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Protocol for detection of *Salmonella* Typhi and *Salmonella* Paratyphi A in Soil

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ABSTRACT

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

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PROTOCOL CITATION

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soil, Salmonella Typhi, Salmonella Paratyphi A, enviroment, enrichment, detection

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MATERIALS TEXT

- Gloves
- 70% ethanol
- Biosafety hood
- Vortex
- 37°C incubator
- Sterile spatula
- Serological pipettes and pipette controller
- Sterile graduated cylinder
- Shaker
- Scale
- PBS (20 mL/sample)
- UP (Universal pre-enrichment) 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.

- Sterile 250 mL flask (1 per sample)
- Sterile 500 mL 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 Soil Sample

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

1.1 Clean your work surface with 70% ethanol.

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

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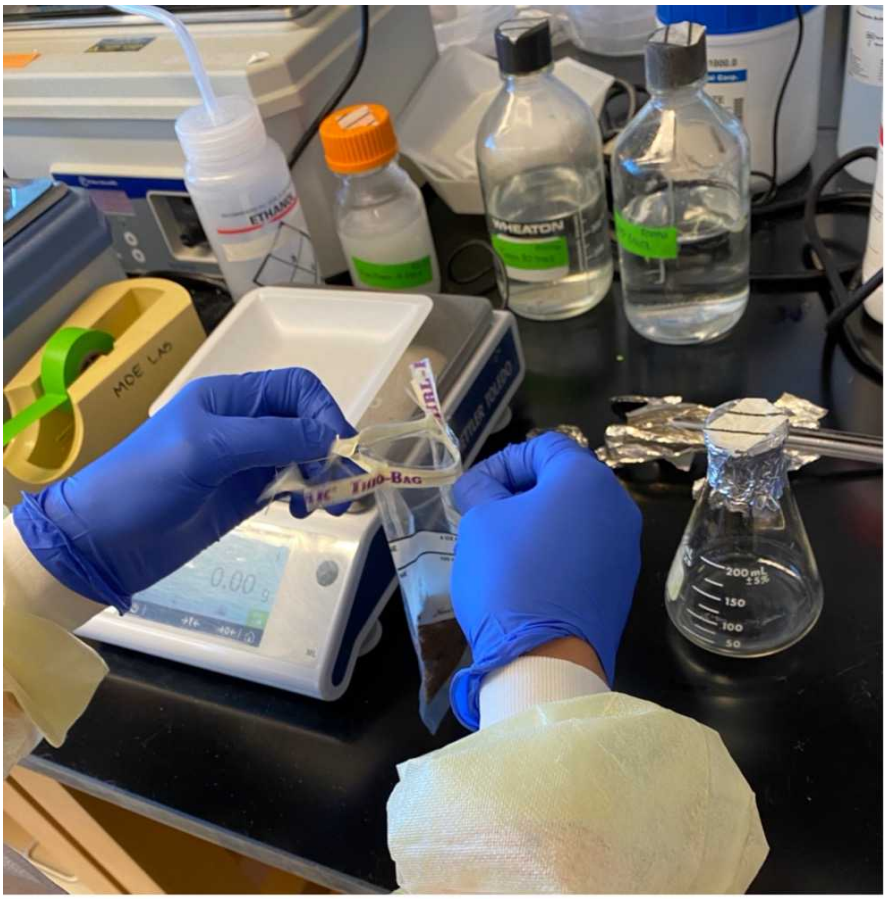
1.3 Spray the outside of the Whirl-Pak bag containing the sample with 70% ethanol and rub it well.

1.4 Rotate the Whirl-Pak bag with the sample five times to mix the sample.

1.5 Open the Whirl-Pak bag by untwisting the ties and pulling gently outwards until the mouth of the bag opens.



1.6 Using a weighing scale and a sterile spatula, measure 10 grams of sample.

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1.7 Transfer the sample to a sterile 250 mL flask.

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1.8 Using a graduated cylinder, add **100 mL** of PBS to the measured sample.

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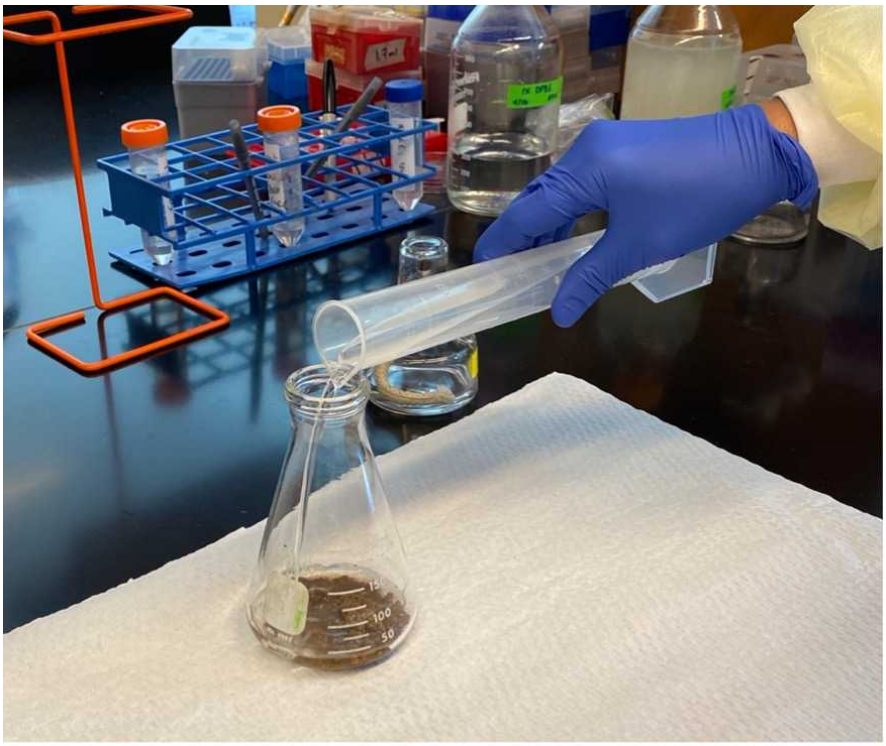

1.9 Shake **vigorously** on a rotator or shaker for **00:30:00** at room temperature. 30m

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If a rotator or shaker is not available, shake manually every 5 minutes for 1 minute, for a total of 30 minutes.

1.10 Let the sample settle for **00:30:00** . 30m

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2. Enrichment culture

2 The following steps are for optional enrichment of the sample. If enrichment is not performed, supernatant from settled sample (after step 1.10) can be used directly for membrane filtration and DNA extraction (Section 3).

2.1 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.

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2.2 With a sterile pipette, carefully remove **20 mL** of supernatant from the top of the 250 mL flask, without sucking up particulate matter and debris.

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2.3 Transfer the supernatant to the flask containing the UP Broth.

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2.4 Incubate the flask at **37 °C** in a shaking incubator 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 1.10 or step 2.4.

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 samples processed without enrichment, use 100 ml of soil suspension from step 1.10 for membrane filtration.

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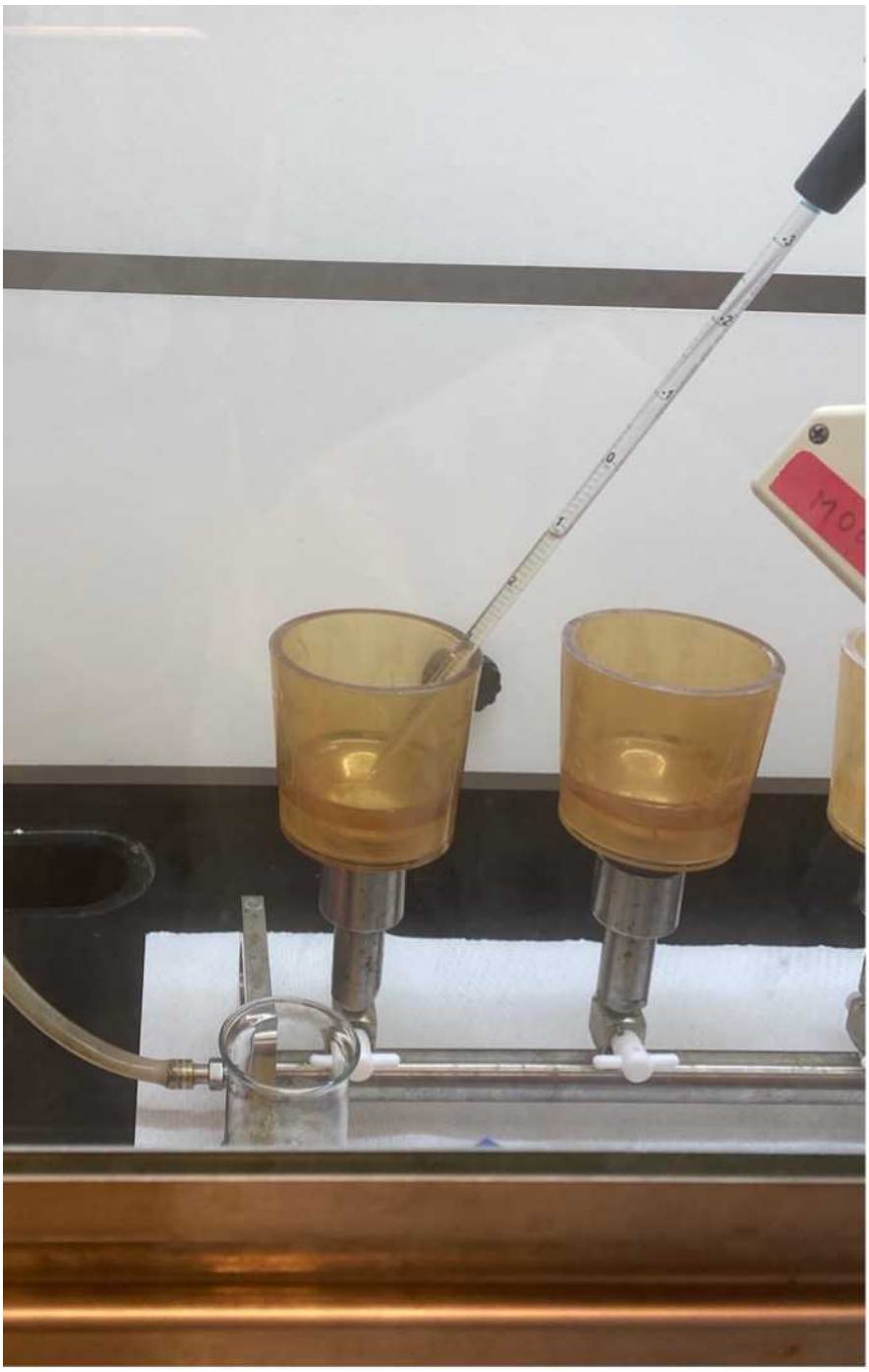

3.5 Allow the sample to filter until liquid is no longer visible on the filter.

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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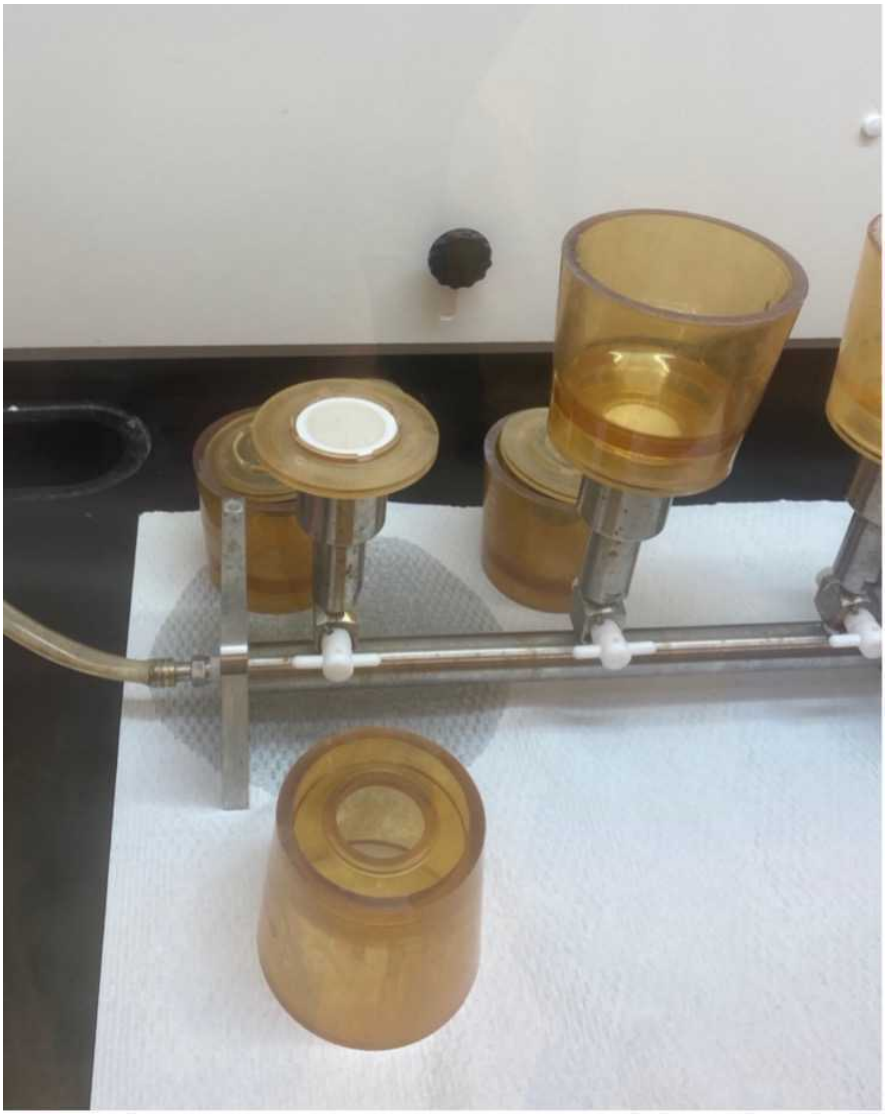

3.7 Using a sterile forcep, remove the filter from the base.

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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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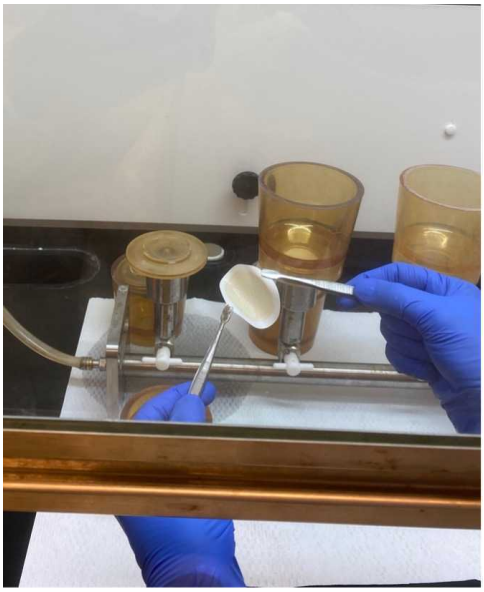
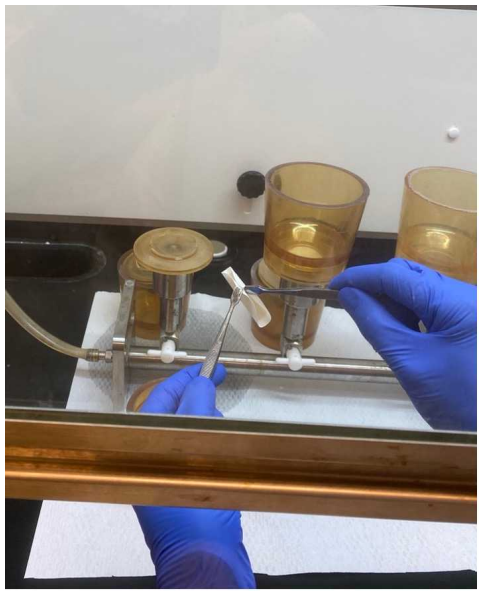
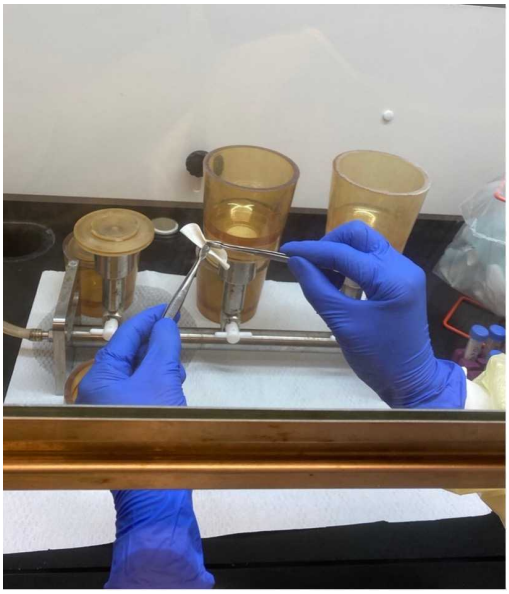




3.9 Fold the filter in half again.



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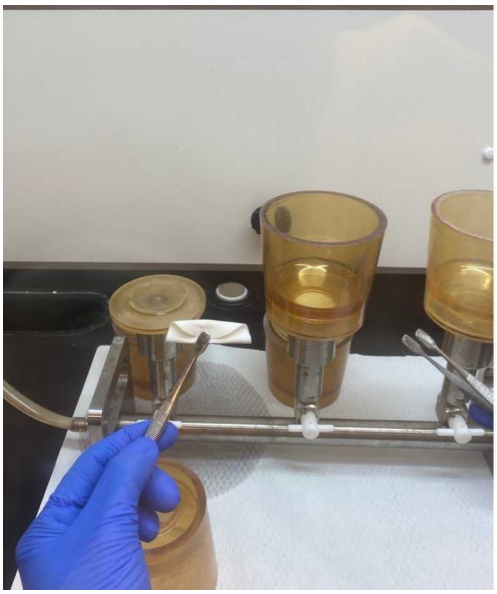

3.10 Transfer the folded filter to a bead tube (from Qiagen DNeasy PowerWater kit).

Bead tube (Qiagen DNeasy PowerWater kit)

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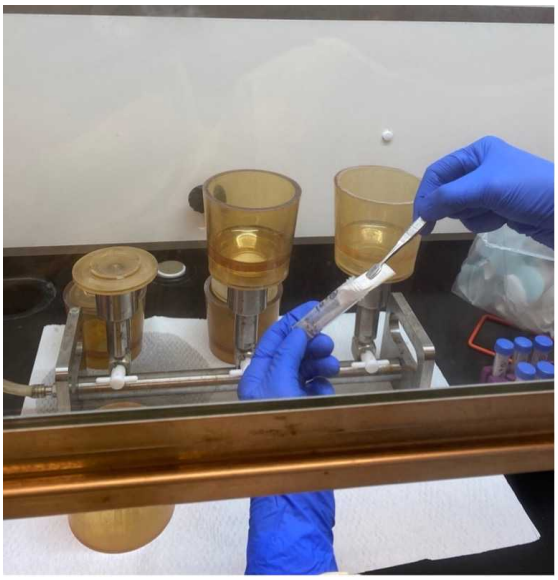

3.11 Add **1 mL** of Buffer PW1 (Qiagen DNeasy PowerWater kit) to the bead tube and vortex for **00:05:00** . 5m

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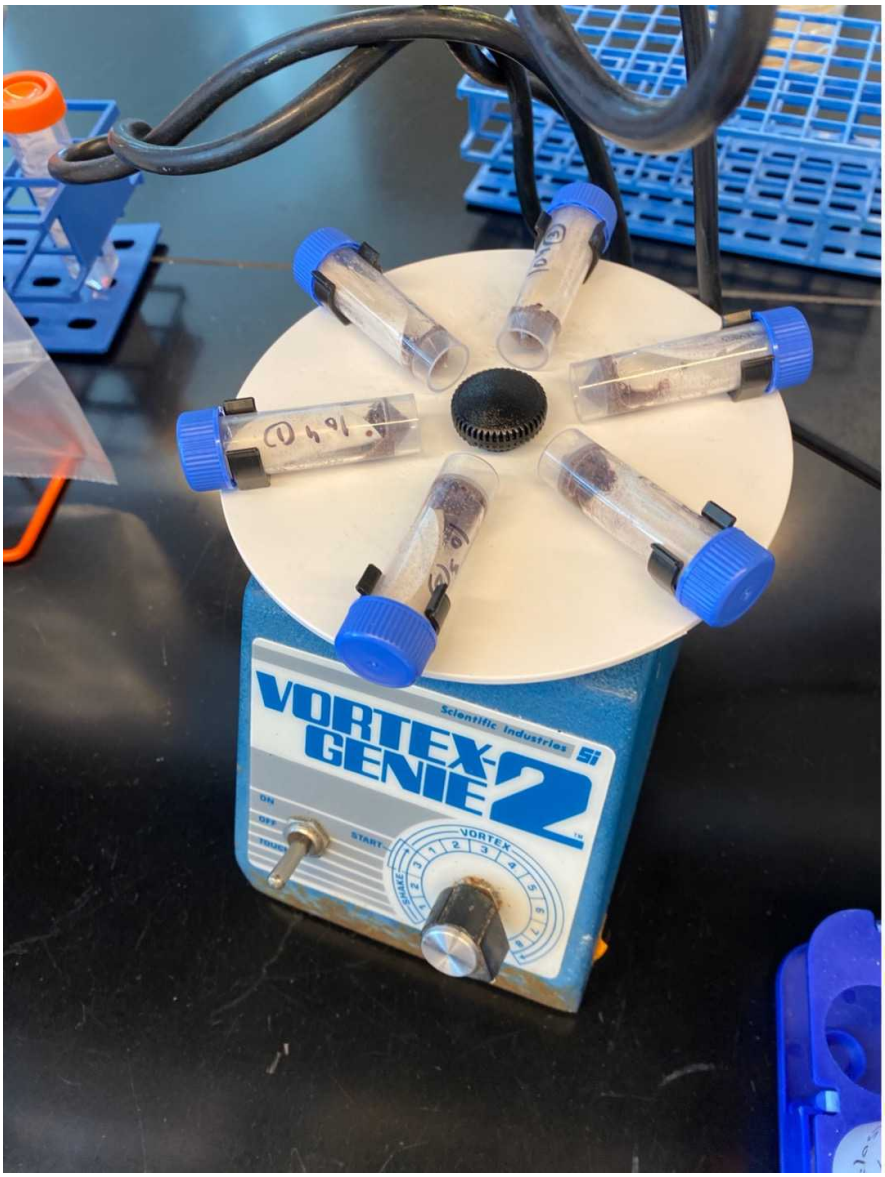

3.12 Proceed with the DNA Extraction according to manufacturer's protocol (Qiagen DNeasy PowerWater kit).

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';
*tvi*B_R 5'-GACTTCCGATACCGGGATAATG-3';

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*tvi*B_P HEX-TGGATGCCGAAGAGGTAAGACGAGA-BHQ1;
*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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