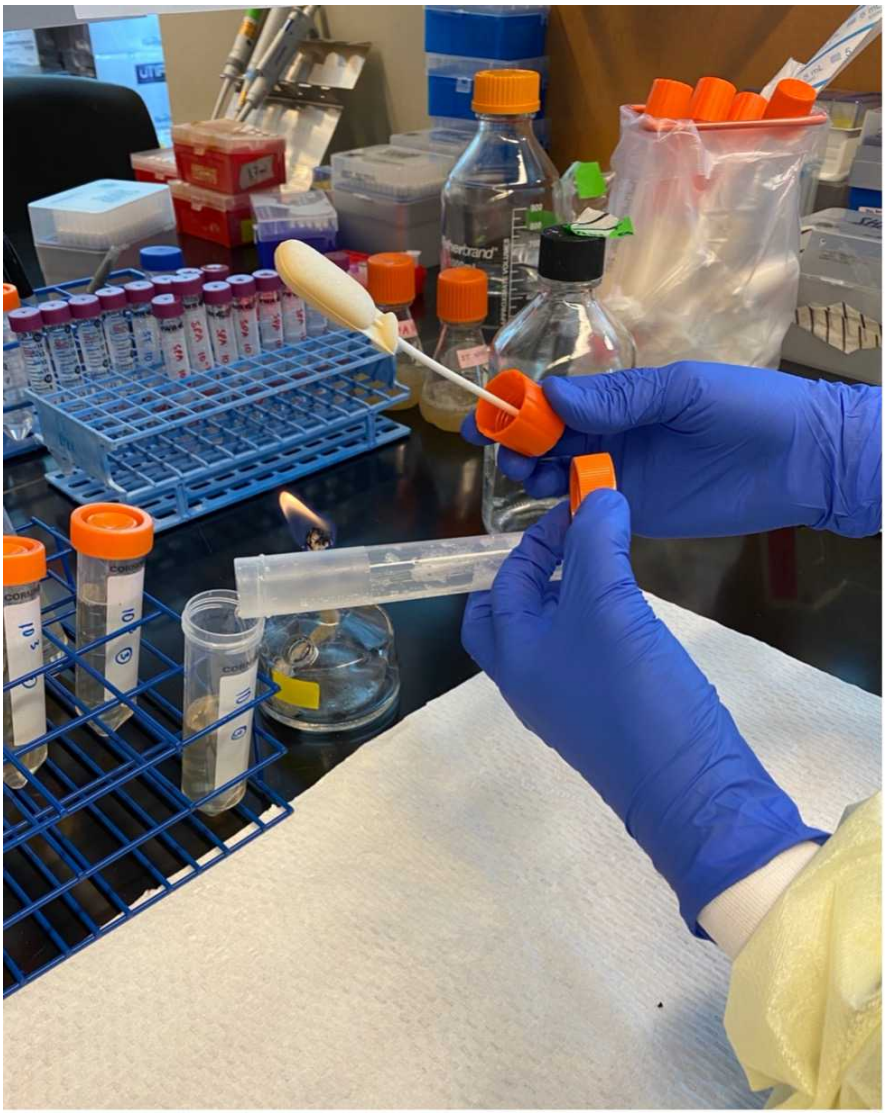

1.11 Wash the swab again with 10 ml of PBST by repeating steps 1.5 through 1.9, then proceed to step 1.12.

1.12 Press the swab against the side of the tube to squeeze out as much remaining wash solution as possible, and transfer into the 50 mL conical tube.

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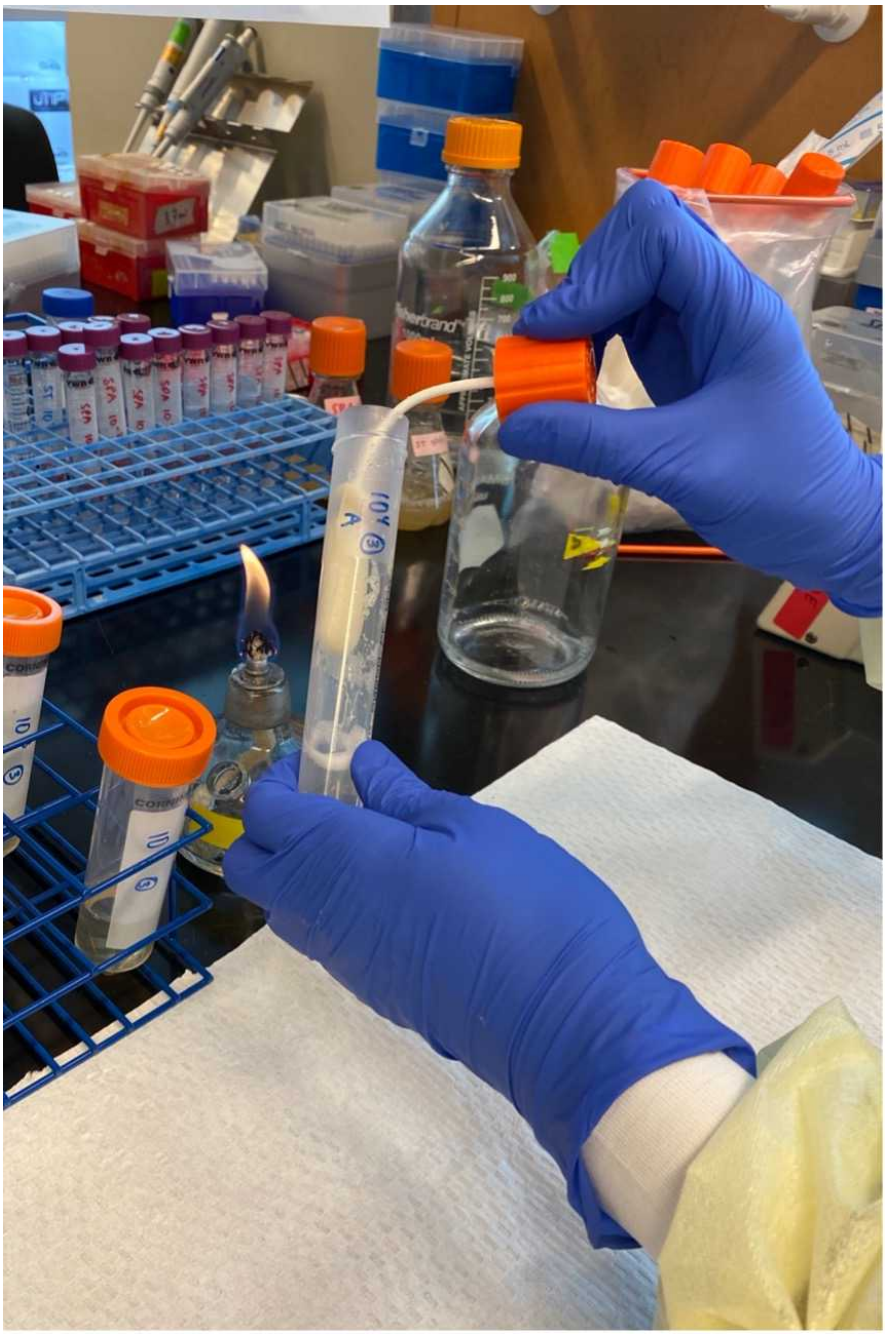

Do not apply too much force, as the swab can break. Just gently press out as much liquid as possible. There will be ~ **15 mL** of swab elute after two washings of the swab.

2. Enrichment culture

2 The following steps are for optional enrichment of the sample. If enrichment is not performed, the processed sample can be used directly for membrane filtration and DNA extraction (Section 3).

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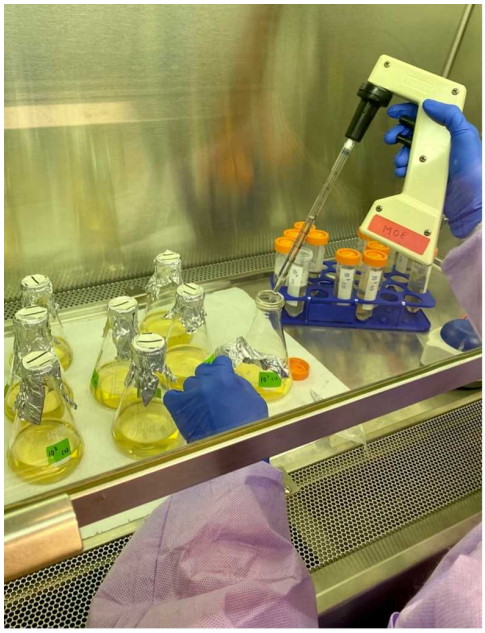
2.1 In a biosafety hood, transfer **180 mL** of Universal Pre-enrichment (UP) Broth (USEPA Standard Analytical Protocol for Salmonella Typhi in Drinking Water) to a sterile 500 mL flask.

2.2 Add **10 mL** of swab elute to the flask containing UP Broth.



2.3 Incubate the flask in shaking incubator **37 °C** overnight.

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3. DNA Extraction 5m

3 The following steps are for membrane filtration and DNA extraction. They can be performed following step 1.12 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.

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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.

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3.4 Add **20 mL** of enriched sample to the cup and turn on the vacuum.

For processing samples without enrichment, use 10 ml of swab elute from step 1.12 for membrane filtration.

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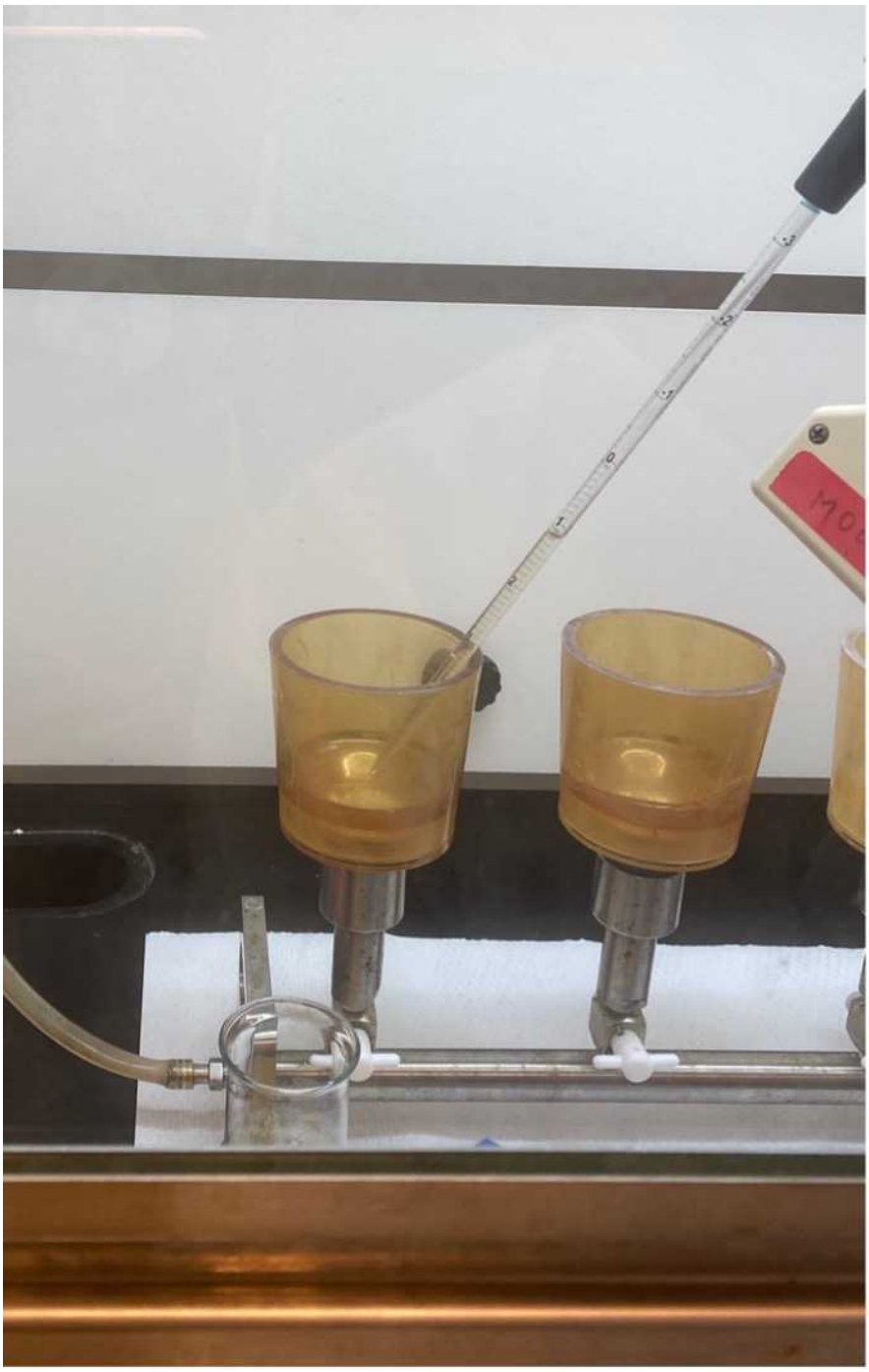

3.5 Allow the sample to filter until liquid is no longer visible on the filter.

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.

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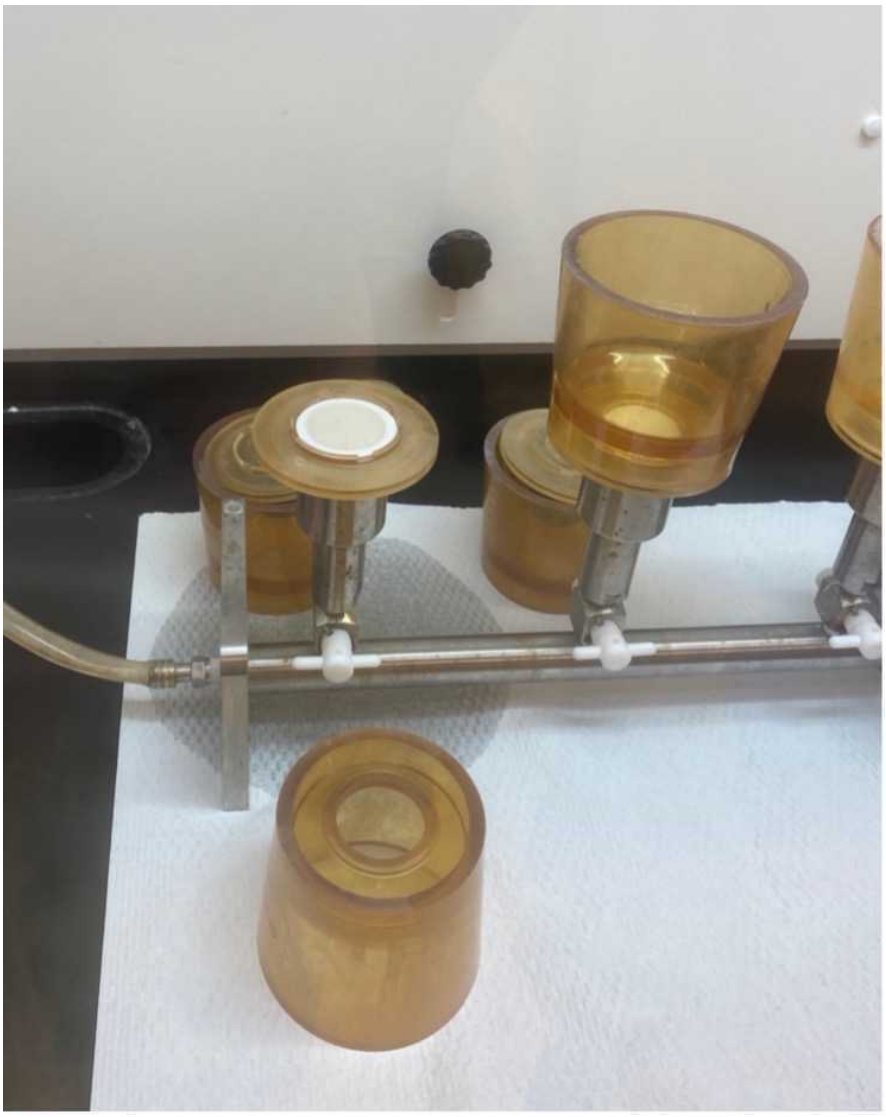

3.7 Using a sterile forcep, remove the filter from the base.

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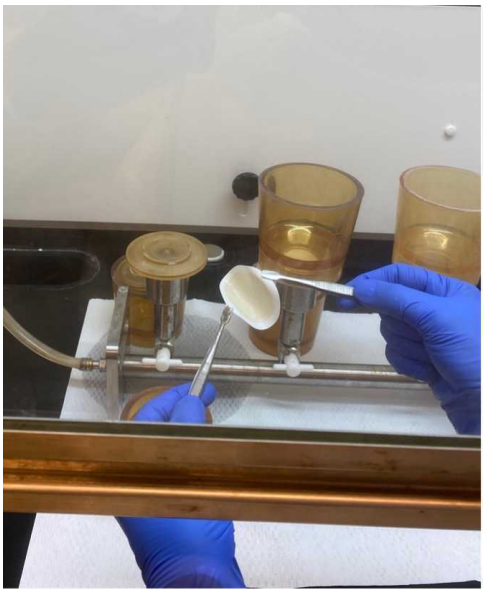
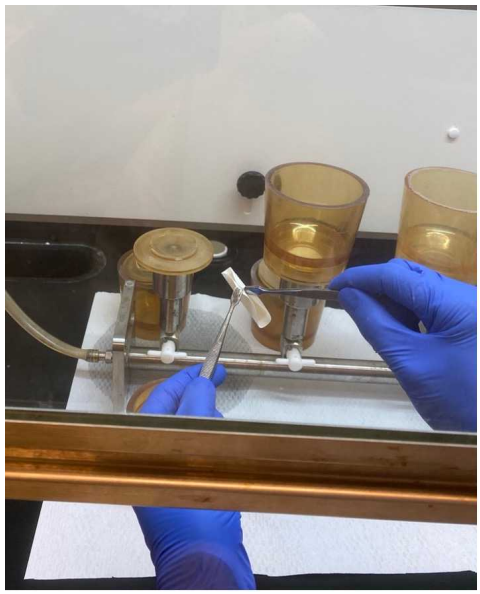
3.8 Using two sterile forceps, fold the filter in half inward, so the cells are now contained inside the folded filter.





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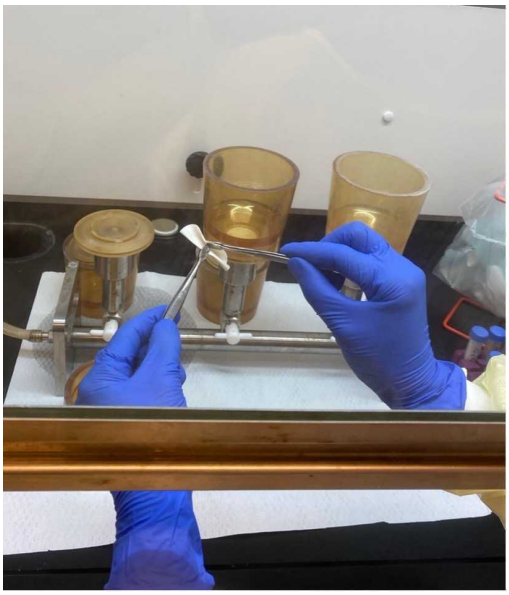
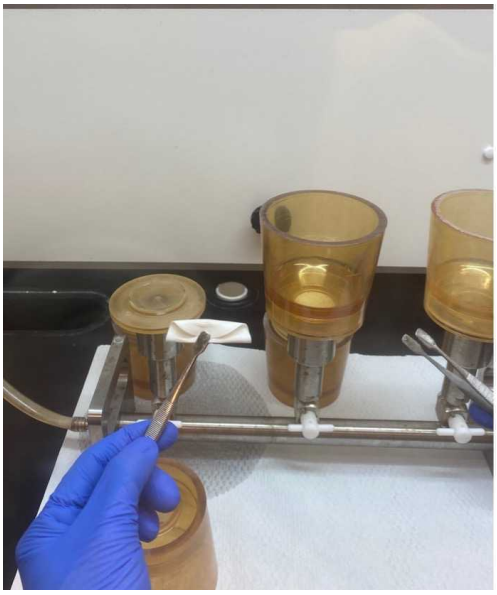
3.9 Fold the filter in half again.





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

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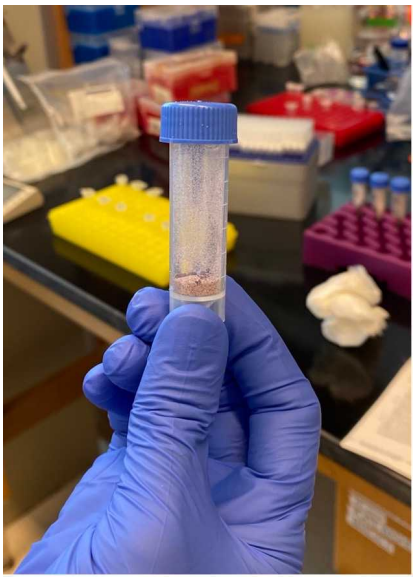
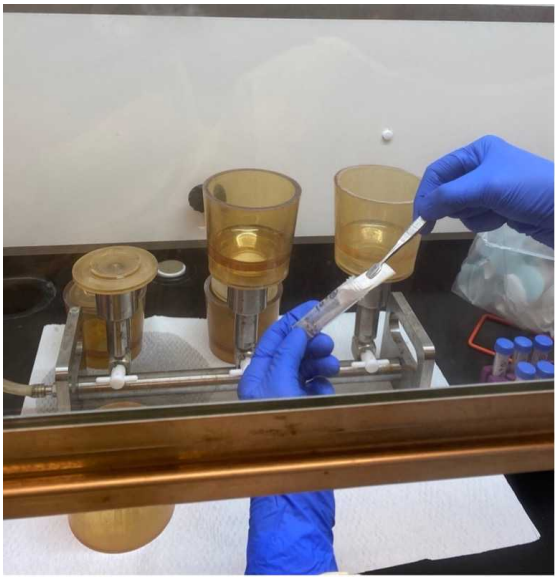


Bead tube provided in Qiagen DNeasy Powerwater kit



3.11 Add **1 mL** of Buffer PW1 (Qiagen DNeasy PowerWater kit) to the bead tube and vortex for **00:05:00** . 5m

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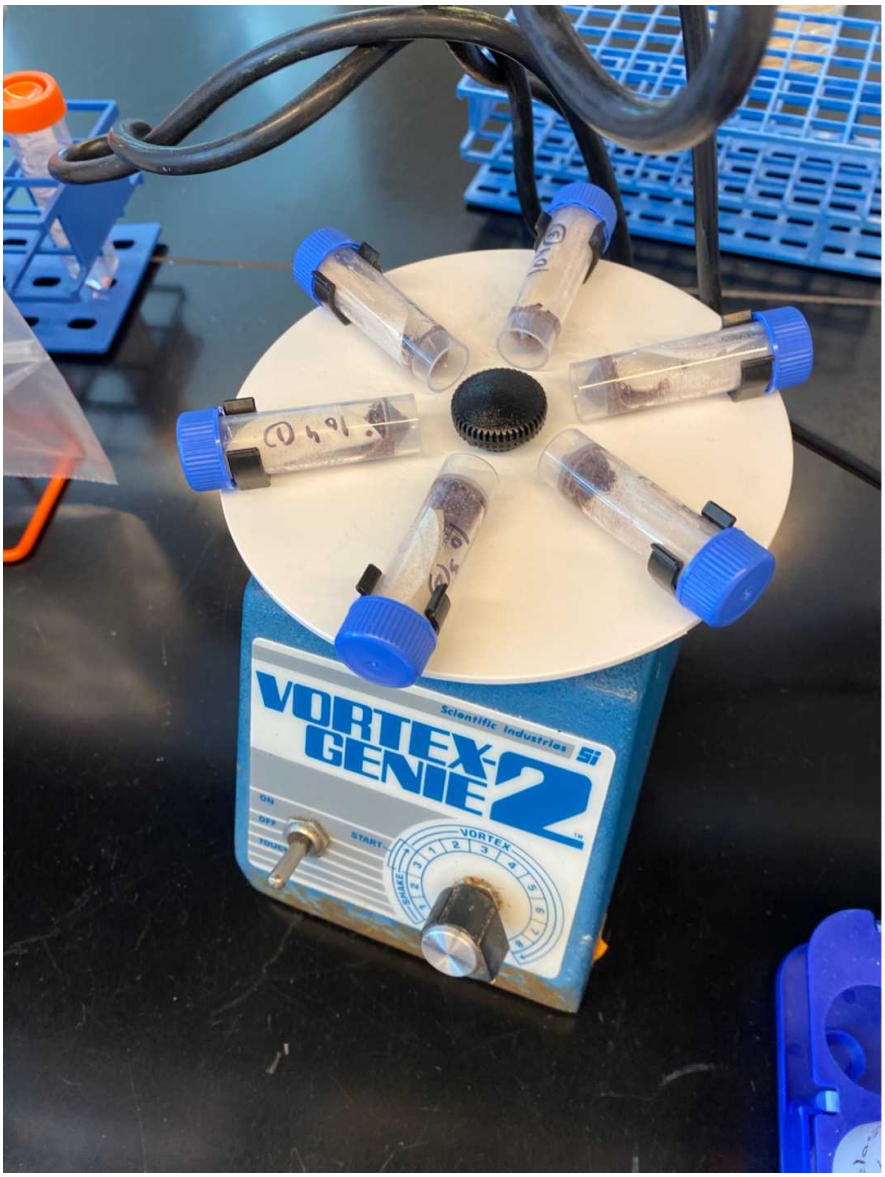

3.12 Proceed with the DNA Extraction according to manufacturer's protocol (Qiagen DNeasy PowerWater kit).

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) uslng primers and probes targeting the *tviB* and *staG* genes (Nair et al., 2019).

*tviB*_F 5'TGTGGTAAAGGAACTCGGTAAA-3';
*tviB*_R 5'-GACTTCCGATACCGGGATAATG-3';
*tviB*_P HEX-TGGATGCCGAAGAGGTAAGACGAGA-BHQ1;

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*sta*G_F 5’- CGCGAAGTCAGAGTCGACATAG-3’;
*sta*G_R 5’-AAGACCTCAACGCCGATCAC-3’;
*sta*G_P FAM-5’-CATTTGTTCTGGAGCAGGCTGACGG-3’-BHQ1

The reaction mixture contain 0.65 µl of tviB_F (20µM), 0.75 µl each of tviB_R (20µM), staG_F(20µM),and staG_R (20µm), 0.5 µl each of the probe tviB_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^{O}C for 5 min, followed by 45 cycles of 95^{O}C 30 sec, 64^{O}C 30 sec, 72^{O}C 10 sec, and final extension at 72^{O}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-CCCATACAATTTCATTCTTATTGAGAATGCGC-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^{O}C for 5 min, followed by 45 cycles of 95^{O}C 30 sec, 60^{O}C 30 sec, 72^{O}C 30 sec, and final extension at 72^{O}C for 10 min.

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