

(Qiagen) on the WBC pellets with modifications to the manufacturer's protocol as described in the *Supplementary Data*. DNA was eluted in 32 μ L of nuclease-free water.

Real-time PCR

Primers and probes used in this study are shown in Table 1. Primers were designed in Primer express version 2.0 (Applied Biosystems). Primers were designed using Primer3 version 4.0.0 [29, 30]. Real-time PCR was performed using a 20- μ L reaction containing 10 μ L TaqMan Fast Universal PCR Master Mix (2 \times) (Life Technologies, Grand Island, New York), 1 μ L probeset mix (900 nM probe, 250 nM forward primer, and 250 nM reverse primer), and 9 μ L template. The instrumentation used was the 7500 FAST Dx Real-time PCR machine (Life Technologies). Initial denaturation was at 95°C for 20 seconds followed by 50 cycles of 95°C denaturation for 3 seconds, and 60°C annealing and extension for 30 seconds. Data was collected during the annealing and extension step. A positive was determined if amplification intersected with the threshold within 50 cycles. Results reported as an undetermined cycle threshold (Ct) were examined for indications of late amplification as described in the *Supplementary Data*. Controls were run with every reaction as described in the *Supplementary Data*.

Table 1. Primers and Probes Used in This Study

Oligo	Sequence (5' to 3') and Fluorophore	Target	Reference
Cloning primers			
ST-Fc	GGAGCTGGCGTTTTAGACA	Salmonella Typhi STY0201	This work
ST-Rc	TCTCTTCAAGCCAGCAGAGAT	Salmonella Typhi STY0201	This work
PA-Fc	AATTGGCGGGGTAGTGATAAG	Salmonella Paratyphi A SSPA2308	This work
PA-Rc	GTGAAGGGGACAGATGTGGAG	Salmonella Paratyphi A SSPA2308	This work
clyA589-F	ATAGTCGCCCGTTCGTTG	Salmonella Typhi clyA	This work
clyA722-R	GGGGCATGATACTTTATTG	Salmonella Typhi clyA	This work
Diagnostic primers and probes			
ST-Probe	FAM-CATTCTGCTGGAGCAGGCTGAGGG-TAMRA	Salmonella Typhi STY0201	[22]
ST-Frt	CGCGAAGTCAGAGTCGACATAG	Salmonella Typhi STY0201	[22]
ST-Rrt	AAAGACCTCAACGGGGATGAC	Salmonella Typhi STY0201	[22]
Pa-Probe	CYS-CCATACAAATTCTATTGAGAATGCGC-BHQ2	Salmonella Paratyphi A SSPA2308	[22]
Pa-Frt	AGGATGATGACTGATTATGAAAC	Salmonella Paratyphi A SSPA2308	[22]
Pa-Rrt	TGAAAGATATCTCAGAGCTGG	Salmonella Paratyphi A SSPA2308	[22]
pHIV-Probe	CYS-TTTTATGTTGGGGCAACATGCGATC-BHQ2	Recombinant pCR TOPO 2.1 gB	[22]
pHIV-Frt	GGGGAAATCACAGATTGAACT	Recombinant pCR TOPO 2.1 gB	[22]
pHIV-Rrt	GCGGTTGAAAGTGACCAA	Recombinant pCR TOPO 2.1 gB	[22]
clyA542-Probe	FAM-CGGTGCTGAGCAGGGATA-TAMRA	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA498-F	TTATTTTCACTGACAGGTGGATAG	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA877-R	CTAGTAAAGAAATTTCGACTGCTTTA	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA383-Probe	FAM-HACTGAAATGAAGCGAAAAATCTCTCGG-TAMRA	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA335-F	CAAGCAGAAAAGACATTCTCATC	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA440-R	GAAGGTTGTTGAAAGCTTGTGAA	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work
clyA508-Probe	FAM-ATTGCTGCCGGCGTGAATTGAAAGGAA-TAMRA	Salmonella Typhi and <i>Salmonella Paratyphi A</i> clyA	This work

In Vitro Evaluation of Specificity

Specificity of primers was tested on clarified boiled lysate of *Salmonella enterica* (serovars Typhi, Paratyphi A, Paratyphi B, Paratyphi C, Typhimurium, Enteritidis, Dublin, Choleraesuis, and Newport) and other bacteria including *S. pneumoniae*, *K. pneumoniae*, *H. influenzae*, *P. aeruginosa*, and *E. coli* (Table 2). Three to 5 colonies were suspended in molecular-grade water and boiled at 95°C for 10 minutes in a thermal cycler. The lysate was centrifuged at maximum speed in a microcentrifuge for 1 minute. The supernatant was transferred into a fresh tube and 2 μ L of a 1:10 dilution of the clarified lysate was tested in a 20- μ L reaction for amplification of the clyA amplicon.

In Vitro Evaluation of Sensitivity

Salmonella Typhi reference strain Ty2 was grown overnight in HS media to approximately 1×10^8 CFU/mL. Serial 1:10 dilutions of bacteria were made in phosphate-buffered saline. A total of 100–200 μ L of bacterial suspension was spiked onto the WBC pellet that was generated following erythrocyte lysis. Total genomic DNA was extracted as described above. Actual CFU used to spike WBC pellets was determined by performing viable counts using the dilutions expected to contain 100–1000 CFU/mL.

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