National Center for Emerging and Zoonotic Infectious Diseases

Environmental Surveillance for Typhoid in Kibera, an Informal Settlement in Nairobi, Kenya

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Environmental Surveillance for *Salmonella* and Antimicrobial Resistance Genes (AMR) Symposium

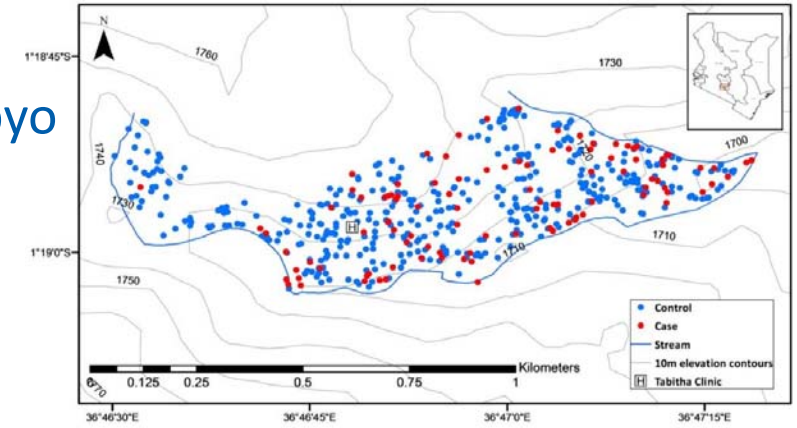
March 26, 2019

Kibera, Nairobi, Kenya

- Large urban informal settlement in Nairobi, Kenya
  - Densely populated
  - Inadequate sanitation
  - Water primarily obtained from vendors with unregulated connections to municipal water pipes

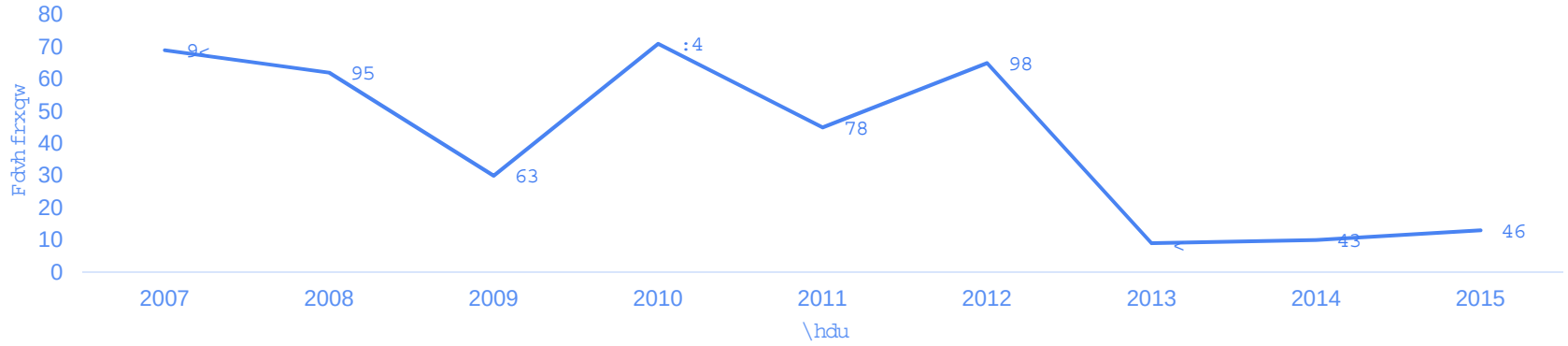
Typhoid fever in Kibera

- Population-based infectious disease surveillance (PBIDS) showed high burden of typhoid fever 03/2007- 02/2009
  - Crude incidence 247/100,000 person-years (pyo)
  - Adjusted 822/100,000 pyo
    - In aged 2-4 years 2,243/100,000 pyo
- Higher risk of disease in lower elevation
  - Among children <10 years



Decline in typhoid fever in Kibera

- In 2013, incidence reduced by ~80%
  - Has remained at low levels
- Reasons for decline unclear
  - Improved water/sanitation? Population dynamics? Shift in strains?



Kibera Typhoid Project

- Aimed at understanding decline in typhoid fever
- Strengthened surveillance
  - Optimize detection of typhoid fever cases
  - Water/sanitation data collected from participating households
- Whole genome sequencing of blood isolates from typhoid fever cases
- Environmental surveillance for *Salmonella*
  - Drinking water and sewage
- Statistical and mathematical modeling

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Why Environmental Surveillance?

- Gain insight in potential environmental exposure pathways
  - Geographic elevation
  - Comparison with case patient strains

- Develop tools for environmental monitoring
  - High genomic resolution for trace back to clinical cases
  - Identifying and assessing rapid remediation strategies

Environmental Surveillance

- 4 Major Objectives
  1. **Collect samples from sewage-impacted drainage streams and drinking water in both low and high elevation areas in Kibera and use both culture-based and molecular-based methods to detect *S.* Typhi.**

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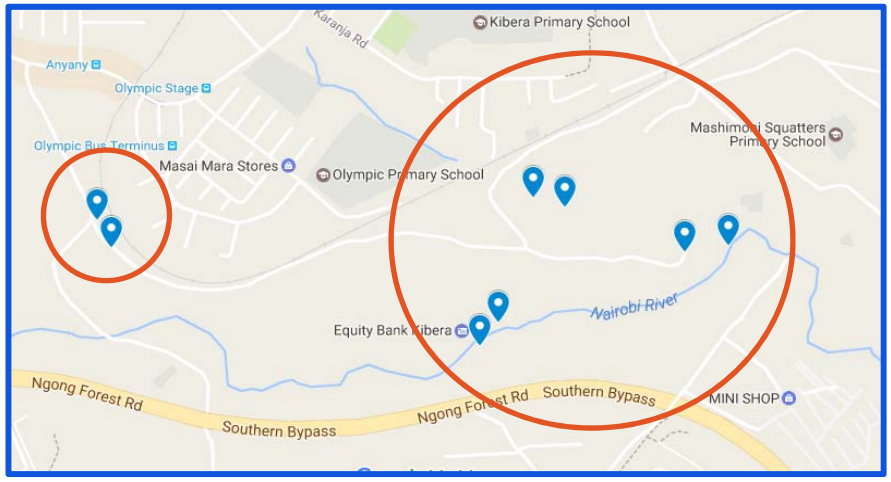
  2. Measure select microbiological and physicochemical parameters, including fecal indicator bacteria, turbidity, pH, temperature, conductivity, and chlorine.
  3. Perform molecular-based analysis of DNA obtained from environmental samples and compare DNA obtained from both patient cases and environmental samples.
  4. **Develop and refine new molecular diagnostic tools for detecting low-concentration pathogens directly from environmental samples.**

Sampling

- Collect samples from in both low (n=3) and high (n=1) elevation sites in Kibera 6 times from November 2017 – December 2018



Open drainage stream

Vended drinking water

Sampling

- Large-volume water samples, in triplicate, via dead-end ultrafiltration (DEUF)
  - Drainage stream: 10 L (or until clogging occurs)
  - Drinking water: 20 L

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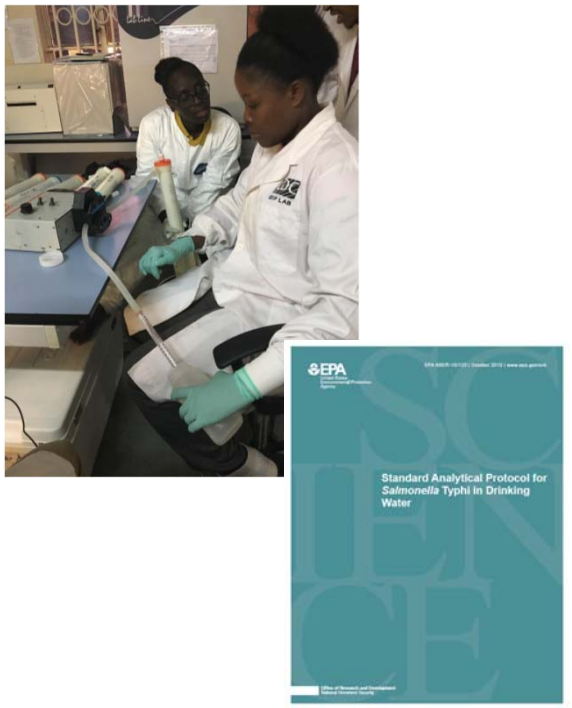
  - Drainage stream: 10 L (or until clogging occurs)
  - Drinking water: 20 L
- Small-volume water samples, in duplicate, for additional water quality parameters

Sample Processing

- In the Kibera laboratory:
  - Ultrafilter backflush → EPA culture method
    - Universal pre-enrichment (UPE) broth
    - Selenite cysteine (SC) broth
    - Bismuth sulfite (BS) agar and xylose lysine deoxycholate (XLD) agar



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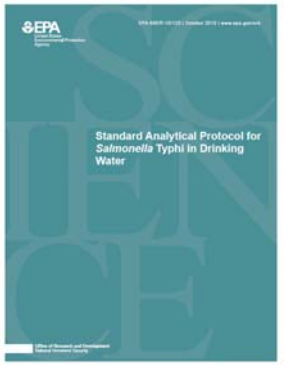
  - Grab samples
    - IDEXX Colilert-18 for *E. coli*
    - IDEXX Enterolert for *Enterococci*



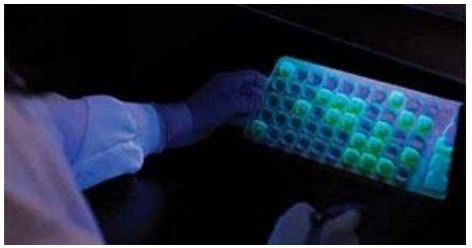
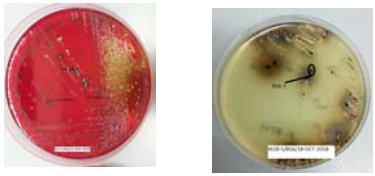


Photo: idexx.co.au

Sample Preservation

Ultrafilter backflush

SC enrichment broth



Presumptive positive *Salmonella* isolates

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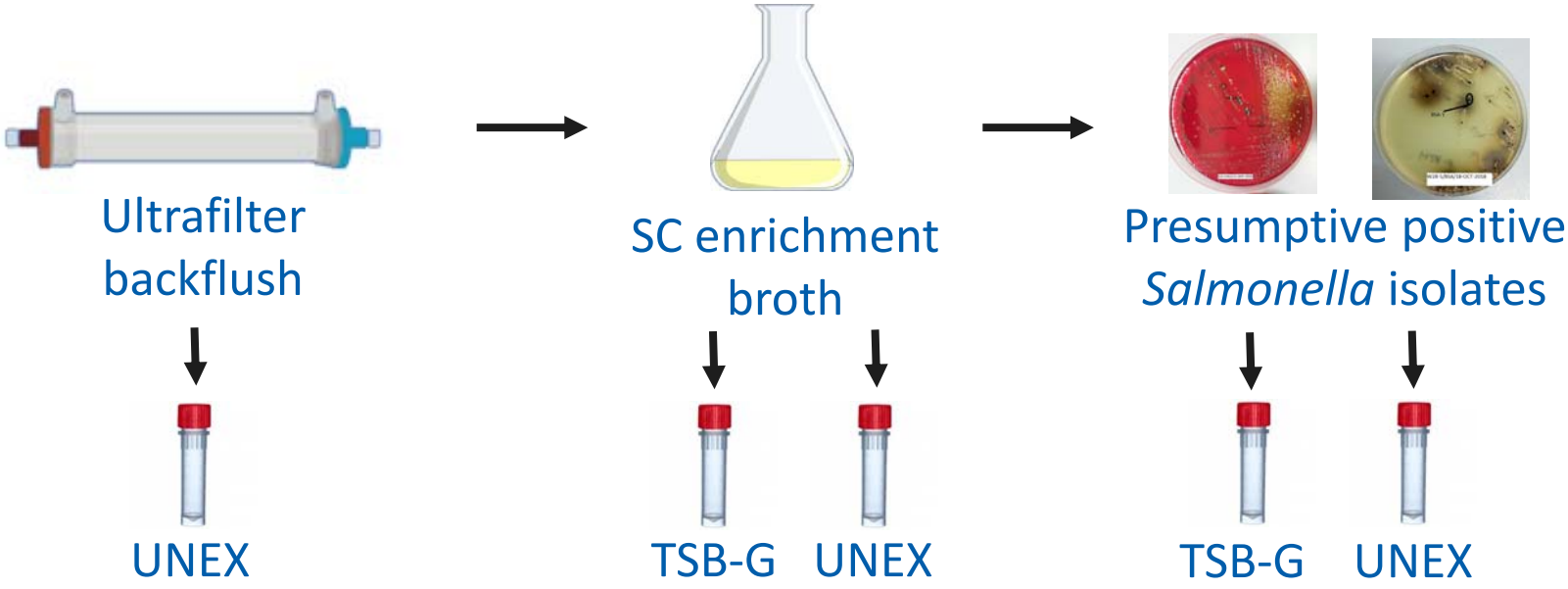

- Universal nucleic acid extraction (UNEX) buffer at ambient temperature
- Tryptic soy broth with 15% glycerol (TSB-G) at -20 °C

Physicochemical Results

Table 1: Median (range) physicochemical water quality measures

	Drainage Water (n=24)	Drinking Water (n=24)
Turbidity (NTU)	673.5 (12.6 - >1000)	0.9 (0.5 - 8.6)
pH	7.8 (6.5 - 8.6)	8.1 (6.5 - 8.6)
Temperature (°C)	21.8 (19.1 - 25.8)	21.8 (17.6 - 26.5)
Conductivity (µS/cm)	1005 (236 - 17800)	97.9 (79.9 - 280.0)
Free chlorine residual (mg/L)	n/a	0.17 (<0.02 - 0.52)

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Fecal Indicator Bacteria Results

Table 2: Fecal indicator bacteria measures

	Drainage Water (n=24)	Drinking Water (n=24)
# Samples *E. coli*-positive* (range, MPN/100 mL)	24 (10^5 - 10^9)	5 (<1.0 - 33.6)
# Samples *Enterococci*-positive* (range, MPN/100 mL)	24 (10^5 - 10^7)	2 (<1.0 - 1.0)

*at least 1 of 2 replicates positive

Presumptive Positive Isolate Testing

- CDC Environmental Microbiology (EM) laboratory currently uses a modified version of the CDC Enterics Laboratory's *S.* Typhi assay for isolates

	***fim*A (*Salmonella* spp)**	***fli*C-d**	***via*B**	***tyv***
***Salmonella* Typhi**	+	+	+	+
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Murphy et al. *Appl Environ Microbiol* 2017;83(23)e01706-17.

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- Presumptive-positive isolates from 83% (60 of 72) of drainage samples
  - Isolates from 90% (26 of 29) of samples tested to-date are PCR-positive for *Salmonella* spp. (*fim*A)

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- Presumptive-positive isolates from 60 (83%) of 72 drainage samples
  - Isolates from 26 (90%) of 29 samples tested to-date are PCR-positive for *Salmonella* spp. (*fim*A)
  - No isolates tested to-date are PCR-positive for *S.* Typhi
- Presumptive-positive colonies isolated from 15% (11 of 72) drinking water samples

Murphy et al. *Appl Environ Microbiol* 2017;83(23)e01706-17.

Environmental Sample PCR Assay Development

- *S*. Typhi is challenging to isolate from the environment
  - Dilution, competition in the culture process, viable but non-culturable state (VBNC)

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- Real time PCR screening → more accurate representation
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- PCR assays developed for clinical samples provide a good starting point for environmental assay development
- However, infected clinical specimens differ considerably from environmental samples
  - Are relatively "clean" (e.g., blood) or well-characterized (e.g., stool) matrices
  - Have high concentration of target nucleic acids
  - Rarely have multiple species or serotypes of the same pathogen

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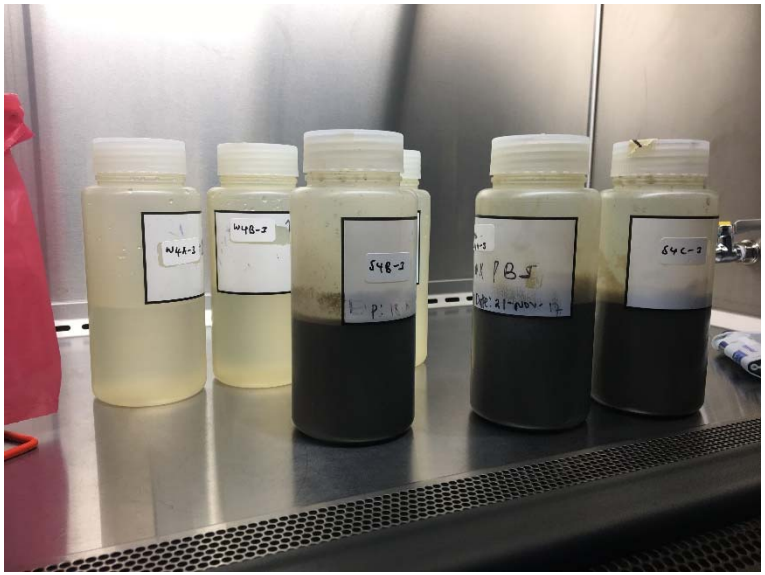
  - Vast array of uncharacterized nucleic acids that may be amplified (false positives)
  - Compounded when assays are multiplexed
    - Primer interactions
    - Relative abundance of targets per cell and across closely-related organisms
    - Competition for reagents within the reaction

Environmental Sample PCR Assay Development

- Ideal environmental PCR assay
  - Singleplex
  - Sensitive and specific within known genomic databases
  - **Thoroughly vetted via performance testing in relevant environmental samples**



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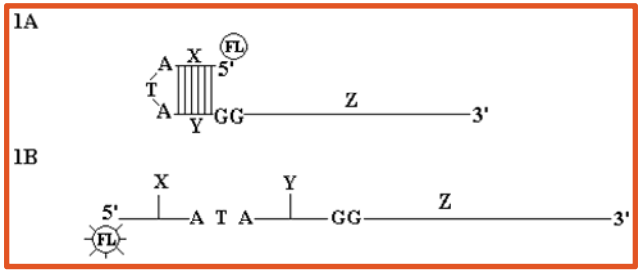
- **Not ideal for environmental samples**
  - Other environmental microorganisms may carry one or more of these genes
  - Relative abundance of different genes in complex matrix can affect interpretation of non-detects

S. Typhi PCR Assay for Environmental Samples

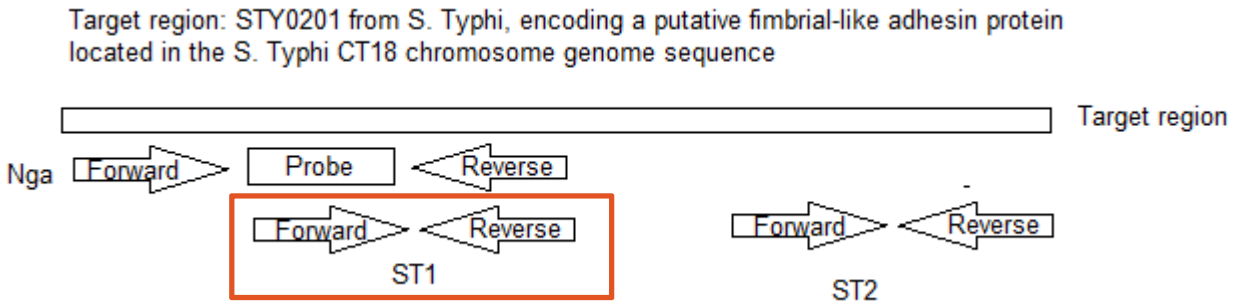
- Started with effective clinical singleplex *S.* Typhi method
- Created photo-induced electron transfer (PET) PCR assay
  - Designed for use in malaria control and elimination programs
  - Self-quenching fluorogenic primers (no internal probes or dyes)
  - Less expensive, less complex
  - Potential for use large scale screening in surveillance and epidemiological studies



Lucchi et al. *PLoS One* 2013. 8(2):e56677

S. Typhi PCR Assay for Environmental Samples

- Modified Nga primers → PET PCR primers

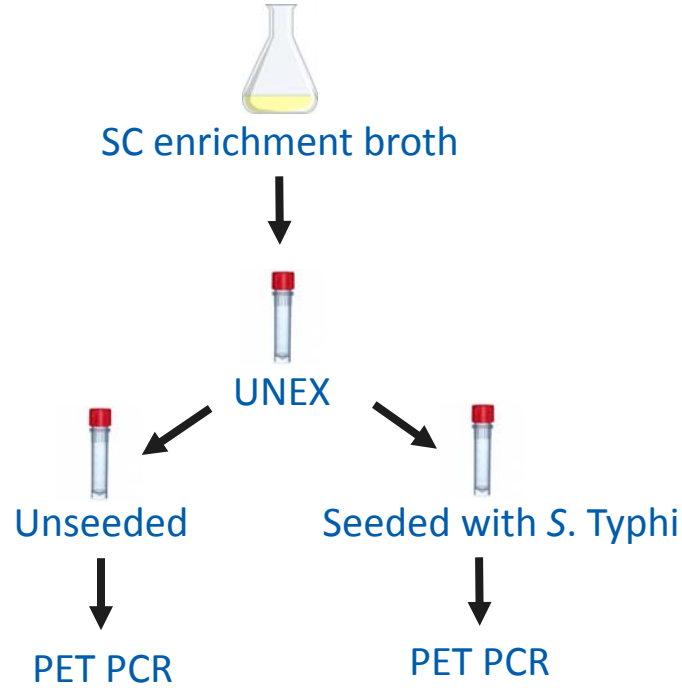


- *In silico* analysis of PET PCR target "ST1"
  - CDC clinical *S.* Typhi genomes
  - CDC *S. enterica* subsp. *enterica* genomes
  - All NCBI bacteria
  - CDC curated human stool database of 330 bacterial genome sequences

Nga et al. *BMC Infect Dis* 2010;10:125.

Performance Testing in Environmental Samples

- 4 Kibera drainage enrichments seeded with *S*. Typhi (91 CFU / PCR reaction)



Seeded Study Results

Table: PET PCR Threshold cycle (Ct) for ST1, unseeded and seeded Kibera enrichments

Sample	ST1 Ct	
	Un	Seeded
1	39.4	37.0
2	36.4	36.1
3	39.6	39.6
4	39.0	36.3
dH_2O	n/a	36.1

- Inhibition apparent in 1 sample (#3)
- ST1 detected in all 4 unseeded enrichments

Seeded Study Results

Table: PET PCR Threshold cycle (Ct) for ST1 and four target assay, unseeded and seeded Kibera enrichments

Sample	ST1 Ct		*fim*A Ct		*tyv* Ct		*via*B Ct		*fli*C-d Ct	
	Un	Seeded	Un	Seeded	Un	Seeded	Un	Seeded	Un	Seeded
1	39.4	37.0	+	+	+	+	-	+	+	+
2	36.4	36.1	+	+	+	+	+	+	+	+
3	39.6	39.6	+	+	-	+	+	+	+	+
4	39.0	36.3	+	+	-	+	+	+	+	+
dH_2O	n/a	36.1	n/a	+	n/a	+	n/a	+	n/a	+

- Enrichment 2 is also positive for each of the four additional assays → likely *S.* Typhi; sequence confirmation needed

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3	39.6	39.6	+	+	-	+	+	+	+	+
4	39.0	36.3	+	+	-	+	+	+	+	+
dH_2O	n/a	36.1	n/a	+	n/a	+	n/a	+	n/a	+

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- Enrichments 1, 3, and 4: unclear (detection limit: 40 cycles)

Next Steps

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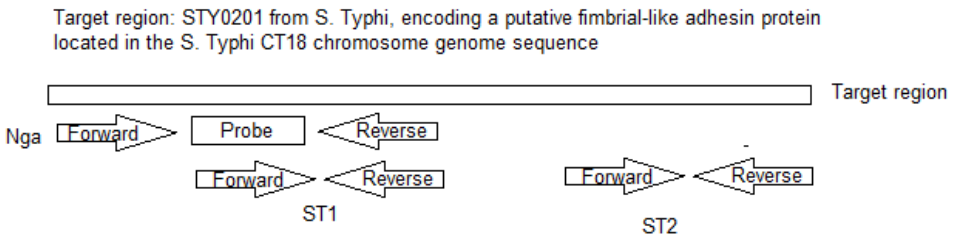
  - Limitation: ST1 is a short target ≠ sequencing



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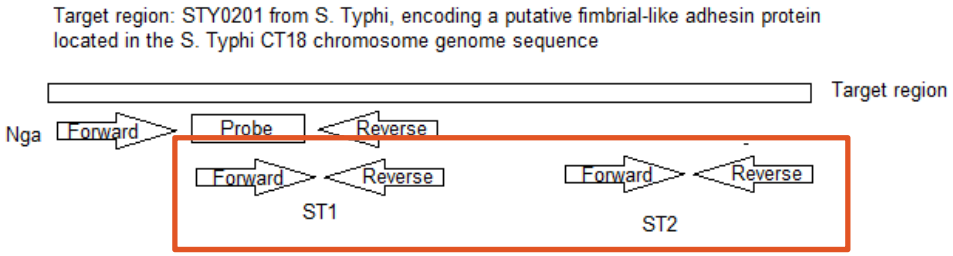
- Complete analyses of presumptive positive isolates
- Continue optimization for PET PCR:
  - Wet lab confirmation of *in silico* sensitivity and specificity
  - Limitation: ST1 is a short target ≠ sequencing
    - "ST2" appears to be highly sensitive and specific
    - Sequence longer amplicon for sequence confirmation



Next Steps

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- **Additional funding for environmental surveillance design method development:**
  - Geographical representative sampling sites (e.g., high vs low elevation)
  - Person-denominator for each sample directing public health action

Acknowledgments

- **KEMRI**
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  - Tricia Akers
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  - Jothikumar Narayanan
  - Mia Mattioli
  - Amy Kirby
  - Ana Lauer
  - Jo Williams
  - Sean Lucking
  - Patti Fields
  - Heather Carleton
  - Matt Mikoleit
- **CDC Kenya**
- **Institute for Disease Modeling**
- **Bill and Melinda Gates Foundation**
- **Henry Jackson Foundation**

Thank you!

Questions?

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov