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

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ABSTRACT

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

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PROTOCOL CITATION

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MATERIALS TEXT

- Gloves
- 70% ethanol
- Biosafety hood
- 37°C incubator
- PBST (500 mL/sample)
- UP (Universal pre-enrichment) Broth (450 mL/sample)

Preparation of 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.

- 2 Sterile 500 mL graduated cylinder
- 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 produce sample 12m

1 The following steps describe processing of produce samples 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 Clean your work surface with 70% ethanol. Steps 3-6 should be done inside a biosafety hood.

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

Inspect the bag to see if liquid can be added without overflowing. If the bag is more than 2/3rds full with produce, open the bag by untwisting the ties and pulling them gently outwards until the

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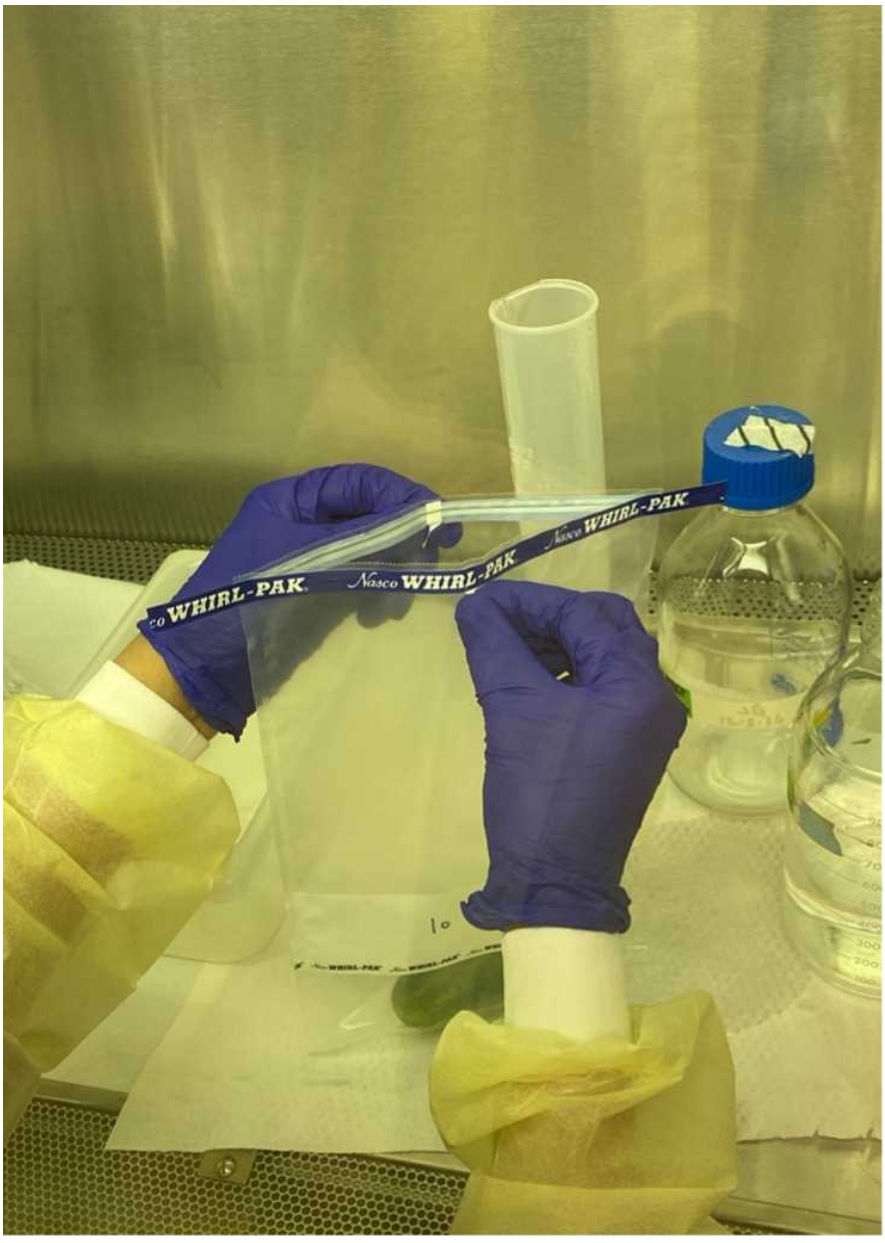
mouth of the bag opens. Remove extra produce items one at a time by pressing upward underneath them from the outside of the bag and move them to the top. Never stick your hands into the bag. Discard removed produce, and only process what remains.

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



1.5 Add **500 mL** PBST to the bag.

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1.6 Seal the bag, trapping minimal amounts of air inside.

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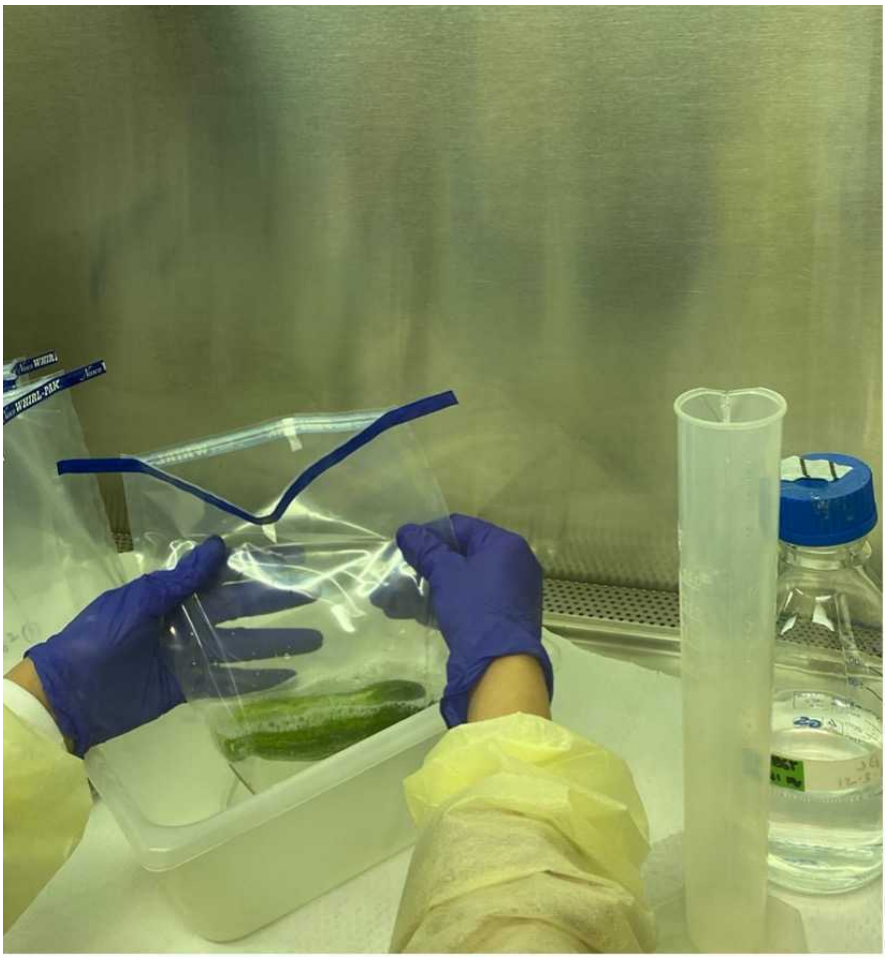

1.7 Incubate the bag for **00:10:00** at 37°C. 10m

1.8 Vigorously shake the bag with the produce for **00:00:30** . 30s

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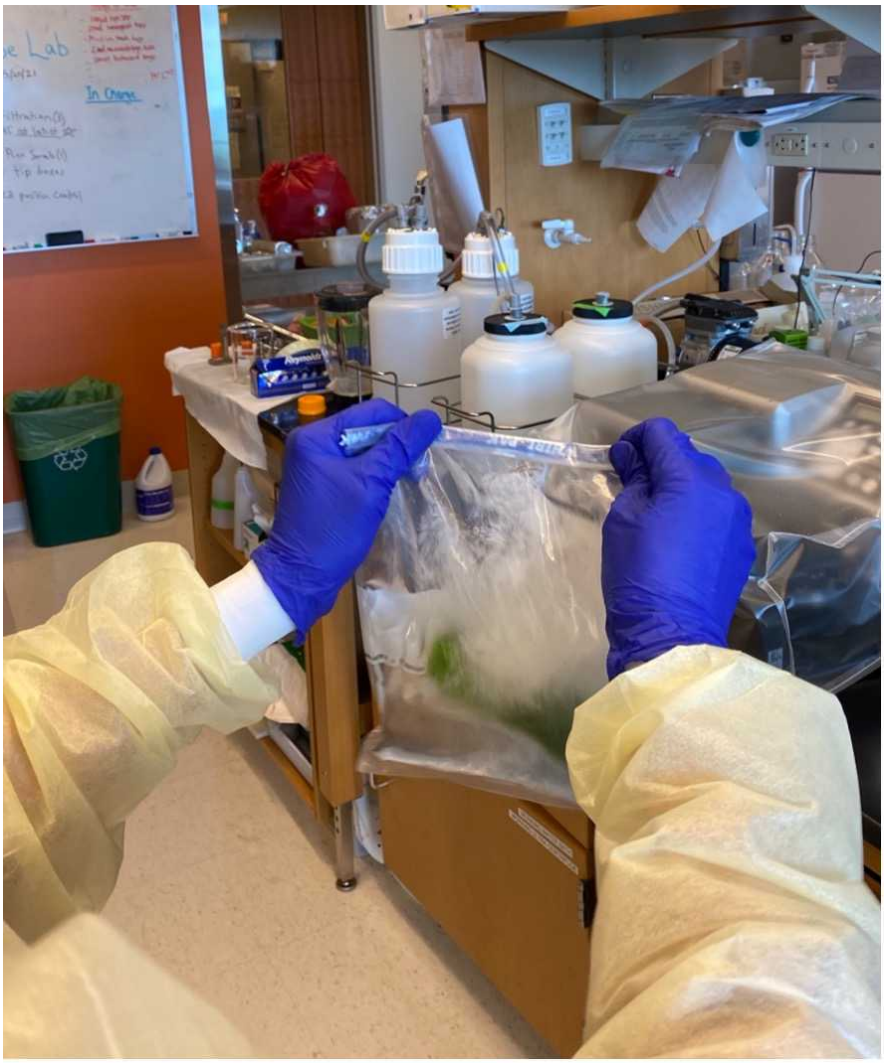

1.9 Gently massage the surface of each piece of produce through the bag for **00:01:00** . 1m

For delicate items like lettuce or onions, try to rub at least the outer leaves. Try not to break open any items.

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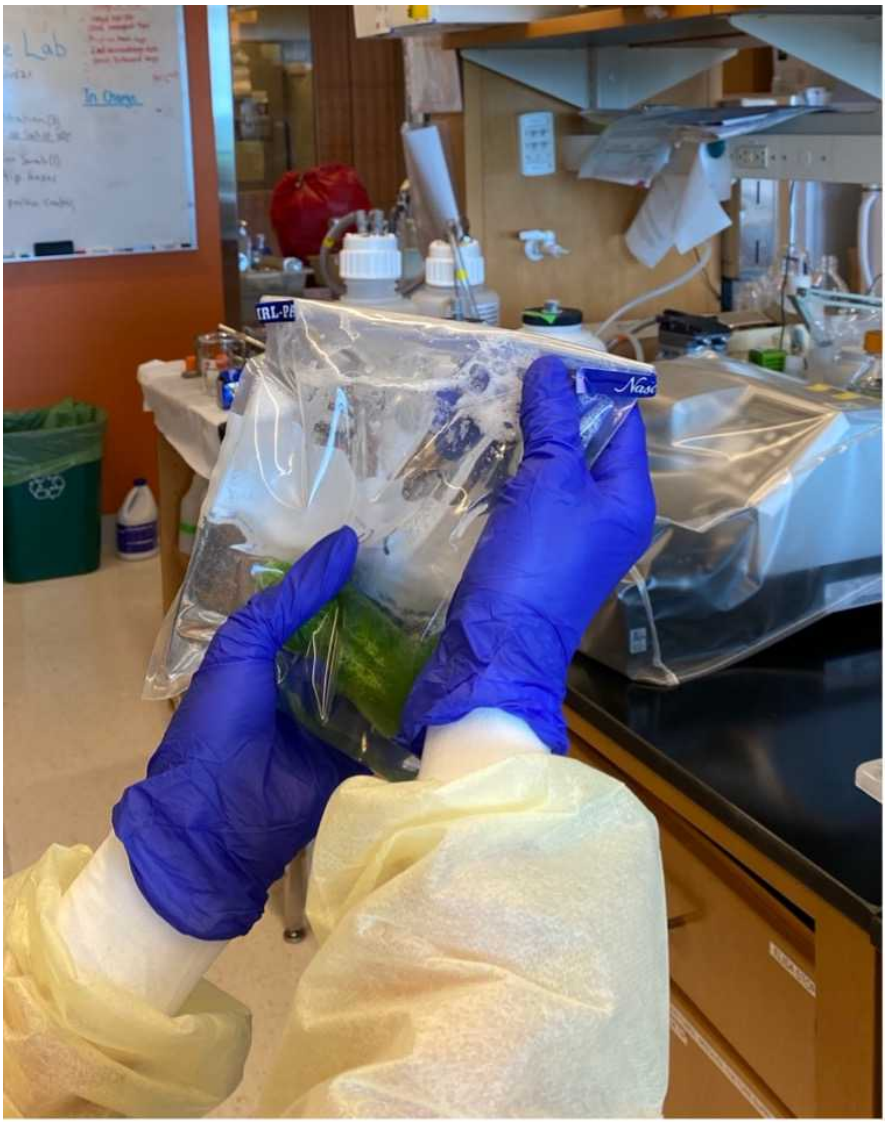

1.10 Shake the bag again for **00:00:30** . 30s

1.11 Open the Whirl-Pak bag.

1.12 Remove the produce from the Whirl-Pak bag by gently pressing upwards underneath each item from the outside of the bag and move it to the top.

Never stick your hands inside the bag. Take care not to lose any water or smash any produce.

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1.13 Set aside produce removed from bag.

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1.14 Close the Whirl-Pak bag containing produce wash.

1.15 Weigh the produce (using aluminum foil) and record the weight.

2. Enrichment culture

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

2.1 Fill a 1 liter flask with **450 mL** of Universal Pre-enrichment (UP) Broth (USEPA Standard Analytical Protocol for Salmonella Typhi in Drinking Water).

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2.2 Add **50 mL** of produce wash to the flask containing 450 mL of UP Broth.

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2.3 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 1.15 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 samples processed without enrichment, use 100 mL of produce wash from step 1.15 for membrane filtration.

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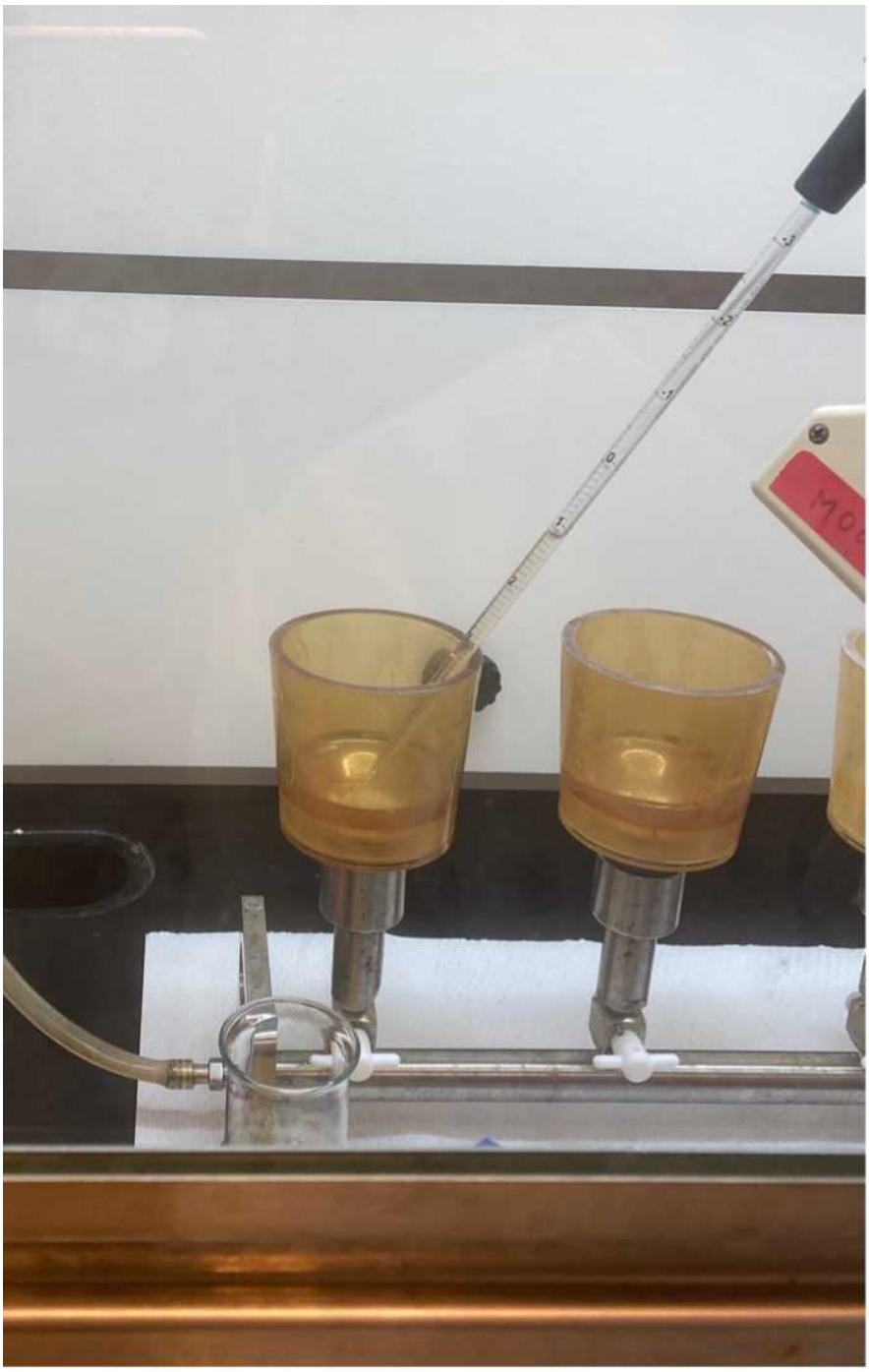

3.5 Allow the sample to filter until liquid is no longer visible on the filter.

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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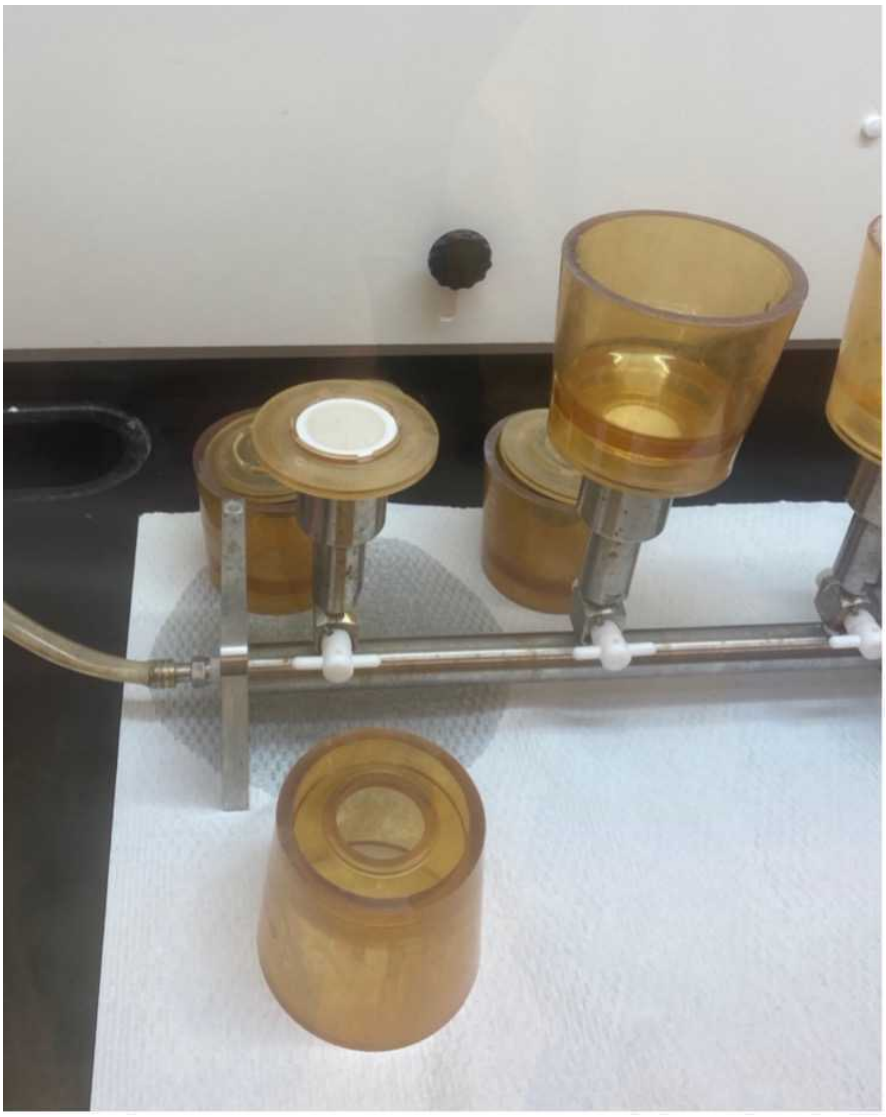

3.7 Using a sterile forcep, remove the filter from the base.

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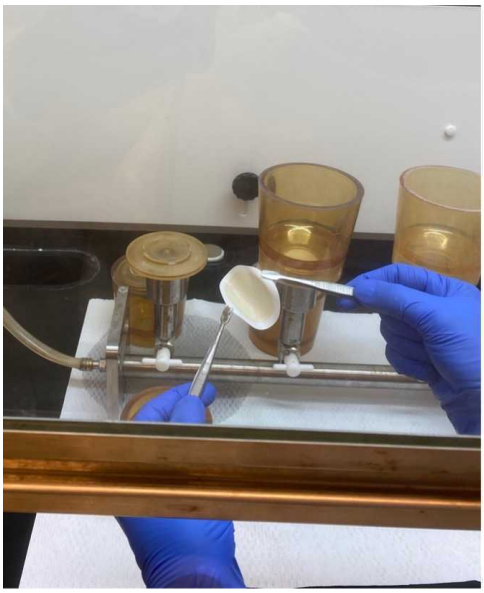
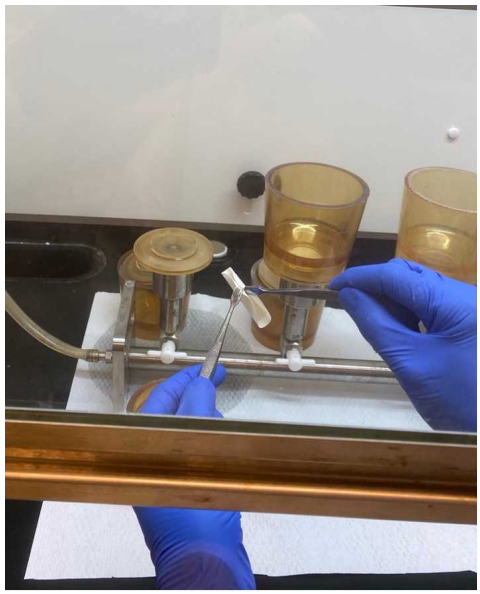
3.8 Using two sterile forceps, fold the filter in half inward, so the cells are now contained inside the folded filter.





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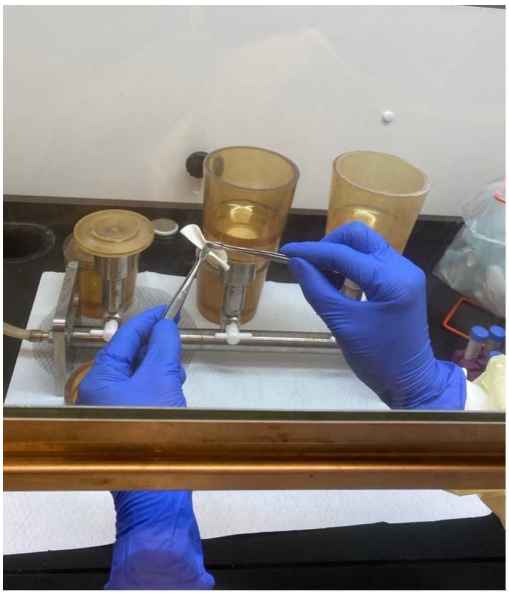
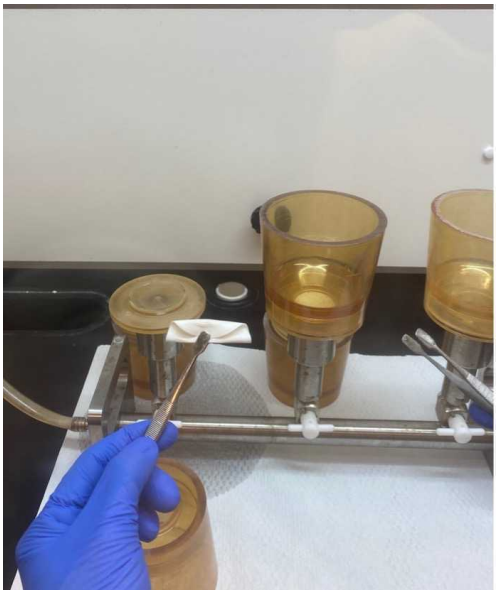
3.9 Fold the filter in half again.





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

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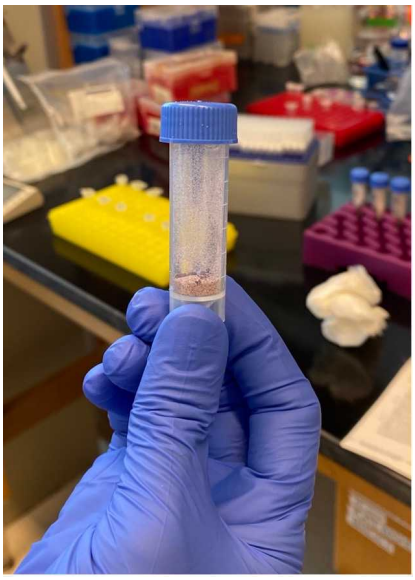
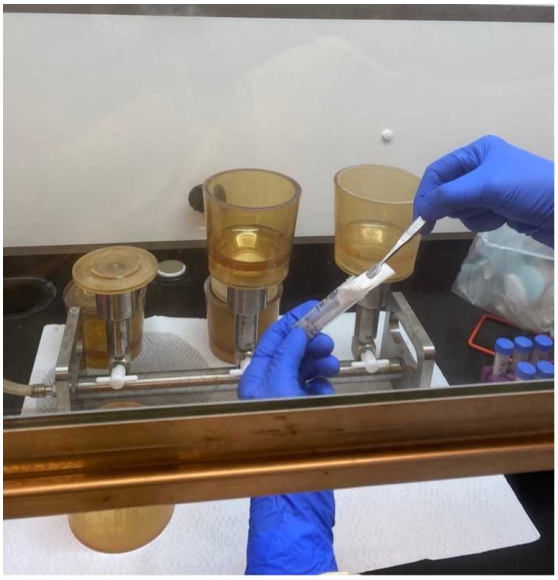


Bead tube (Qiagen DNeasy PowerWater kit)



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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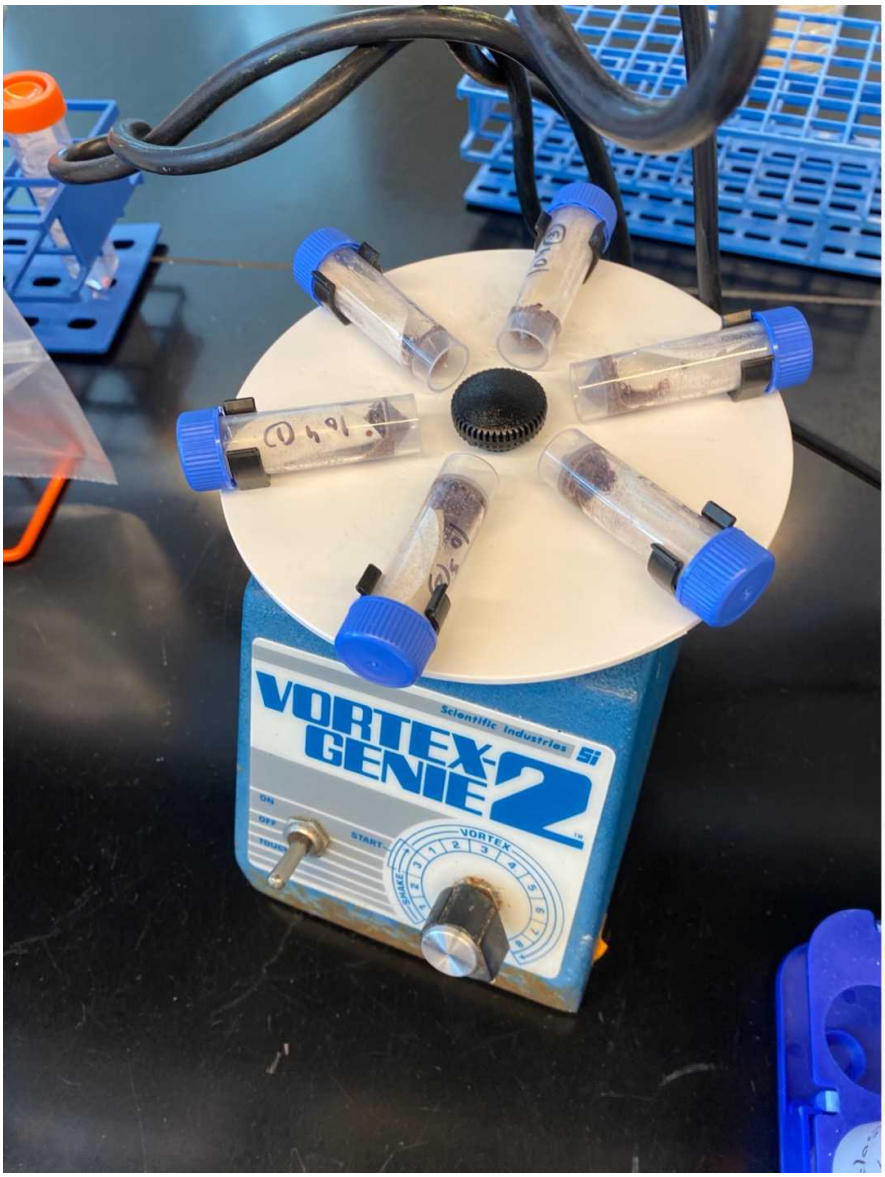

3.12 Proceed with the DNA Extraction according to manufacturer's protocol (Qiagen DNeasy PowerWater kit).

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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