Aug 23, 2021

Protocol for detection of *Salmonella* Typhi and *Salmonella* Paratyphi A in Street Food

Renuka Kapoor[1], Christine Moe[1]

[1]Center for Global Safe Water, Sanitation and Hygiene, Rollins School of Public Health, Emory University

dx.doi.org/10.17504/protocols.io.bv7zn9p6

Emory University

Renuka Kapoor

ABSTRACT

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

DOI

dx.doi.org/10.17504/protocols.io.bv7zn9p6

PROTOCOL CITATION

Renuka Kapoor, Christine Moe 2021. Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

FUNDERS ACKNOWLEDGEMENT

Bill & Melinda Gates Foundation
Grant ID: OPP1150697

KEYWORDS

street food, Salmonella Typhi, Salmonella Paratyphi A, environment, detection, enrichment

CREATED

Jun 30, 2021

LAST MODIFIED

Aug 23, 2021

PROTOCOL INTEGER ID

51161

MATERIALS TEXT

- Gloves
- 70% ethanol
- Biosafety hood
- 37°C incubator
- BagMixer 400 CC lab blender (Interscience, Cat. No. 024 230)
- BagPage + full-page filter bags (Interscience, Cat. No. 122 025)
- PBST (225 mL/sample)
- UP (Universal pre-enrichment) Broth (90 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.

- 2 Sterile 250 mL graduated cylinder
- Serological pipettes and pipette controller
- Sterile 250 mL flask (1 per sample)
- 0.45 µM membrane filter and filtration unit
- Sterile forceps
- Sterile spatula
- PowerWater DNA extraction kit (Qiagen, Cat. No.: 14900-100-NF)

1. Processing of Street food

1 The following steps describe processing of street food 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(s) with 70% ethanol.

Weigh 25 grams of street food.

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

1.3

If the street food has multiple ingredients, garnishes, or sauce, mix everything together well before weighing.

1.4 Use a sterile spatula to transfer the sample to a BagPage+ full-page filter bag (Interscience Inc.).

Make sure to add all the sample to the front side of the bag.

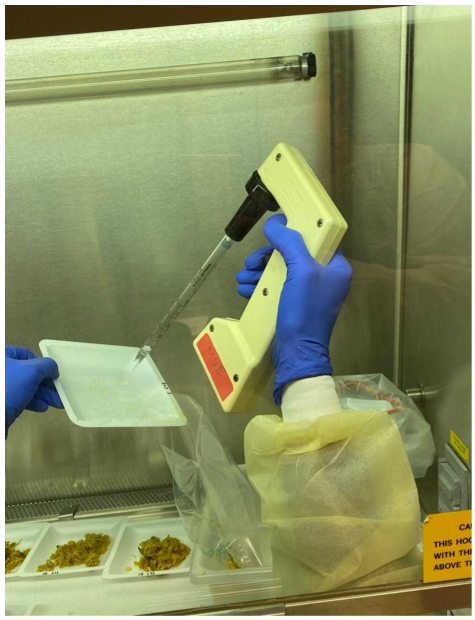
Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

- BagPage+ full-page filter bag (Interscience, Cat. No. 122 025)

If BagPage + full-page filter bags are not available, BagFilter P lateral filter bags (Interscience Cat # 111425) can be used.

1.5 Rinse spatula and weigh boat with **25 mL** of 1x PBST to add to the sample bag.

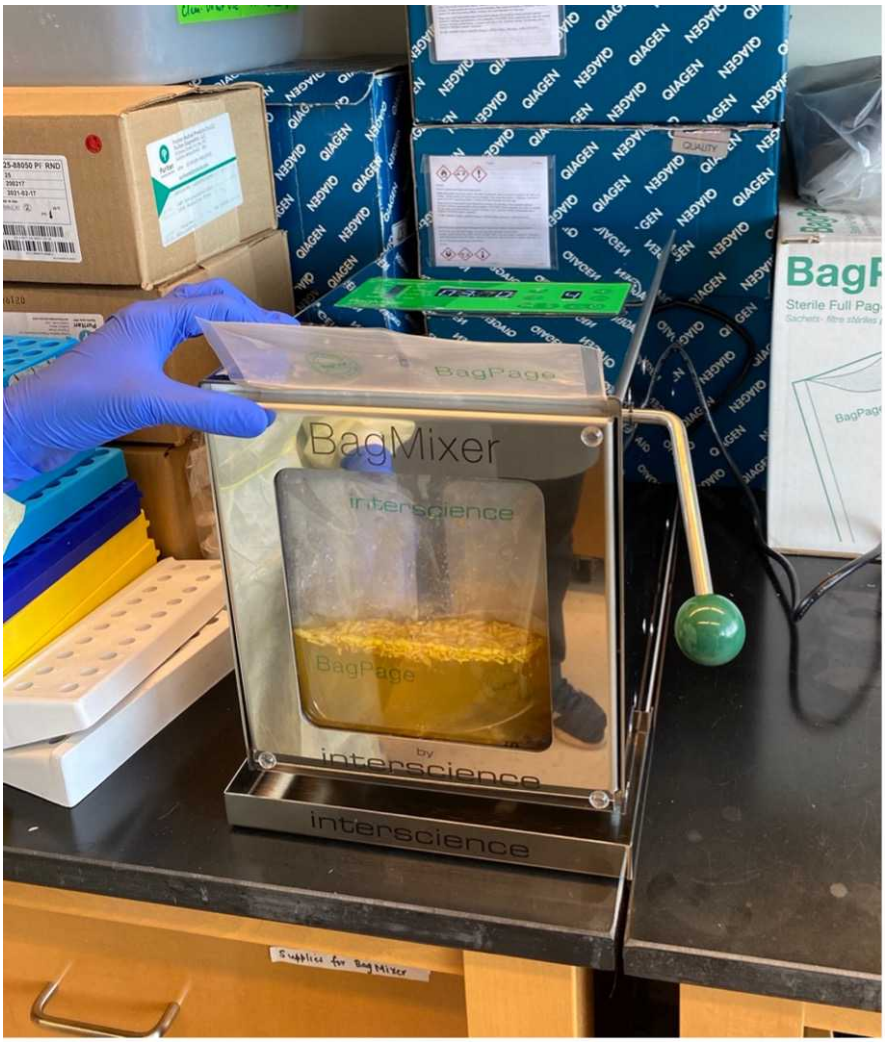
Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6



1.6 Add **200 mL** of 1x PBST to the sample in the bag.

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

1.7 Place bag into the BagMixer 400 CC and mix for **00:03:00** at settings of speed 4 with gap at -3 mm. 3m



2. Enrichment culture

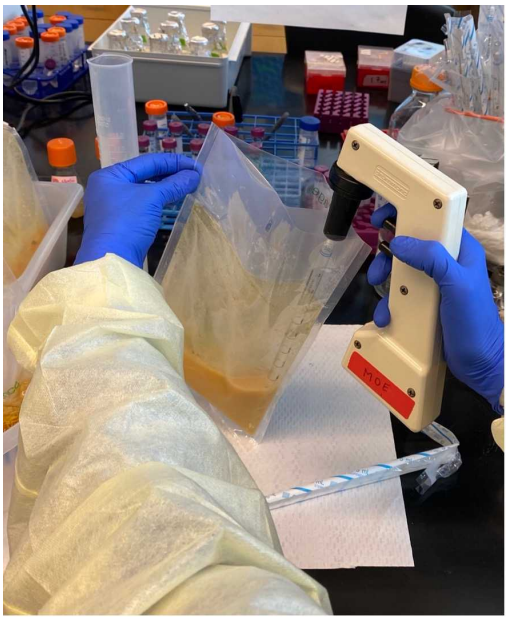
2 The following steps describe enrichment culture process for street food samples

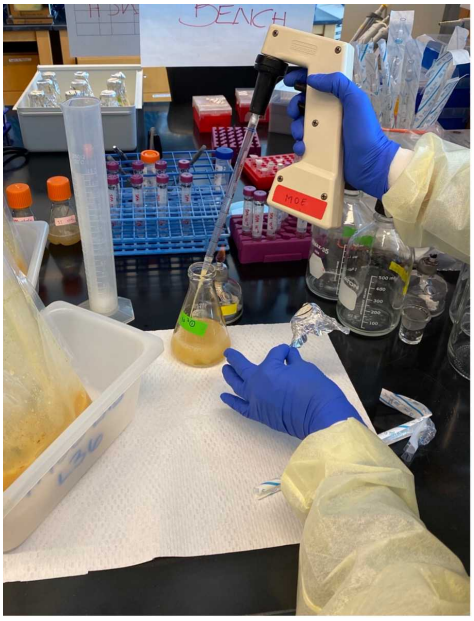
2.1 Add **90 mL** of Universal Pre-enrichment (UP) Broth (USEPA Standard Analytical Protocol for Salmonella Typhi in Drinking Water) to a 250 mL flask.

It is recommended to do this step ahead of time or while the sample is mixing in the BagMixer so the sample can be immediately transferred into the broth.

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

2.2 Using a sterile disposable pipette, immediately transfer **10 mL** of homogenized solution from the filtered side of the bag (do not allow particles to settle) to 250 ml flask containing 90 mL of UP broth.





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

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

3. DNA Extraction 5m

3 The following steps are for membrane filtration and DNA extraction.

3.1 Clean your workspace and set up your filter units.

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

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

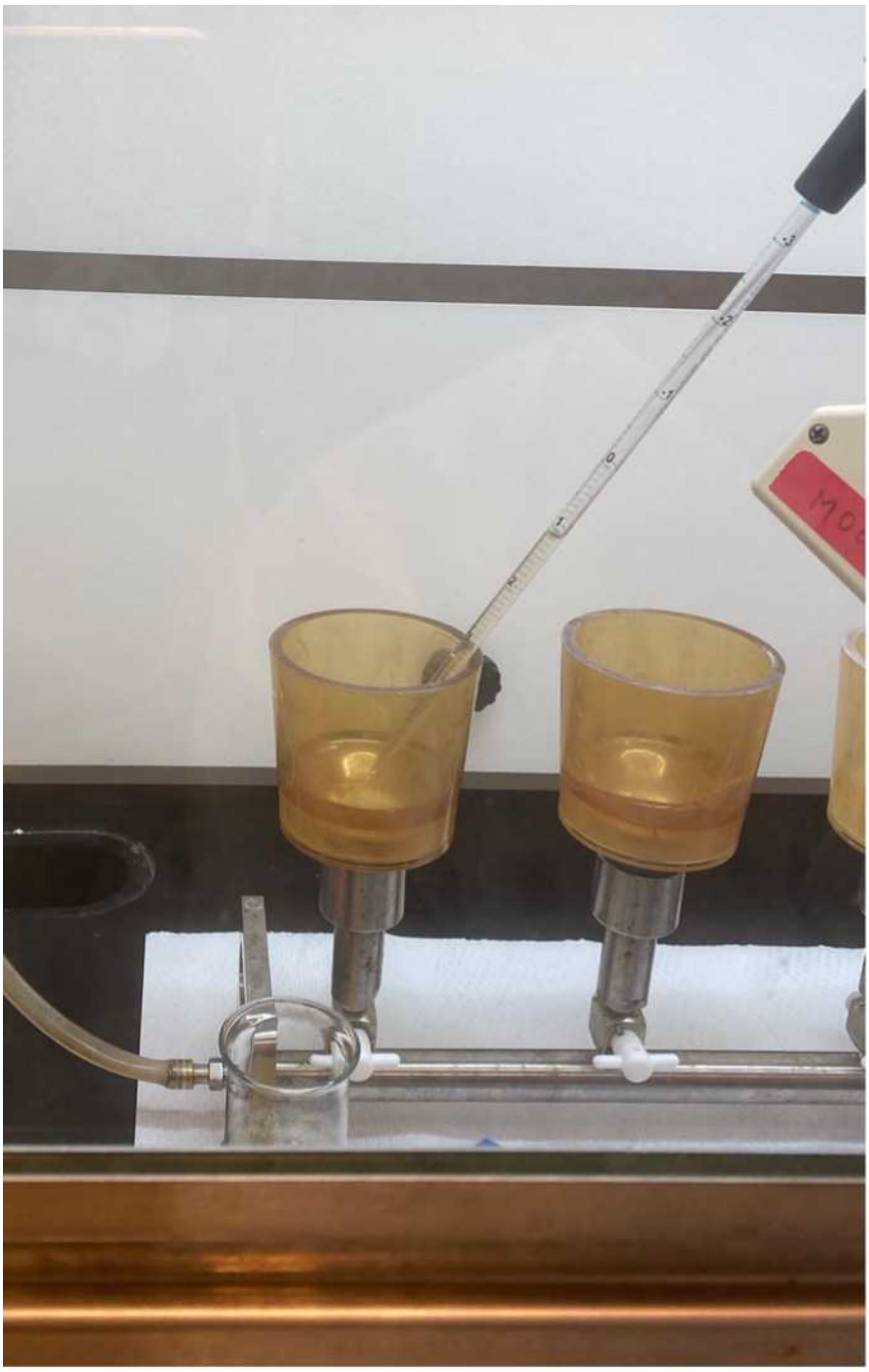
3.3 Place the cup on top of the filter.

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

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

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

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

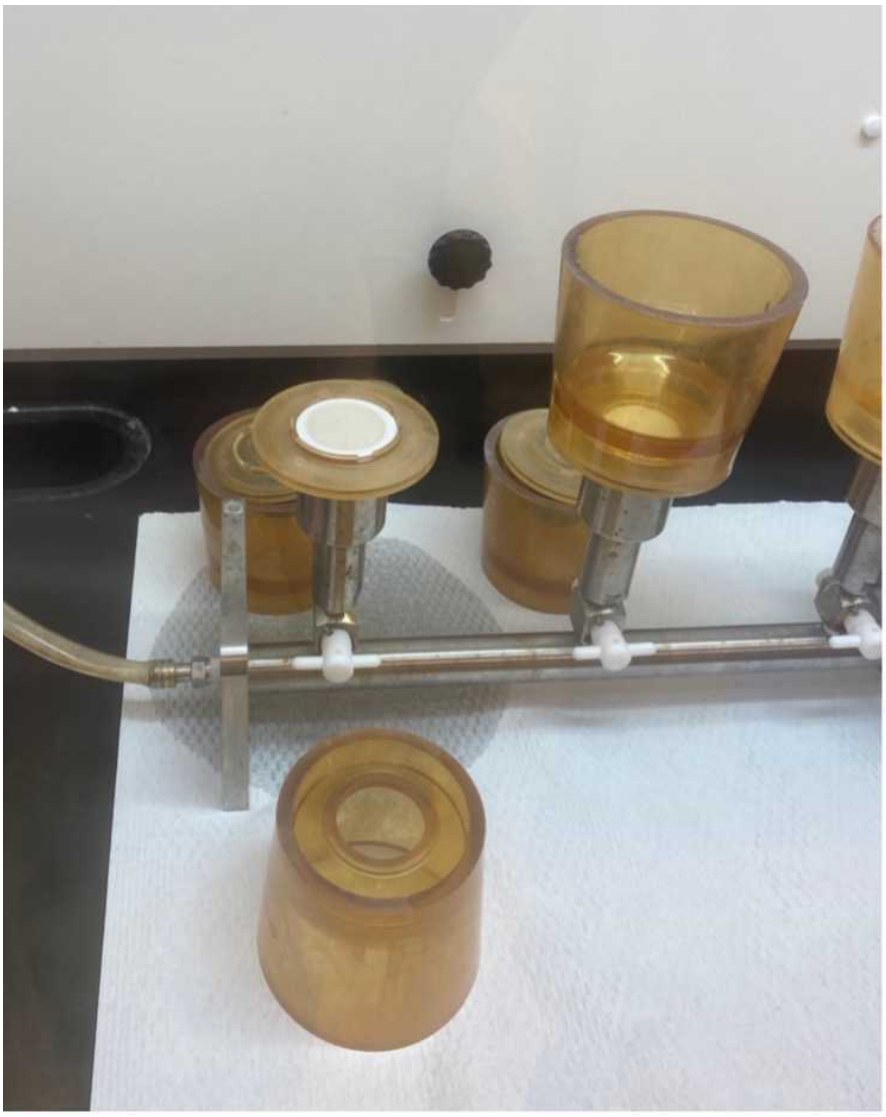


3.5 Allow the sample to filter until liquid is no longer visible on the filter.

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.

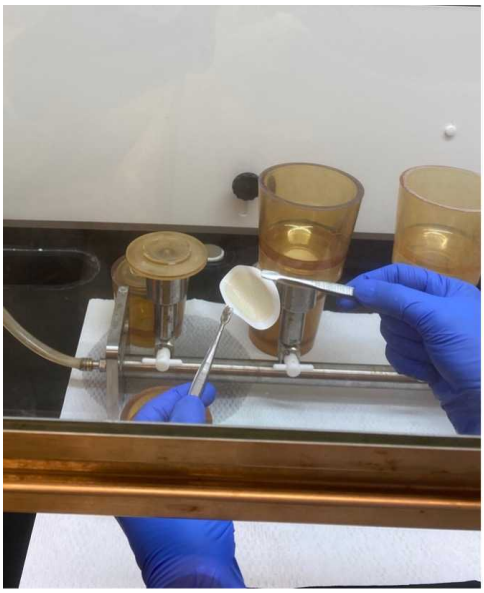
Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

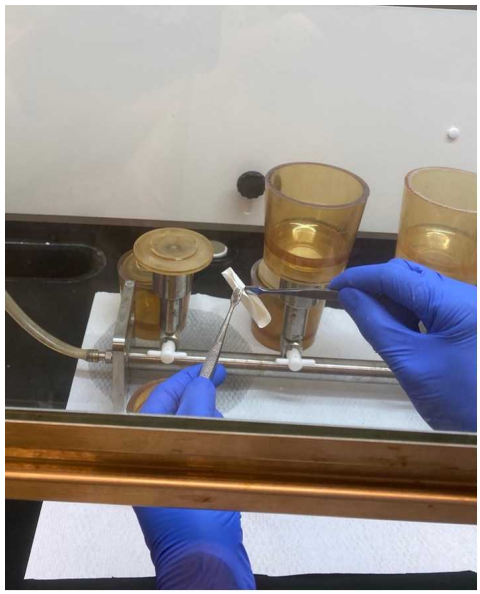


3.7 Using a sterile forcep, remove the filter from the base.

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

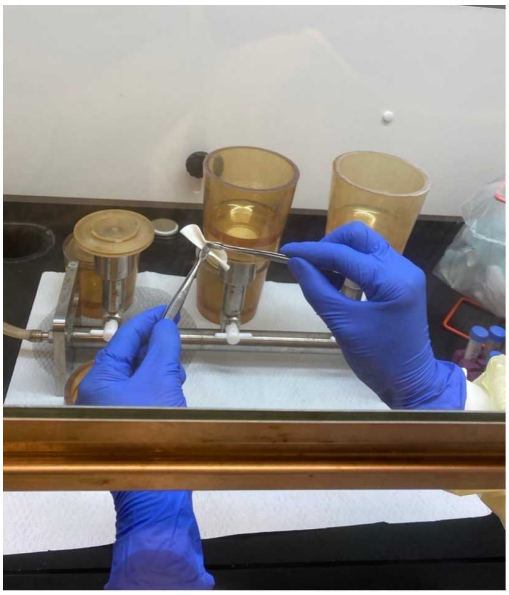
3.8 Using two sterile forceps, fold the filter in half inward, so the cells are now contained inside the folded filter.

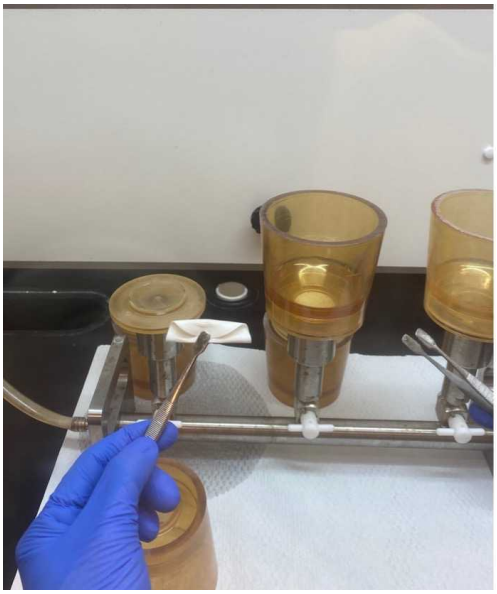




Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

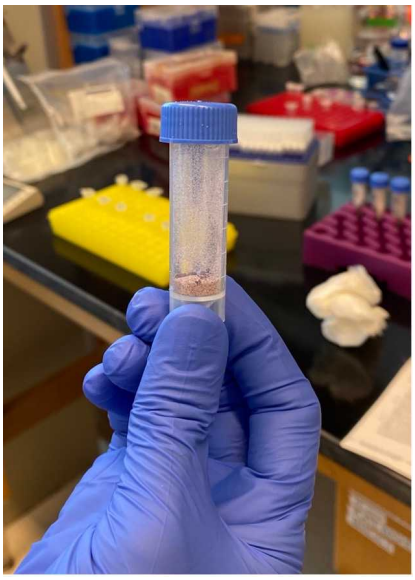
3.9 Fold the filter in half again.



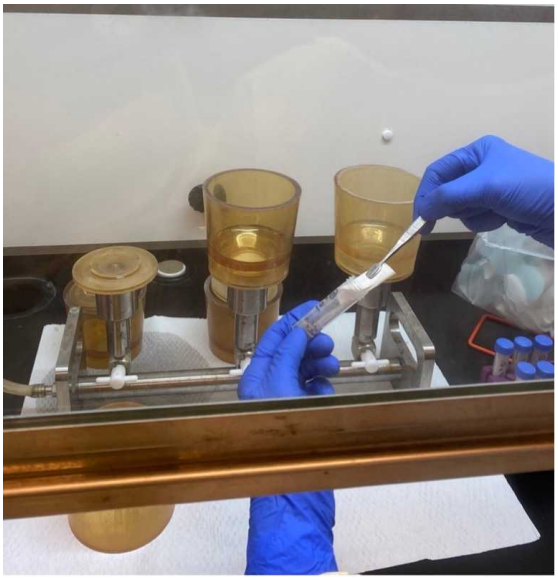


3.10 Transfer the folded filter to a bead tube (from Qiagen DNeasy PowerWater kit).

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

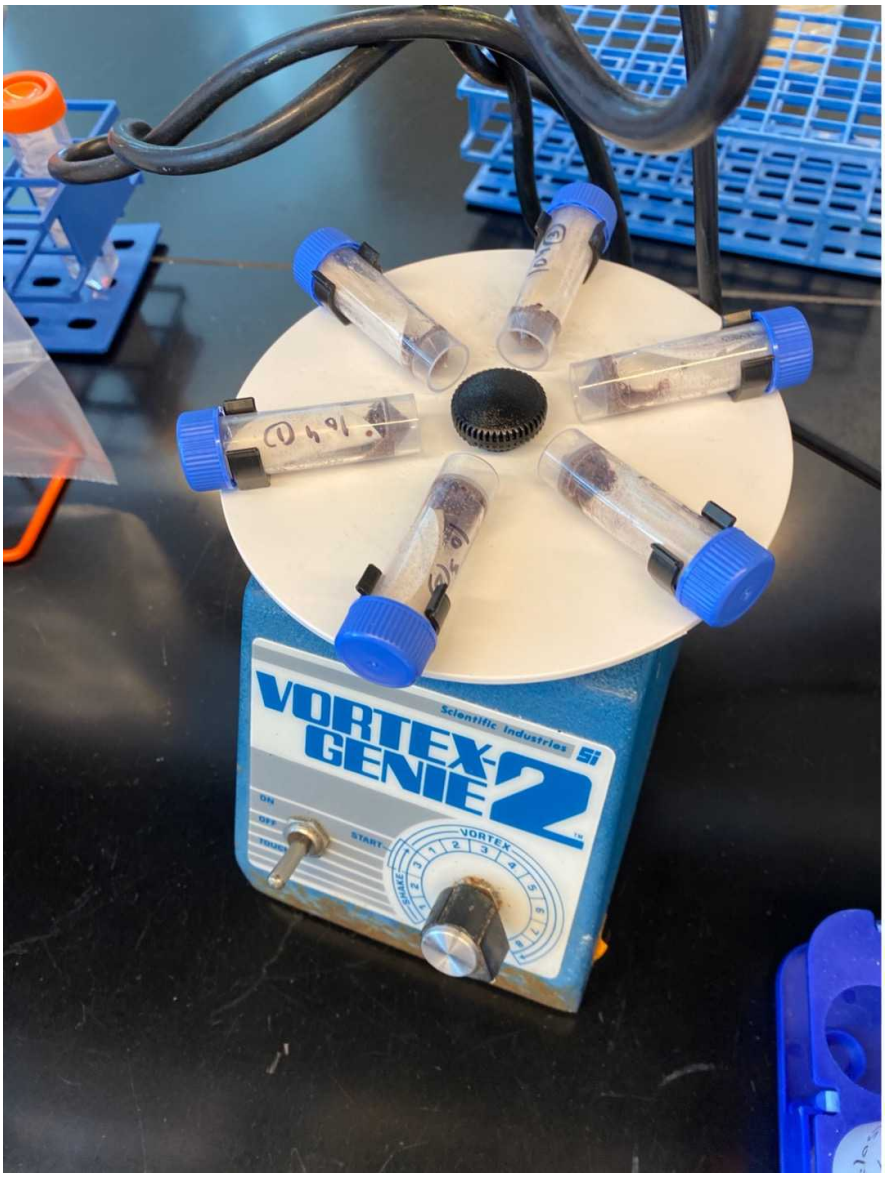


Bead tube (Qiagen DNeasy PowerWater kit)



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

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6



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';
*tvi*B_P HEX-TGGATGCCGAAGAGGTAAGACGAGA-BHQ1;

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6

*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.

Citation: Renuka Kapoor, Christine Moe (08/23/2021). Protocol for detection of Salmonella Typhi and Salmonella Paratyphi A in Street Food. https://dx.doi.org/10.17504/protocols.io.bv7zn9p6