

(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](#). Probes 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](#).

In Vitro Evaluation of Specificity

Specificity of primers was tested on clarified 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 *clpA* 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.

Table 1. Primers and Probes Used in This Study

Oligo	Sequence (5' to 3') and Fluorophore	Target	Reference
Cloning primers			
ST-Fc	GGAGTCGCCGTTTITAGACA	<i>Salmonella</i> Typhi STY0201	This work
ST-Rc	TCCTTCAGCCAGCAGAGAAT	<i>Salmonella</i> Typhi STY0201	This work
PA-Fc	AATTGGCGGCGTAGTGATAG	<i>Salmonella</i> Paratyphi A SSPA2308	This work
PA-Rc	GTGAGGGGACAGATGTGGAG	<i>Salmonella</i> Paratyphi A SSPA2308	This work
clpA559-F	ATAGTCGCCCGGTCCGTTTG	<i>Salmonella</i> Typhi <i>clpA</i>	This work
clpA722-R	GCCGCATCGATATCTTTATTCG	<i>Salmonella</i> Typhi <i>clpA</i>	This work
Diagnostic primers and probes			
ST-Probe	FAM-CATTGTGTTCTGGAGCCAGGCTGACGG-TAMRA	<i>Salmonella</i> Typhi STY0201	[22]
ST-Frt	CGCGAAGTCAGAGTCGACATAG	<i>Salmonella</i> Typhi STY0201	[22]
ST-Rrt	AAGACCTCAACGCCGATCAC	<i>Salmonella</i> Typhi STY0201	[22]
Pa-Probe	CYS-CCCATACAATTTTCATTCTTATTGAGAAATGCGC-BHQ2	<i>Salmonella</i> Paratyphi A SSPA2308	[22]
Pa-Frt	ACGATGAGTACTGATTTCGGAAC	<i>Salmonella</i> Paratyphi A SSPA2308	[22]
Pa-Rrt	TGAAAAGATATCTCTCAGAGCTGG	<i>Salmonella</i> Paratyphi A SSPA2308	[22]
pHHV-Probe	CYS-TTTTATGTGTCCGCCACCATCTGGATC-BHQ2	Recombinant pCR TOPO 2.1 gB	[22]
pHHV-Frt	GGGCGAATCACAGATTGAATC	Recombinant pCR TOPO 2.1 gB	[22]
pHHV-Rrt	GCGGTTCCAAACGTACCAA	Recombinant pCR TOPO 2.1 gB	[22]
clpA542-Probe	FAM-CCGGTGCTGCAGCAGGCATA-TAMRA	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA498-F	TTATTTCCAGTCACAGGTGGATAG	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA577-R	CTAGTAAAGAAATTTTGCACCTGCTTTTA	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA383-Probe	FAM-AACTGAATGAAGCGCAAAAATCTCTCTCTGG-TAMRA	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA335-F	CAQCGCAGAAAGACATTTCTCATC	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA440-R	GAAGCGTTGTTGAACCTTTGTGAA	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work
clpA598-Probe	FAM-ATTGCTGCGGGCGGTGATTGAAGGGA-TAMRA	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi A <i>clpA</i>	This work

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Table of Contents Organisms Infect Dis Words Cd

1. Understanding the eBook Organisms Infect Dis Words Cd
 - The Rise of Digital Reading Organisms Infect Dis Words Cd
 - Advantages of eBooks Over Traditional Books
2. Identifying Organisms Infect Dis Words Cd
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Organisms Infect Dis Words Cd
 - User-Friendly Interface
4. Exploring eBook Recommendations from Organisms Infect Dis Words Cd
 - Personalized Recommendations
 - Organisms Infect Dis Words Cd User Reviews and Ratings
 - Organisms Infect Dis Words Cd and Bestseller Lists
5. Accessing Organisms Infect Dis Words Cd Free and Paid eBooks
 - Organisms Infect Dis Words Cd Public Domain eBooks
 - Organisms Infect Dis Words Cd eBook Subscription Services
 - Organisms Infect Dis Words Cd Budget-Friendly Options
6. Navigating Organisms Infect Dis Words Cd eBook Formats
 - ePub, PDF, MOBI, and More
 - Organisms Infect Dis Words Cd Compatibility with Devices
 - Organisms Infect Dis Words Cd Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Organisms Infect Dis Words Cd
 - Highlighting and Note-Taking Organisms Infect Dis Words Cd
 - Interactive Elements Organisms Infect Dis Words Cd
8. Staying Engaged with Organisms Infect Dis Words Cd

- Joining Online Reading Communities
- Participating in Virtual Book Clubs
- Following Authors and Publishers Organisms Infect Dis Words Cd
- 9. Balancing eBooks and Physical Books Organisms Infect Dis Words Cd
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Organisms Infect Dis Words Cd
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Organisms Infect Dis Words Cd
 - Setting Reading Goals Organisms Infect Dis Words Cd
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Organisms Infect Dis Words Cd
 - Fact-Checking eBook Content of Organisms Infect Dis Words Cd
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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