

Evaluation of Sampling and Concentration Methods for *Salmonella enterica* Serovar Typhi Detection from Wastewater

Nicolette Zhou, Angelo Ong, Christine Fagnant-Sperati, Joanna Harrison, Alexandra Kossik, Nicola Beck, Jeffrey Shirai, Elisabeth Burnor, Rachael Swanstrom, Bethel Demeke, Suhani Patel, the Typhoid Environmental Surveillance Working Group, and John Scott Meschke*

Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington

Abstract. *Salmonella enterica* serovar (*Salmonella* Typhi) is the causative bacterial agent of typhoid fever. Environmental surveillance of wastewater and wastewater-impacted surface waters has proven effective in monitoring various pathogens and has recently been applied to *Salmonella* Typhi. This study evaluated eight sample collection and concentration methods with 12 variations currently being developed and used for *Salmonella* Typhi surveillance globally to better understand the performance of each method based on its ability to detect *Salmonella* Typhi and its feasibility. *Salmonella* Typhi strains Ty21a and Ty2 were seeded to influent wastewater at known concentrations to evaluate the following methods: grab sampling using electropositive filters, centrifugation, direct enrichment, or membrane filtration and trap sampling using Moore swabs. Concentrated samples underwent nucleic acid extraction and were detected and/or quantified via quantitative polymerase chain reaction (qPCR). Results suggest that all methods tested can be successful at concentrating *Salmonella* Typhi for subsequent detection by qPCR, although each method has its own strengths and weaknesses, including the *Salmonella* Typhi concentration it is best suited for, with a range of positive detections observed as low as 0.1–0.001 colony-forming units (CFU) Ty21a/mL and 0.01 CFU Ty2/mL. These factors should be considered when identifying a method for environmental surveillance and will greatly depend on the use case planned.

INTRODUCTION

The WHO estimates the annual global death toll from typhoid fever to be between 128,000 and 161,000 people.¹ The pathogen responsible for typhoid fever is the gram-negative bacterium *Salmonella enterica* serovar Typhi (*Salmonella* Typhi). Humans are the only known natural host and reservoir for *Salmonella* Typhi, which is spread fecally through water, food, or objects contaminated with feces of an infected individual.² Typhoid fever is a human health risk particularly in low- and middle-income countries where access to clean water and adequate sanitation can be a challenge.^{3,4} Typhoid fever is endemic in most South Asian and sub-Saharan African countries; however, the incidence varies by season, and localized outbreaks can occur.^{5,6} Although the annual estimated number of cases ranges between 11 and 21 million people,¹ this global burden estimate is based primarily on limited clinical blood culture surveillance data. However, surveillance based on diagnostic clinical microbiology is expensive, requires specialized facilities, is approximately 50–60% sensitive, and depends on symptomatic individuals presenting to healthcare facilities and on clinicians performing a diagnostic test.⁷ Often, clinical treatment is not sought.^{8,9} Additionally, asymptomatic carriers or pauci-symptomatic individuals do not tend to have diagnostic tests performed, though these cases can still shed the pathogen in feces.⁶ For these reasons, the global typhoid burden is likely underestimated.¹⁰

Given the challenges with conventional approaches to typhoid surveillance, novel strategies are necessary. One strategy that has proven effective in monitoring pathogens is environmental surveillance (ES). Environmental surveillance is the collection of soil, water, air, or other environmental

samples and analysis for pathogens. Because *Salmonella* Typhi is shed through feces, the organism is expected to be in wastewater or wastewater-impacted surface waters of locations with outbreaks or endemic transmission.^{11,12} Environmental surveillance for *Salmonella* Typhi may inform on disease burden in the population and help identify typhoid hot spots. Environmental surveillance has been shown to support the reduction of diseases and prevention of outbreaks of various enteric pathogens.^{13–16} The WHO first recommended typhoid conjugate vaccine (TCV) in March 2018,¹⁷ but introduction has been limited to locations at highest risk and greatest burden because of limited availability and funding options. Thus, ES can be used to help guide TCV deployment decisions and later to monitor the effectiveness of intervention strategies such as TCV introduction.

Multiple collection and concentration methods have recently been developed and applied globally for downstream analysis to detect *Salmonella* Typhi in water sources; this study was focused on wastewater.¹² Environmental surveillance methods for pathogen surveillance have used grab sampling or trap sampling. These techniques generally avoid collecting large sediments by sampling the wastewater or wastewater-impacted water surface or by prefiltering the water through a coarse filter. The volumes typically processed in grab sample methods vary from 50 mL to 20 L,^{18–22} and samples are collected from outlet pipes, open sewer channels, surface waters, or other sewage streams. Grab samples are either concentrated in-field via gravity filtration or are transported to a laboratory with a proper cold chain for subsequent concentration, elution, and/or enrichment.^{10,18,23–28} Sample concentration enables a larger volume of wastewater or wastewater-impacted water to be assayed, which is important when low concentrations are anticipated, as is typically the case in environmental samples. Methods for sample concentration also vary. Some samples are filtered through an electropositive ViroCap filter, 0.45- μ m membrane filters, or ultrafilters; other methods include centrifugation.^{12,18–22,26,29} Trap sample methods for *Salmonella* Typhi have historically used the Moore swab method.^{30,31}

*Address correspondence to John Meschke, Department of Environmental and Occupational Health Sciences, University of Washington, 4225 Roosevelt Way NE, Ste. 100, Seattle, WA 98105. E-mail: jmeschke@uw.edu

The Moore swab, or Moore cotton tampon, is suspended into the water source for up to 6 days^{29,32–37} to allow for bacteria flowing through water to adsorb on the swab, but not for large debris to get trapped. Swabs are then enriched using different growth/enrichment media. After processing, samples are analyzed using culture-based detection methods and/or quantitative polymerase chain reaction (qPCR). Although culture-based methods have historically been used to isolate *Salmonella* Typhi, recovery has been inconsistent and culturing is challenging; therefore, qPCR is increasingly used for detection of *Salmonella* Typhi in wastewater.^{10,29,38} Further, when enrichment is not used in sample processing (i.e., unless most probable number methodologies are used), qPCR enables quantification of the organism's concentration in the original sample and allows for detection of viable but not culturable *Salmonella* Typhi, whereas culture confirms the viability of an organism and permits comparative genomic analysis.

Of the methods used for sampling and detection of *Salmonella* Typhi from environmental wastewater and wastewater-impacted surface water samples, none would be uniformly appropriate as a stand-alone method for all study designs, sampling locations, and wastewater matrices. The aim of this study was to evaluate various sample collection and concentration methods currently being developed and used for *Salmonella* Typhi surveillance globally to better understand the performance of each method based on its ability to detect *Salmonella* Typhi and its feasibility. Eight methods with 12 variations were evaluated in total for the detection of *Salmonella* Typhi in a wastewater matrix. Additional ES methods for *Salmonella* Typhi, including dead-end ultrafiltration and hollow fiber ultrafiltration, were not included in this study because of time and resource constraints. The methods evaluated consisted of grab sampling using electropositive filters, centrifugation, direct enrichment, or membrane filtration and trap sampling using Moore swabs. Concentrated samples underwent nucleic acid extraction and were analyzed via qPCR for *Salmonella* Typhi. The detection of *Salmonella* Typhi, each method's feasibility (active time, total time, safety, key processing supplies per sample, and key laboratory equipment), and potential use cases were also evaluated.

MATERIALS AND METHODS

Organism culture and enumeration. Two commonly used strains of *Salmonella* Typhi were used as positive controls in this study, Ty21a and Ty2. *Salmonella* Typhi strain Ty21a is used in the oral, live attenuated typhoid vaccine and is negative for the Vi antigen. *Salmonella* Typhi strain Ty2 is a well-characterized, reference strain that is positive for the Vi antigen. To confirm the presence of the Vi antigen in Ty2 prior to experiments, a Vi antigen agglutination test was performed using Difco™ *Salmonella* Vi Antiserum (Becton, Dickinson and Company, Franklin Lakes, NJ). *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) was used as a negative control in the agglutination test. Ty21a (33459; American Type Culture Collection, Manassas, VA), Ty2, and *Salmonella* Typhimurium were obtained from Dr. Stephen Libby (University of Washington). For each strain, 10 µL serum and 10 µL overnight culture were combined on a glass slide and examined for agglutination after 15 minutes. Agglutination indicated the presence of the Vi antigen in Ty2.

Throughout this work, Ty21a was grown using LB-Miller broth (IBI Scientific, Dubuque, IA), and Ty2 was grown in the dark using LB-Miller broth with a supplemental aromatic amino acid mix and 50 ng/mL ferrioxamine E (Millipore, Burlington, MA). The aromatic amino acid mix was prepared in a 100× stock consisting of L-Phenylalanine (4 mg/mL) (TCI America™, Portland, OR), L-Tryptophan (4 mg/mL) (Acros Organics, Fair Lawn, NJ), 2,3-dihydroxybenzoic acid (1 mg/mL) (TCI America), and para-aminobenzoic acid (1 mg/mL) (Sigma-Aldrich, St. Louis, MO), which were dissolved in deionized water and filter sterilized. To improve our understanding of Ty21a and Ty2 growth and therefore ensure that experiments were seeded during exponential growth, growth curves were determined for Ty21a and Ty2 and measured via optical density at 600 nm and spot plating of 100 µL of relevant dilutions³⁹ on LB-Miller agar or LB-Miller agar with a supplemental aromatic amino acid mix and 50 ng/mL ferrioxamine E. Ty21a and Ty2 inocula for experiments were prepared by growing the organisms for a specified period of time at 37°C, monitoring for exponential growth phase, and storing single-use aliquots of the cultures in 30% glycerol until use (−80°C). Prior to planned experiments, 20 µL of the frozen Ty21a or Ty2 glycerol stock was inoculated in 15 mL of liquid media and incubated with shaking (200 rpm, 37°C, 12–16 h).

Study design. Primary influent wastewater grab samples were collected from a local wastewater treatment plant in Seattle, WA, that processes 90 million gallons per day (mgd) during the dry season and can process more than 300 mgd during the rainy season,⁴⁰ resulting in matrix variability. Grab samples (7–14 L per carboy) were stored at 4°C until processing (conducted within 72 hours of collection). Varying amounts of Ty21a or Ty2 were seeded to 10 mL 1× phosphate-buffered saline, vortexed (30 seconds), and seeded into varying volumes of wastewater to reach the target concentrations. The final concentration of Ty2 or Ty21a in the seeded wastewater varied depending on the experiment and ranged from approximately 0.001 to 10,000 colony-forming units (CFU) per milliliter (Supplemental Figure 1). Methods tested at the same concentration level used primarily the same initial wastewater matrix with replicates of three or six to enable comparison between the methods. The seeded concentrations were assessed in parallel for each experiment via spread plating of 100 µL of relevant dilutions on LB-Miller agar (Ty21a) or LB-Miller agar with a supplemental aromatic amino acid mix and 50 ng/mL ferrioxamine E (Ty2). The seeded wastewater was thoroughly mixed and then distributed using a peristaltic pump while continuously shaken for processing by 1) filter cartridge, 2) differential centrifugation, 3) grab enrichment, 4) membrane filtration, and 5) Moore swab methods (Figure 1). The methods were evaluated in a laboratory setting, and factors considered included feasibility of performing these methods in other settings and *Salmonella* Typhi detection by qPCR. Feasibility was based on processing time, equipment, and supplies needed. Active time was defined as the total hands-on time required to conduct an individual method, excluding time in which personnel could complete other tasks. These periods could include incubation, shaking, and filtration.

Methods evaluated. *Two-inch filter cartridge method.* Two variations of the 2-inch filter cartridge method were tested, mentioned hereafter as FC1-D and FC2-D. The main differences between the two methods were the input

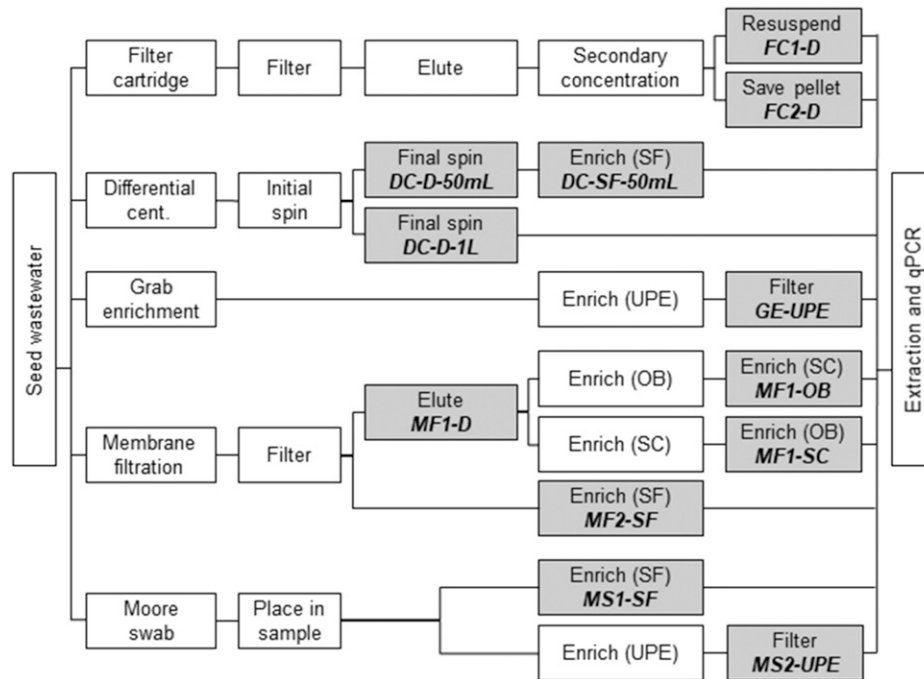


FIGURE 1. Workflow of method evaluations. Shaded boxes indicate where samples were collected for DNA extraction. cent. = centrifugation; D = direct; OB = ox bile; qPCR = quantitative polymerase chain reaction; SC = selenite cystine broth; SF = selenite F broth; UPE = universal pre-enrichment broth. Italicized, bolded text indicates method names.

for DNA extraction (half of the resuspended pellet versus the full sample pellet) and the resulting difference in effective volume assayed (Table 1, Supplemental Information).^{20,21,41}

Differential centrifugation. Three versions of differential centrifugation methods were used: DC-D-50 mL, DC-SF-50 mL, and DC-D-1 L. All methods involved centrifugation for 1 minute at 1,000 ×g, 4°C, followed by transfer of the supernatant centrifuging again (15 minutes, 4,000 ×g, 4°C). Additional details are provided in the Supplemental Information.

Grab enrichment. For grab enrichment (hereafter, GE-UPE), 20 mL of seeded wastewater was added to 180 mL of Universal Pre-Enrichment broth (UPE; Becton, Dickinson and Company) and incubated at 37°C (24 hours). After incubation, 20 mL of the enriched sample was membrane filtered through one mixed cellulose ester (MCE) filter (0.45-μm pore size, 47-mm diameter; Millipore). The membrane filter

was cut into 6–10 pieces using sterile scissors, placed in a 2-mL screw top tube, and stored at –20°C prior to DNA extraction.

Membrane filtration methods. Multiple variations of vacuum membrane filtration were tested: MF1-D, MF1-OB, MF1-SC, and MF2-SF (Figure 1). MF1 methods were prefiltered using a paper cone-shaped coffee filter placed on top of a filtration cup with an MCE filter, whereas the MF2 methods did not involve prefiltration. Additional details are provided in the Supplemental Information.

Moore swab methods. Two Moore swab methods were tested, with one enriched using Selenite F broth (SF) (hereafter, MS1-SF) and another using UPE broth (hereafter, MS2-UPE) (Supplemental Information).

DNA extraction and qPCR. DNA extraction was performed on the samples using the QIAamp PowerFecal Pro

TABLE 1
Volumes processed and effective volume entering qPCR for each method evaluated

Method type	Method name	Enrichment	Initial volume	Average volume processed (±95% CI)	Average effective volume entering qPCR* (±95% CI)	Organism tested
Filter cartridge (FC)	FC1-D	N/A	6 L	5.6 ± 0.6 L	85 ± 22 mL	Ty21a
	FC2-D	N/A	6 L	4.6 ± 0.5 L	123 ± 27 mL	Ty21a, Ty2
Differential centrifugation (DC)	DC-D-50 mL	N/A	50 mL	50 mL	1.7 ± 0.3 mL	Ty21a
	DC-SF-50 mL	SF	50 mL	50 mL	0.145 ± 0.022 mL	Ty21a
	DC-D-1 L	N/A	1 L	1 L	9.7 ± 2.1 mL	Ty2
	GE-UPE	UPE	20 mL	20 mL	0.104 ± 0.016 mL	Ty21a
Grab enrichment (GE)	MF1-D	N/A	1 L	423 ± 19 mL	2.0 ± 0.2 mL	Ty21a, Ty2
	MF1-OB	OB to SC	1 L	440 ± 28 mL	0.112 ± 0.014 mL	Ty21a
	MF1-SC	SC to OB	1 L	465 ± 28 mL	0.124 ± 0.015 mL	Ty21a
	MF2-SF	SF	1 L	418 ± 33 mL	2.3 ± 0.5 mL	Ty21a
	MS1-SF	SF	5 L	N/A†	N/A†	Ty21a, Ty2
Moore swab (MS)	MS2-UPE	UPE	5 L	N/A†	N/A†	Ty21a, Ty2

OB = ox bile; qPCR = quantitative polymerase chain reaction; SC = selenite cystine broth; SF = selenite F broth; UPE = universal pre-enrichment.

* Calculated values assume no benefit from enrichment and no losses throughout the concentration process.

† Moore swab volume not quantifiable as the processed volume is unknown.

DNA Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, with the following modifications. The input was pelleted 1-mL aliquots of the samples (FC1-D, DC-D-50 mL, DC-SF-50 mL, DC-D-1 L, MF1-D, MF1-OB, MF1-SC, MF2-SF, and MS1-SF), pelleted secondary concentrates (FC2-D), or sliced membrane filters (GE-UPE and MS2-UPE). The DNA was eluted in 60 μ L and aliquoted into two 30- μ L volumes or eluted in 120 μ L and aliquoted into three 40- μ L volumes and stored at -20°C prior to qPCR.

Samples were analyzed for Ty21a and Ty2 via a qPCR assay targeting the *staG* gene commonly used for *Salmonella* Typhi detection in human clinical samples.³⁸ The reaction was carried out with 0.4 μM of the forward (5'-CGCGAAGTCAGAGTCGACATAG-3') and reverse (5'-AAGACCTCACGCCGATCAC-3') primers, 0.15 μM of the probe (5'-FAM-CATTTGTTCTGGAGCAGGCTGACGG-BHQ), and 1 \times iTaq Universal Probes Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). A DNA extract input volume of 5 μL was used with a 25- μL reaction. Each DNA extract was analyzed undiluted and at a 10-fold dilution to monitor for PCR inhibition effects. Unseeded wastewater controls, no template controls, and a positive control dilution series (standard curve) of Ty21a or Ty2 were analyzed with each qPCR run. Unseeded wastewater controls were positive in 17 of 56 wells throughout the study, with a minimum cycle threshold (Ct) value of 34.1 and maximum Ct value (considered positive) of 39.0. No template controls were negative throughout the course of the study. Standard curve efficiencies averaged 104%, and R^2 values were > 0.96 . The input for the standard curves was prepared by centrifuging (10 minutes, 10,000 $\times g$) a 1-mL volume of the Ty21a or Ty2 overnight culture harvested during exponential growth (quantified via spread plating of 100 μL of relevant dilutions on LB-Miller agar [Ty21a] or LB-Miller agar with a supplemental aromatic amino acid mix and 50 ng/mL ferrioxamine E [Ty2]), removing the supernatant, and performing DNA extraction as described above. All samples and controls were tested in duplicate or triplicate.

Samples positive for *Salmonella* Typhi were defined as those that amplified with a Ct of 40 or lower in one or two of the two technical replicates with an appropriately shaped curve. Samples with a Ct > 40 were assumed to be negative because of the potential for spurious artifacts to interfere with detection. The limit of detection (LOD) was determined to be a Ct of 37 (corresponding to approximately 100 CFU/mL of the original culture) for both Ty21a and Ty2 and was defined as 95% samples positive for this assay on this qPCR instrument used (Bio-Rad CFX96 Instrument; Bio-Rad Laboratories, Inc.).⁴² The limit of quantification was determined to be a Ct of 33 (corresponding to approximately 1,000 CFU/mL of the original culture) based on a coefficient of variation below 35%.⁴³ As samples above the LOD but below a Ct of 40 have a higher likelihood of false negatives, samples were determined to be positive if they amplified at a Ct of 40 or lower.

Data analysis. Methods were primarily evaluated for their rate of *Salmonella* Typhi positivity. This was determined irrespective of the application of enrichment steps and the sample volume processed, as larger sample volumes do not inherently yield greater positivity. However, the effective volume assayed for the various methods was determined as shown below. Methods were not able to be evaluated for

recovery efficiency because of the use of enrichment steps in several methods and the use of Moore swabs (collecting an unknown amount of volume).

The concentration factor (Eq 1) and effective volume assayed were calculated (Eq 2) using Microsoft Excel[®] 2016.

$$CF = \frac{V_P}{V_{Cf}} \times \frac{V_{D0}}{V_{Df}}, \quad (\text{Eq 1})$$

where CF is the concentration factor, V_P is the sample volume processed, and V_{Cf} is the final concentrate volume before DNA extraction, V_{D0} is the volume entering DNA extraction, and V_{Df} is the final concentrate volume after DNA extraction.

$$V_{\text{eff}} = CF \times V_{\text{PCR0}}, \quad (\text{Eq 2})$$

where V_{eff} is the effective volume assayed and V_{PCR0} is the volume entering the PCR reaction.

RESULTS

Method feasibility. The feasibility of these methods depends on a variety of factors such as timing, volumes concentrated, supplies and equipment required, and safety (Tables 1 and 2). The active time for each method is < 3 hours. The DC-D-50 mL, DC-SF-50 mL, GE-UPE, and MS1-SF methods each require approximately 60 minutes or less of active personnel time. The total time to yield concentrates for DNA extraction can be obtained for most methods within 48 hours, including active and inactive time. The exception to this timing was for Moore swabs, which require 3–6 days for the concentrate owing to the swab holding time in wastewater and the incubation time. The sample volume concentrated affects the processing time, with larger volumes requiring more processing time (i.e., filter cartridge, DC-D-1 L, and membrane filtration). Procurement of supplies is simple, as all required items are accessible commercially in the United States, though this may vary in other locations. For example, selenite-based enrichment broths can be difficult to obtain in other countries because of chemical safety concerns. These selenite-based enrichment broths are used in the DC-SF-50 mL, MF1-OB, MF1-SC, MF2-SF, and MS1-SF methods. Supply and equipment costs vary between methods and depend heavily on already existing supplies and equipment in other laboratory settings.

Ty21a detection. Eight methods with 11 variations total were tested for their ability to concentrate wastewater seeded with varying concentrations of Ty21a for detection via qPCR (Table 1).

When seeded at high concentrations (10,000 and 100 CFU/mL), all methods consistently detected Ty21a (100%; Table 3). Four methods maintained a high frequency of positive detection ($> 75\%$) at all concentrations tested: FC1-D, FC2-D, MF1-D, and MS1-SF, whereas other methods yielded fewer positive detections at lower seeded Ty21a concentrations. For DC-D-50 mL, a high frequency of positive detection (100%) was maintained to a seeded concentration of 0.1 CFU/mL compared with DC-SF-50 mL samples, which were minimally detected at or below a seeded concentration of 1 CFU/mL. Positive detection in GE-UPE samples also decreased at a seeded concentration of 1 CFU/mL. MF1-OB samples maintained a high frequency of positive detection to 0.01 CFU/mL compared with MF1-SC samples, which

TABLE 2
Details of collection and concentration methods tested affecting feasibility

Method	Active time (minutes)*	Total time (days)	Safety†	Key processing supplies per sample, (quantity)‡	Key reagents	Key laboratory equipment§
FC1-D	125	1–2	+	(1) Sampling and elution kit	Skimmed milk	Centrifuge (50 mL); shaker table
FC2-D	110	1–2	+	(6) 50 mL conical (1) 10-mL serological pipet	Beef extract Glycine PBS	
DC-D–50 mL	30	1	+	(2) 50 mL conical (1) 25-mL serological pipet (1) 10-mL serological pipet	PBS PBS	Centrifuge (50 mL)
DC-SF–50 mL	45	2	–	(2) 50 mL conical (1) 25-mL serological pipet (1) 10-mL serological pipet	PBS SF	Centrifuge (50 mL); incubator
DC-D–1 L	90	1	+	(42) 50 mL conical (1) 25-mL serological pipet (1) 10-mL serological pipet	PBS	Centrifuge (50 mL)
GE-UPE	60	2	+	(1) MCE filter (2) 25-mL serological pipet	UPE	Incubator; vacuum filter unit
MF1-D	115	1	+	(5) MCE filter (5) Coffee filter (1) Whirl-Pak® bag	Ringer's lactate	Vacuum filter unit
MF1-OB	130	2	–	(5) MCE filter	Ringer's lactate	Incubator; vacuum filter unit
MF1-SC	130	2	–	(5) Coffee filter (1) Whirl-Pak® bag (Nasco Sampling, Madison, WI)	OB SC	
MF2-SF	115	2	–	(2) 15 mL conical (5) MCE filter (1) 25-mL serological pipet (1) 10-mL serological pipet (1) 15 mL conical	SF	Incubator; vacuum filter unit
MS1-SF	30	6	–	Moore swab	SF	Incubator; vacuum filter unit; peristaltic pump
MS2-UPE	90	3	+	Moore swab, (1) MCE filter (1) 25-mL serological pipet	UPE	Incubator; vacuum filter unit; peristaltic pump

D = direct; DC = differential centrifugation; FC = filter cartridge; GE = grab enrichment; MCE = mixed cellulose ester; MF = membrane filtration; MS = Moore swab; OB = ox bile; PBS = phosphate-buffered saline; SC = selenite cystine broth; SF = selenite F broth; UPE = universal pre-enrichment.

* Time estimated does not include time for media preparation, dishes, etc.

† Worker safety/exposure and hazardous material disposal concerns due to use of selenite: +, no selenite; –, selenite.

‡ Supplies are for the versions of the methods evaluated in this study (not field applied); assumes the following supplies are available for all methods: personal protective equipment, autoclave bags, reusable items (i.e., permanent marker, collection bottles, graduated cylinders), DNA extraction items, or qPCR items.

§ Equipment is for the laboratory (not field) versions of these methods; assumes the following equipment is available for all methods: microcentrifuge (1-mL capacity), vortex, autoclave, biosafety cabinet, –20°C freezer, pipettor, balance, hot stir plate, and glassware.

maintained a high frequency of positive detection to 0.1 CFU/mL. MF2-SF and MS2-UPE samples yielded similar results with a high frequency of positive detection down to 0.01 CFU/mL. Of the seven methods that maintained a high rate of positivity (> 75%) at a seeded concentration of 0.01 CFU/mL or lower, four of the methods included an enrichment step.

In general, as the concentration of Ty21a seeded in the samples decreased, the Ct value increased linearly until the lowest Ty21a concentrations were tested, which had similar Ct values to the next lowest concentration (Supplemental Figure 2). Exceptions to this included MF1-OB, which had very low Ct values at a concentration of 0.01 CFU/mL, and MS1-SF and MS-UPE, which had fairly consistent Ct values

TABLE 3
Positive detection of Ty21a in seeded samples measured via qPCR with a Ct ≤ 40

Method*	CFU Ty21a/mL seeded					
	10,000	100	1	0.1	0.01	0.001
FC1-D	3/3 (100%)	3/3 (100%)	–	–	6/8 (75%)	3/4 (75%)
FC2-D	3/3 (100%)	3/3 (100%)	–	–	7/8 (88%)	4/4 (100%)
DC-D–50 mL	3/3 (100%)	3/3 (100%)	6/6 (100%)	6/6 (100%)	3/6 (50%)	–
DC-SF–50 mL	3/3 (100%)	3/3 (100%)	0/6 (0%)	4/6 (67%)	1/6 (17%)	–
GE-UPE	3/3 (100%)	3/3 (100%)	2/6 (33%)	3/6 (50%)	1/6 (17%)	–
MF1-D	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	6/6 (100%)	5/6 (83%)
MF1-OB	3/3 (100%)	3/3 (100%)	3/3 (100%)	2/3 (67%)	6/6 (100%)	1/6 (17%)
MF1-SC	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	0/6 (0%)	–
MF2-SF	3/3 (100%)	3/3 (100%)	–	–	6/6 (100%)	2/6 (33%)
MS1-SF	3/3 (100%)	3/3 (100%)	3/3 (100%)	–	5/6 (83%)	3/3 (100%)
MS2-UPE	3/3 (100%)	3/3 (100%)	–	–	3/3 (100%)	1/3 (33%)

– = no data available; CFU = colony-forming units; D = direct; DC = differential centrifugation; FC = filter cartridge; GE = grab enrichment; MF = membrane filtration; MS = Moore swab; OB = ox bile; qPCR = quantitative polymerase chain reaction; SC = selenite cystine broth; SF = selenite F broth; UPE = universal pre-enrichment.

* Naming convention shown in Table 1.

at all concentrations tested, likely because of the enrichment step used. With a seeded concentration of 10,000 and 100 CFU/mL, all samples yielded Ct values < 40, with less than a 1-log variation in Ct values between replicates for a majority of sample types (82%, 9/11 and 73%, 8/11 for 10,000 and 100 CFU/mL, respectively).

Ty2 detection. Five methods were further tested with Ty2: FC2-D, DC-D-1 L, MF1-D, MS1-SF, and MS2-UPE. When seeding 0.1 CFU Ty2/mL, four methods yielded high detection rates: FC2-D, DC-D-1 L, MS1-SF, and MS2-UPE (> 89%; Table 4). When the seeding concentration was decreased to 0.01 CFU/mL, DC-D-1 L, MF1-D, MS1-SF, and MS2-UPE had high detection rates (> 67%; Table 4). In general, seeding 0.1 CFU/mL resulted in a lower average Ct value and greater frequency of positivity compared with seeding 0.01 CFU/mL (Supplemental Figure 3).

DISCUSSION

Ty21a and Ty2 detection. This study examined the detection and percent positivity of Ty21a by 11 ES concentration methods and Ty2 by five ES concentration methods. Methods were not optimized or evaluated for recovery efficiency, but were rather chosen based on percent positivity and feasibility. As anticipated, when the concentration seeded into the wastewater decreased, the positive detection rate generally decreased. Exceptions to this were seen with decreased recovery of Ty21a at an intermediate concentration in FC1-D, FC2-D, and MS1-SF samples, which could be due to variability between experiments, as the wastewater matrix was collected at different times for each experiment, and variability in the Ty21a strain, as it was attenuated. Typically, methods that assayed larger volumes of the initial sample generally yielded a higher positive detection rate at low-seeded concentrations than did methods that assayed smaller volumes of seeded wastewater. A high percentage of recovery was measured at low Ty21a seeding concentrations for FC1-D and FC2-D methods, which assayed the largest volume of the initial sample (85 and 123 mL, respectively; Table 1), and for MS1-SF samples. The Moore swab methods are inherently non-volumetric. The hold-up volume of the Moore swabs was approximately 60 mL (amount of liquid the Moore swabs absorbed), and they were exposed to 5 L of recirculated sample in this study. Methods that assayed < 1 mL of the original sample included DC-SF-50 mL (minimal detection at 0.01 CFU Ty21a/mL), MF1-OB (minimal detection at 0.001 CFU Ty21a/mL), MF1-SC (no detection at 0.01 CFU

Ty21a/mL), and GE-UPE (minimal detection at 1 and 0.1 CFU Ty21a/mL and no detection at 0.01 CFU Ty21a/mL). This information was factored into the decision to exclude these methods from further experiments conducted with Ty2.

For all methods tested with Ty2, when the seeded concentration decreased, the positive detection rate decreased. Additionally, for methods with a calculable volume of the original sample assayed, high volumes processed yielded more positive detection, with DC-D-1 L (9.7 mL) yielding the highest rate of positive detection, followed by FC2-D (123 mL) and MF1-D (2 mL). Finally, where the same assay was used, Ty21a was detected at lower seeded concentrations than Ty2. This could be due to differences in assay sensitivity to Ty21a and Ty2, variability in experiments or wastewater matrix used, differences in the organisms captured by these methods, or differences in the stability of these organisms in the wastewater matrix throughout processing.

Method feasibility and use case scenarios. Method selection depends on field logistics, laboratory constraints, project design, and budgetary considerations; an appropriate ES method will operate within these confines while maintaining effective performance. Local field conditions and infrastructure impact appropriate surveillance sites (e.g., sewer, wastewater treatment plant pumping station, river, pit latrine) and therefore the appropriate matrix. These matrices will vary by available sample volume, total solids, and solid characteristics (e.g., sediments, debris), which in turn will inform appropriate sampling and concentration method selection. For example, matrices with high solids content may be a challenge for filter cartridge and membrane filtration methods because of filter clogging, whereas methods such as GE-UPE, differential centrifugation, and Moore swabs are able to process samples with high solids content. Filter cartridge methods rely on the adsorption of negatively charged bacteria onto positively charged filters with a pore size of 2–3 μm ; thus, these methods may be less applicable in turbid waters (e.g., high-strength wastewater or pit latrine waste) as less volume may be able to be filtered, which will affect recovery. Additionally, sampling sites with very low flows may not be applicable for methods that process large volumes, such as filter cartridge, DC-D-1 L, or membrane filtration.

Site access and field-worker safety are critical considerations for sample collection and in-field processing. If sample shipment between the collection site and processing laboratory is required, then this may impact the sample volume (requiring smaller volume samples, as with DC-D-50 mL, DC-SF-5 mL, or GE-UPE), the need to perform primary concentration at the field site (e.g., with the filter cartridge methods or the in-field version of Moore swabs), and/or the ability to maintain sample integrity over the period of transport. It is important to note that the Moore swabs methods tested in this evaluation were not representative of what happens in their intended use case, as the experimental Moore swab was held in a recirculating system with multiple exposures to the same seeded wastewater rather than placed in a drainage with exposure only to new wastewater throughout the holding period.

Available laboratory equipment and supplies, physical space, and personnel time also impact the ability to conduct methods. For example, although the filter cartridge methods use a commercialized kit, making procurement simple, they also require a large centrifuge and shaking table. Centrifuge capacity is also a challenge for DC-D-1 L, particularly if the centrifuge does not contain a cooling mechanism or if multiple samples must be

TABLE 4
Positive detection of Ty2 in seeded samples measured via qPCR with a Ct \leq 40

Method*	CFU Ty2/mL seeded	
	0.1	0.01
FC2-D	11/12 (92%)	3/6 (50%)
DC-D-1 L	8/9 (89%)	7/9 (78%)
MF1-D	6/18 (33%)	6/6 (100%)
MS1-SF	2/2 (100%)	2/3 (67%)
MS2-UPE	5/5 (100%)	7/9 (78%)

CFU = colony-forming units; D = direct; DC = differential centrifugation; FC = filter cartridge; GE = grab enrichment; MF = membrane filtration; MS = Moore swab; qPCR = quantitative polymerase chain reaction; SF = selenite F broth; UPE = universal pre-enrichment.

* Naming convention shown in Table 1.

processed in 1 day. Access to a house vacuum or strong vacuum pump could also be a challenge preventing use of GE-UPE, membrane filtration methods, and MS2-UPE. Laboratorian safety is critical when choosing an appropriate ES method. Both selenite cystine broth (SC) (used for MF1-OB and MF1-SC) and SF (used for DC-SF-50 mL, MF2-SF, and MS1-SF) broths must be prepared and used under a biosafety cabinet capable of chemical protection (e.g., Class II B2) because of their acute toxicity and teratogenicity. Selenite-based enrichment broths cannot be autoclaved and must be disposed of as hazardous chemical waste because of their aquatic toxicity. Therefore, these chemical hazards are important considerations in settings where selenite-based compounds cannot be contained or disposed of appropriately.

Finally, study design, time to results, and associated budgetary considerations necessarily impact method selection. In cases where results are needed rapidly, filter cartridge methods, DC-D-50 mL, DC-D-1 L, or MF1-D may be ideal, as results can be obtained in < 24 hours as no overnight incubation steps are required. This combination of field, laboratory, study, and performance considerations results in a complex decision tree where one outcome is not appropriate for all applications. Thus, it is necessary to have the flexibility to select an appropriate method that meets all or a majority of these needs. Here, different use case scenarios are outlined with potentially appropriate methods (Table 5).

TCV campaign location selection. Environmental surveillance data can be used by decision makers for selecting high-burden locations to implement TCV campaigns using either qualitative or quantitative methods. If spread of typhoid is anticipated to be high within a population, then effective volume assayed and sensitivity will be less critical to delineate true positive results, and results may be quantifiable. However, in lower prevalence areas, an understanding of the lower limit of detection is important to determine fit for purpose. Methods that involve an enrichment step could be used with a substantial increase in the sample number to improve understanding of the generated results. For this use case, all methods would be appropriate (Table 5).

TCV campaign monitoring. After a vaccine campaign is implemented, it is critical to continue ES as a quality control check on effective implementation by observing an anticipated

decrease in the wild-type organism. A low concentration of wild-type *Salmonella* Typhi would be anticipated because of vaccine efficacy; therefore, an ES method with high sensitivity and a high effective volume assayed would be appropriate. Additionally, a quantitative method (e.g., a discrete volume processed and no enrichment) could best inform on *Salmonella* Typhi presence before and after vaccine distribution. Although a qualitative method could be used, this would require a substantial increase in sample number (via Moore swab) or splitting of a sample and enriching at multiple input volumes to see an effect via a most probable number approach. Appropriate methods may include filter cartridges, high-volume differential centrifugation without enrichment, and membrane filtration without enrichment (Table 5).

Low concentration detection/early outbreak detection. Prior to the outset of community spread, low or no concentrations of *Salmonella* Typhi would be anticipated in wastewater or wastewater-impacted surface waters. Consequently, an ES method aimed at early outbreak detection should have a high effective volume assayed and low limit of detection to look for a relative increase in concentration and percent positivity. Additionally, if a presence/absence result was adequate to alert health centers of an impending outbreak, then a qualitative ES method (e.g., non-discrete volume processed or enrichment) would be appropriate and might increase the likelihood of detection. Moore swabs allow a large volume to pass through the swab over time, thus increasing the potential for samples to collect the target organism. Enrichment increases the copy numbers of the target organism prior to assay via qPCR or culturing, subsequently increasing the potential to capture the organism in the volume assayed as well as informing on the viability of enriched bacteria. Potential methods include filter cartridges, differential centrifugation, membrane filtration, and Moore swabs (Table 5). This use case is aspirational, as it would require routine surveillance to be successful.

Disease surveillance. Routine surveillance to monitor geographic disease spread within the context of an active outbreak could be conducted using either qualitative or quantitative methods. In poorly resourced health systems, ES might also act as a trigger to scale up bacteremia surveillance to confirm the etiology of the outbreak and establish the antimicrobial susceptibility of the strain causing the outbreak. An active outbreak would indicate higher anticipated *Salmonella* Typhi concentrations, making method sensitivity potentially less critical in these instances. Further, routine or intense targeted surveillance can be costly, and so incorporating a less expensive method that still yields results may be a sustainable solution. Appropriate methods may include differential centrifugation, grab enrichment, membrane filtration, and Moore swabs (Table 5).

Limitations. Limitations of this study include variability between and within experiments, the number of replicates for different methods and seeding levels evaluated, the assay chosen for detection, and the methods evaluated. Variability between experiments could result from the wastewater matrix used. Although the background matrix was collected from the same local wastewater treatment plant for every experiment, the experiments were conducted over a 1-year time frame owing to laboratory capacity to process samples. Additionally, wastewater could not be collected once and stored for all experiments, as wastewater characteristics change with storage time and limited storage space was

TABLE 5
Appropriate use cases for ES methods

Method name	TCV campaign location selection	TCV campaign monitoring	Low concentration detection	Disease surveillance
FC1-D	X	X	X	–
FC2-D	X	X	X	–
DC-D-50 mL	X	–	X	X
DC-SF-50 mL	X*	–	–	X
DC-D-1 L	X	X	X	X
GE-UPE	X	–	–	X
MF1-D	X	X	X	X
MF1-OB	X*	–	–	X
MF1-SC	X*	–	–	X
MF2-SF	X*	X*	X	X
MS1-SF	X*	X*	X	X
MS2-UPE	X*	X*	X	X

D = direct; DC = differential centrifugation; ES = environmental surveillance; FC = filter cartridge; GE = grab enrichment; MF = membrane filtration; MS = Moore swab; OB = ox bile; SC = selenite cystine broth; SF = selenite F broth; TCV = typhoid conjugate vaccine; UPE = universal pre-enrichment; X = an appropriate method for the indicated use case.

* An appropriate method for the indicated use case with an increase in sample size or a most probable number approach.

available. Thus, there may have been variability in the wastewater matrix composition due to precipitation events prior to or during certain sample collections, as the treatment plant is a combined sewer overflow system. Additionally, although glycerol stocks of the same initial culture of Ty21a or Ty2 were used to prepare the organisms for seeding and growth curves were determined, there was minor variation in the length of the overnight cultures, potentially introducing variability in the anticipated amount of Ty21a or Ty2 seeded. Overnight cultures were quantified via spread plating, with differences from the anticipated amount ranging from 8% to 156% for Ty21a and 11% to 498% for Ty2. There may also have been variability within an experiment, as it was difficult to fully homogenize large sample volumes. The effect of this may have been amplified at low seeding levels. Although the extraction method used was consistent across all sample types, the extraction efficiency of the different sample types may have varied, though this was not evaluated in this study. Finally, when SF and SC broths were used, the length of the overnight culture in the selenite broths varied from 12 to 18 hours and 20 to 21 hours, respectively. Longer enrichment in selenite-based media can overtax the media, leading to breakthrough of organisms other than the target, *Salmonella* Typhi.

Variability in the number of replicates per method and per experiment makes comparisons across methods and seeding levels challenging, and only a limited number of replicates were generated for the methods evaluated. Differences in the number of replicates for the different methods were due to the complexity and feasibility of processing multiple replicates using a certain method, as some methods required less wastewater or personnel time than others. For example, the large variability in Ct values seen at 0.01 CFU Ty2/mL compared with 0.1 CFU Ty2/mL with DC-D-1 L could be due to differences in the number of experiments (three were conducted at 0.01 CFU/mL and one conducted at 0.1 CFU/mL), the number of replicates, and challenges with homogenizing a low concentration of Ty2 throughout a sample. Additionally, we expected to see large variation between replicates at low concentrations that approached the assay limit of detection.

This study used the Nga et al.³⁸ primer and probe set for detection of *Salmonella* Typhi. This assay was originally designed for clinical samples and was recently applied for detection in environmental samples. However, wastewater contains a variety of organisms as well as both known and unknown DNA in the samples, which could interfere with this assay and result in cross reactivity.⁴⁴ The ability of the concentration methods to detect *Salmonella* Typhi using this assay may also vary, as some methods concentrate larger volumes, resulting in greater concentration of potential qPCR inhibitors. To minimize these effects on reported results, undiluted and 10-fold sample dilutions were assayed to screen for evidence of inhibitors. Future studies should examine improved qPCR assays for ES samples specifically as well as digital qPCR assays. Archived samples from this study could be retested with the optimized method.

Finally, this study evaluated multiple *Salmonella* Typhi ES methods, though some other ES methods were not included. For example, additional ES methods such as dead-end ultrafiltration and hollow fiber ultrafiltration were not included in this study because of time and resource constraints. Future studies should expand on the types of methods evaluated.

This study evaluated eight methods with 12 formats for their applicability to conduct ES using *Salmonella* Typhi Ty21a and Ty2. Results suggest that all methods tested can be successful at concentrating *Salmonella* Typhi for subsequent detection by qPCR, although each method has its own strengths and weaknesses, including the *Salmonella* Typhi concentrations for which they are applicable. These factors should be considered when identifying a method for *Salmonella* Typhi ES and will greatly depend on the use case planned. Future studies could benefit from examining additional ES methods not used here and conducting side-by-side evaluations with field samples.

Received June 24, 2022. Accepted for publication October 13, 2022.

Published online February 6, 2023.

Note: Supplemental information and figures appear at www.ajtmh.org.

Acknowledgments: Typhoid Environmental Surveillance Working Group: Christian Medical College Vellore (Gagandeep Kang, Sidhartha Giri, Venkata Raghava, and Dilip Abraham); Emory University and SaniPath (Christine Moe, Renuka Kapoor, Yuke Wang, and Pengbo Liu); Malawi Liverpool Wellcome Research Programme (Nicholas Feasey, Jonathan Rigby, and Yohane Dines); Liverpool School of Tropical Medicine (Nicholas Feasey, Jonathan Rigby, and Nicola Elviss); UK Health Security Agency (Nicola Elviss); Massachusetts Institute of Technology (Eric Alm, Katya Moniz, and Amy Xiao); Center for Molecular Dynamics Nepal (Dibesh Karmacharya, Rajindra Napit, and Ajit Poudel); National Institute of Health Pakistan (Salman Muhammad and Zurva Ashraf); PATH (David Boyle); Stanford University (Jason Andrews, Kristen Aiemjoy, and Christopher LeBoa); Dhulikhel-Kathmandu University Hospital (Dipesh Tamrakar, and Sneha Shrestha); Institute for Research in Science and Technology (Jivan Shakya); US Centers for Disease Control and Prevention (Jennifer L. Murphy, Jothikumar Narayanan, and Travis Wayne Brown); University of Virginia (Mami Taniuchi); International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) (Md Ohedul Islam); Imperial College (Isobel Blake).

Financial support: This study was supported by the Bill & Melinda Gates Foundation (grants INV-000508 and INV-026705) and the University of Washington, National Institute of Environmental Health Sciences (NIEHS)-sponsored Biostatistics, Epidemiologic and Bioinformatics Training in Environmental Health Training Grant (NIEHS T32ES015459).

Authors' addresses: Nicolette Zhou, Angelo Ong, Christine Fagnant-Sperati, Alexandra Kossik, Nicola Beck, Jeffrey Shirai, Rachael Swanson, Bethel Demeke, and John Meschke, University of Washington, Seattle, WA, E-mails: nicolette.zhou@gmail.com, angelo50@uw.edu, cfagnant@uw.edu, alexandrakossik@gmail.com, nkj2@uw.edu, jshirai@uw.edu, rachswan@uw.edu, bethelademeke@gmail.com, and jmeschke@uw.edu. Joanna Harrison, University of Washington, Seattle, WA, and Arizona State University, Tempe, AZ, E-mail: jcharr19@asu.edu. Elisabeth Burnor, University of Washington, Seattle, WA, and California Department of Health, Los Angeles, CA, E-mail: elisabeth.burnor@gmail.com. Suhani Patel, University of Washington, Seattle, WA, and University of Washington, New York, NY, E-mails: spatel41@gmail.com.

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

1. World Health Organization. *Typhoid*. Available at: <https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/typhoid>. Accessed December 5, 2022.
2. World Health Organization. *Typhoid Fever*. Available at: https://www.who.int/health-topics/typhoid#tab=tab_1. Accessed December 5, 2022.

3. Yajima A, Koottatep T, 2010. Assessment of *E. coli* and *Salmonella* spp. infection risks associated with different fecal sludge disposal practices in Thailand. *J Water Health* 8: 355–364.
4. Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale W, Ramani E, Kim YE, Park JK, Wierzb TF, 2014. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. *Lancet Glob Health* 2: e570–e580.
5. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ, 2002. Typhoid fever. *N Engl J Med* 347: 1770–1782.
6. Obaro SK, Iroh Tam P-Y, Mintz ED, 2017. The unrecognized burden of typhoid fever. *Expert Rev Vaccines* 16: 249–260.
7. Crump JA, Youssef FG, Luby SP, Wasfy MO, Rangel JM, Taalat M, Oun SA, Mahoney FJ, 2003. Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg Infect Dis* 9: 539–544.
8. Muti M, Gombe N, Tshimanga M, Takundwa L, Bangure D, Mungofa S, Chonzi P, 2014. Typhoid outbreak investigation in Dzivaresekwa, suburb of Harare City, Zimbabwe, 2011. *Pan Afr Med J* 18: 309.
9. Andrews JR et al., 2020. Healthcare utilization patterns for acute febrile illness in Bangladesh, Nepal, and Pakistan: results from the Surveillance for Enteric Fever in Asia Project. *Clin Infect Dis* 71: S248–S256.
10. Saha S, Tanmoy AM, Andrews JR, Sajib MSI, Yu AT, Baker S, Luby SP, Saha SK, 2019. Evaluating PCR-based detection of *Salmonella typhi* and Paratyphi A in the environment as an enteric fever surveillance tool. *Am J Trop Med Hyg* 100: 43–46.
11. Mom CP, Schaeffer CO, 1940. Typhoid bacteria in sewage and in sludge: an investigation into the hygienic significance of sewage purification in the Tropics with regard to typhoid fever. *Sewage Work J* 12: 715–737.
12. Matrajt G, Lillis L, Meschke JS, 2020. Review of methods suitable for environmental surveillance of *Salmonella typhi* and Paratyphi. *Clin Infect Dis* 71: S79–S83.
13. Hindiyeh MY, Moran-Gilad J, Manor Y, Ram D, Shulman LM, Sofer D, Mendelson E, 2014. Development and validation of a real time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay for investigation of wild poliovirus type 1-South Asian (SOAS) strain reintroduced into Israel, 2013 to 2014. *Euro Surveill* 19: 20710.
14. Manor Y et al., 2014. Intensified environmental surveillance supporting the response to wild poliovirus type 1 silent circulation in Israel, 2013. *Euro Surveill* 19: 20708.
15. Shulman LM et al.; for the GPI (Genotype–Phenotype Identification) Group, GPI Genotype-Phenotype Identification Group, 2015. Genetic analysis and characterization of wild poliovirus type 1 during sustained transmission in a population with >95% vaccine coverage, Israel 2013. *Clin Infect Dis* 60: 1057–1064.
16. Hellmer M, Paxeus N, Magnus L, Enache L, Arnholm B, Johansson A, Bergstrom T, Norder H, 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. *Appl Environ Microbiol* 80: 6771–6781.
17. World Health Organization, 2019. Typhoid vaccines: WHO position paper, March 2018 – recommendations. *Vaccine* 37: 214–216.
18. Murphy JL, Kahler AM, Nansubuga I, Nanyunja EM, Kaplan B, Jothikumar N, Routh J, Gómez GA, Mintz ED, Hill VR, 2017. Environmental survey of drinking water sources in Kampala, Uganda, during a typhoid fever outbreak. *Appl Environ Microbiol* 83: e01706–e01717.
19. Zacheus OM, Lehtola MJ, Korhonen LK, Martikainen PJ, 2001. Soft deposits, the key site for microbial growth in drinking water distribution networks. *Water Res* 35: 1757–1765.
20. Zhou NA et al., 2018. Evaluation of the bag-mediated filtration system as a novel tool for poliovirus environmental surveillance: results from a comparative field study in Pakistan. *PLoS One* 13: e0200551.
21. Fagnant-Sperati CS et al., 2020. Validation of the bag-mediated filtration system for environmental surveillance of poliovirus in Nairobi, Kenya. *J Appl Microbiol*. 130: 971–981.
22. American Public Health Association, 2012. *Standard Methods for the Examination of Water and Wastewater*, 22nd edition. Rice EW, Baird RB, Eaton AD, Clesceri LS, eds. Washington, DC: American Public Health Association, Inc.
23. Nienie AB, Sivalingam P, Laffite A, Ngelinkoto P, Otamonga J-P, Matand A, Mulaji CK, Biey EM, Mpiana PT, Poté J, 2017. Microbiological quality of water in a city with persistent and recurrent waterborne diseases under tropical sub-rural conditions: the case of Kikwit City, Democratic Republic of the Congo. *Int J Hyg Environ Health* 220: 820–828.
24. Oguntoke O, Aboderin OJ, Bankole AM, 2009. Association of water-borne diseases morbidity pattern and water quality in parts of Ibadan City, Nigeria. *Tanzan J Health Res* 11: 189–195.
25. Ako AA, Nkeng GE, Takem GEE, 2009. Water quality and occurrence of water-borne diseases in the Douala 4th District, Cameroon. *Water Sci Technol* 59: 2321–2329.
26. Isobe KO, Tarao M, Chiem NH, Minh LY, Takada H, 2004. Effect of environmental factors on the relationship between concentrations of coprostanol and fecal indicator bacteria in tropical (Mekong Delta) and temperate (Tokyo) freshwaters. *Appl Environ Microbiol* 70: 814–821.
27. Brick T, Primrose B, Chandrasekhar R, Roy S, Muliyl J, Kang G, 2004. Water contamination in urban south India: household storage practices and their implications for water safety and enteric infections. *Int J Hyg Environ Health* 207: 473–480.
28. Steinman AD, Biddanda B, Chu X, Thompson K, Rediske R, 2007. Environmental analysis of groundwater in Mecosta County, Michigan. *Environ Monit Assess* 134: 177–189.
29. Liu P, Ibaraki M, Kapoor R, Amin N, Das A, Miah R, Mukhopadhyay AK, Rahman M, Dutta S, Moe CL, 2021. Development of Moore swab and ultrafiltration concentration and detection methods for *Salmonella typhi* and *Salmonella* Paratyphi A in wastewater and application in Kolkata, India and Dhaka, Bangladesh. *Front Microbiol* 12: 684094.
30. Sikorski MJ, Levine MM, 2020. Reviving the “Moore swab”: a classic environmental surveillance tool involving filtration of flowing surface water and sewage water to recover typhoidal *Salmonella* bacteria. *Appl Environ Microbiol* 86: e00060-20.
31. Moore B, 1948. The detection of paratyphoid carriers in towns by means of sewage examination. *Mon Bull Minist Health Public Health Lab Serv* 7: 241–248.
32. Moore B, 1951. The detection of enteric carriers in towns by means of sewage examination. *J R Sanit Inst* 71: 57–60.
33. McEgan R, Rodrigues CAP, Sbodio A, Suslow TV, Goodridge LD, Danyluk MD, 2013. Detection of *Salmonella* spp. from large volumes of water by modified Moore swabs and tangential flow filtration. *Lett Appl Microbiol* 56: 88–94.
34. Shinohara N, Tanaka H, Saito T, Deguchi J, Soda K, Sugiyama T, Ishimaru Y, Sonoda S, 1983. Surveillance for typhoid fever in Matsuyama city during 1974–1981 and detection of *Salmonella typhi* in sewage and river waters. *Jpn J Med Sci Biol* 36: 191–197.
35. al-Quarawi SN, el Bushra HE, Fontaine RE, Bubshait SA, el Tantawy NA, 1995. Typhoid fever from water desalinated using reverse osmosis. *Epidemiol Infect* 114: 41–50.
36. Lendon NC, Mackenzie RD, 1951. Tracing a typhoid carrier by sewage examination. *Monthly Bull Ministry of Health 6 Pub Health Lab Service (directed by Med Res Council)* 10: 23–27.
37. Moore B, Pery EL, Chard ST, 1952. A survey by the sewage swab method of latent enteric infection in an urban area. *J Hyg (Lond)* 50: 137–156.
38. Nga TVT et al., 2010. The sensitivity of real-time PCR amplification targeting invasive *Salmonella* serovars in biological specimens. *BMC Infect Dis* 10: 125.
39. Beck NK, Callahan K, Nappier SP, Kim H, Sobsey MD, Meschke JS, 2009. Development of a spot-titer culture assay for quantifying bacteria and viral indicators. *J Rapid Methods Automation Microbiol* 17: 455–464.
40. King County. *West Point Treatment Plant*. Available at: <https://www.kingcounty.gov/sites/depts/dnrp/wtd/system/west.aspx>. Accessed December 5, 2022.

41. Fagnant CS et al., 2017. Development of an elution device for ViroCap virus filters. *Environ Monit Assess* 189: 574.
42. Nutz S, Döll K, Karlovsky P, 2011. Determination of the LOQ in real-time PCR by receiver operating characteristic curve analysis: application to qPCR assays for *Fusarium verticillioides* and *F. proliferatum*. *Anal Bioanal Chem* 401: 717–726.
43. Klymus KE et al., 2020. Reporting the limits of detection and quantification for environmental DNA assays. *Environ DNA* 2: 271–282.
44. Nair S, Patel V, Hickey T, Maguire C, Greig DR, Lee W, Godbole G, Grant K, Chattaway MA, 2019. Real-time PCR assay for differentiation of typhoidal and nontyphoidal *Salmonella*. *J Clin Microbiol* 57: e00167-19.