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We use this protocol and it's working

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River water laboratory processing Protocol

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ABSTRACT

These are the qPCR conditions used for the S. Typhi and Paratyphi qPCR used for environmental surveillance by our lab team

MATERIALS

Microbiology Laboratory:
(Amount of materials required for a month of sampling)
1. 0.5% sodium hypochlorite
2. 70% ethanol
3. Sterile D/W
4. 15ml Falcon tubes (48 tubes)
5. 50ml Falcon tubes (24 tubes)
6. Sterile, clear glass tubes
7. 1ml sterile tips (2 boxes) and pipette
8. 200µl sterile tips (2 boxes) and pipette
9. Selenite F broth (10ml in each tube, 29 tubes)

10. Sterile 1.5ml eppendorf tubes (116 eppendorfs)
a. DNA Zap solution A and B
11. DNA extraction kit (DNEasy Blood and Tissue Kit Cat# 69504)

Molecular Laboratory:
1. 0.5% sodium hypochlorite
2. 70% ethanol
3. Master mix (TaqMan Environmental Mastermix)
4. Primer F (ST/SPTA)
5. Primer R (ST/SPTA)
6. Probe (ST/SPTA)
7. dH2O
8. Template DNA
9. Sterile eppendorf
10. PCR strips
11. 10µl tips (3 boxes) and pipette
12. 20µl sterile tips (1 box) and pipette
13. 200µl sterile tips (1 box) and pipette
14. 1ml sterile tips (1 box) and pipette

qPCR Conditions

List of primer and probes used:
Salmonella Typhi:
Salmonella Typhi Forward Primer-5' CGCGAAGTCAGAGTCGACATAG 3'
Salmonella Typhi Reverse Primer -5' AAGACCTCAACGCCGATCAC 3'
Salmonella Typhi Probe-5' FAM-CATTTGTTCTGGAGCAGGCTGACGG-BHQ1a-Q3'

Salmonella Paratyphi-A:
Salmonella Paratyphi-A Forward Primer - 5'ACGATGATGACTGATTTATCGAAC 3'
Salmonella Paratyphi-A Reverse Primer - 5' TGAAAAGATATCTCTCAGAGCTGG 3'
Salmonella Paratyphi-A Probe-5' FAM-
CCCATACAATTTCATTCTTATTGAGAATGCGC-BHQ1a-Q 3'

Master mix setup
Salmonella Typhi/Paratyphi-A qPCR:

	A	B
	Master mix (TaqMan Environmental Mastermix) (2x)	10 µl
	Primer F (10µM)	0.80 µl (400nM)
	Primer R(10µM)	0.80 µl (400nM)
	Probe (10µM)	0.4 µl (200nM)

	A	B
	Nuclease Free Water	4 µl (DNase/RNase-Free)
	Template	
	Total	20 ul

Cycling parameters:

Encode these temperatures onto qPCR machine

15 min at 95°C (Holding stage)

30 secs at 95°C (Cycling Stage)

30 secs at 60°C (Cycling Stage)

	Initial Denaturation	15 min at 95C	
	Dentaturation	30 secs at 95C	45 cycles
	Annealing and Extension (Fluorescence Capture Step)	30 sec at 60C	

PCR Result:

1. Export Ct value results from qPCR machine
2. Upload results into excel file.
3. Upload the file to the RedCap app.

Turbidity Testing

1 2ml of sample (from 5ml separated for Phage Screening) is taken in a sterile glass tube

2 Turbidity is measured by densitometry.

Differential Centrifugation

3 Spin down 45ml of collected water sample at 2000rpm for 1 min to separate out larger debris.

4 Transfer the supernatant to a new sterile 50ml tube.

5 Centrifuge at 4000rpm for 25mins.

6 Discard the supernatant and resuspend the pellet in 0.5ml of sterile D/w.

Enrichment and DNA extraction

7 Transfer 0.5ml of resuspended pellet into 10ml of Selenite F broth.

8 Transfer 1ml of this suspension to a sterile 1.5ml tube for DNA extraction for T0.

9 Incubate the inoculated Selenite F broth overnight (16hrs) at 37°C.

10 At the end of incubation, transfer 1ml of the o/n growth to 1.5ml tube for DNA extraction at T16.

DNA extraction (DNEasy Blood and Tissue Kit Cat# 69504)

11 Transfer 1ml of bacterial suspension in SF broth to 1.5ml tube.

12 Centrifuge at 2000rpm for 2mins.

13 Discard the Supernatant.

14 Resuspend pellet in 200 µl PBS.

15 Add 20 µl proteinase K.

16 Add 200 µl buffer AL. Mix thoroughly by vortexing.

17 Incubate for 10mins at 55°C.

18 Add 200 µl ethanol (96-100%) and mix thoroughly by vortexing.

19 Transfer the mixture into a DNeasy Mini spin column placed in a 2ml collection tube. Centrifuge at 8000rpm for 1min. Discard the flow-through and replace a new collection tube.

20 Add 500 µl Buffer AW1 to the column and centrifuge at 8000rpm for 1 min. Discard flow-through and replace a new collection tube.

21 Add 500 µl Buffer AW2 to the column and centrifuge at 14000 rpm for 3 mins. Discard flow-through.

22 Transfer the collection tube to a new 1.5ml microcentrifuge tube.

23 Elute the DNA by adding 60 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature. Centrifuge at 8000 rpm for 1 min.

Real time PCR (Adapted from Tran Vu Thieu Nga)

24 **Primer preparation**

Primers/probes are shipped in lyophilized form and need to be constituted with TE buffer or nuclease
free dH2O. Make the primary primer/probe stock solution of 100 µM.
Amount of nuclease free dH2O= 10 µl × X ng
Here, X is the amount of primer/probe provided by the manufacturer.
Generally, use the following formula to find unknown volume or concentration.
C1*V1 = C2*V2
Where,
C1 = Initial concentration.
V1 = Initial volume.
C2 = Final concentration.
V2 = Final volume.
Here, for this protocol, we used a portion of the 100 µM primers/probes to make 10 µM/µl solutions
to use as working solutions. Aliquot the working solution into 5 separate tubes and store at -20 °C to
avoid contamination and multiple freeze-thawing cycles.

25 **Master mix setup**

Master mix hi/Paratyphi-A qPCR:

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