

A 16S ribosomal RNA TaqMan real-time reverse transcription PCR for specific detection of Salmonella in blood

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Research article

Keywords: Salmonella, 16S rRNA, TaqMan RT-PCR, diagnosis, bloodstream infection

Posted Date: January 28th, 2020

DOI: <https://doi.org/10.21203/rs.2.22017/v1>

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Abstract

Background : The Gram -negative bacterium *Salmonella enterica* is an important human pathogen causing a huge public health burden worldwide. Reference diagnosis of *Salmonella* bloodstream infections (BSI) in patients is based on in vitro blood culture followed by biochemical and serotype identification. We have developed a TaqMan real-time reverse transcription PCR (RT-PCR) assay for the specific detection of *Salmonella* 16S rRNA molecules. The specificity was confirmed on a bacterial test panel grown in 5 ml BBL TM culture medium broth, comprising of six *Salmonella enterica* serovars (Typhi, Paratyphi A, Typhimurium, Enteritidis, Heidelberg and Weltevreden) and 10 non-*Salmonella* bacterial species that are known to cause human BSI.

Results : The limit of detection (LOD) of the assay in serial dilutions of purified *Salmonella enterica* serovar Typhimurium RNA was 40 pg RNA per reaction, corresponding to ~2500 colony forming units (CFU). When applied directly in blood experimentally spiked with *Salmonella* Typhimurium, the assay showed an LOD of 10⁵ CFU/ml which is 100x more sensitive than 16S rDNA detection using the same primers and probe set. In 10 ml whole blood spiked with *Salmonella* Typhimurium at 5 CFU/ml followed by incubation in an automatic blood culture instrument, the assay detected 16S rRNA after 10 hours incubation compared to 12 hours for 16S rDNA detection.

Conclusion : Although prior in vitro incubation of blood samples is required, we here delivered the proof-of-concept of specific detection of *Salmonella* in clinical specimens by targeting its 16S rRNA molecule.

Background

Salmonella bloodstream infections (BSI), invasive salmonellosis, cause high morbidity and mortality mainly in developing countries [1]. *Salmonella* serovars causing bloodstream infections are classified into typhoidal and invasive non-typhoidal *Salmonella* (iNTS) serovars [2, 3]. Typhoidal *Salmonella* serovars, causing enteric fever, include *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C and are considered to be restricted to humans without any animal reservoir [4, 5]. Because enteric fever is spread through the faecal-oral route, most cases occur in the developing world and are associated with poor sanitation and hygiene conditions [1]. Typhoidal serovars cause 26.9 million cases and approximately 200,000 deaths worldwide are reported each year [6]. The dominant iNTS serovars are *Salmonella* Typhimurium and *Salmonella* Enteritidis [7]. In developed countries these serovars cause self-limiting gastroenteritis. However, in sub-Saharan Africa, the same serovars are dominant causes of BSI in children and patients co-infected with malaria and HIV in [8, 9]. Globally, iNTS cause approximately 3.4 million invasive infections and 681,000 deaths per year [1].

Given the public health importance of *Salmonella* BSI, rapid and specific detection of *Salmonella enterica* in blood samples is crucial for patient diagnosis and disease control [10]. Current reference methods for *Salmonella* detection in blood rely on conventional blood culture followed by microbiological work-up for

species and serotype identification. Microbiological methods are labour-intensive and generally take 6 to 48 hours before positive growth [11]. Various selective media for the identification of *Salmonella* have been developed and are commercially available. Unfortunately, the use of these media is limited as they target individual *Salmonella* serovars and generally have low analytical sensitivity and specificity [12–16]. To overcome such disadvantages of microbiological culture, various molecular methods based on amplification of *Salmonella*-specific nucleic acid sequences by the polymerase chain reaction (PCR) have been developed [17]. They include monoplex, multiplex, nested, conventional and quantitative real-time PCR formats applied either on grown blood cultures or directly applied to blood samples. They are rapid but present moderate sensitivity when applied directly to biological samples without prior in vitro bacterial culture steps [17]. The 16S ribosomal RNA (rRNA) is a component of the 30S small subunit of the prokaryotic ribosome and is among the most abundant nucleic acid molecules in the bacterial cell. We here report the design and proof-of-concept of a novel molecular assay targeting *Salmonella* 16S rRNA ribosomal RNA molecules in blood with high specificity and repeatability.

Results

Assay performance, analytical specificity and sensitivity

The *Salmonella* 16S rRNA TaqMan real-time RT-PCR assay's efficiency as calculated from its slope was 91% and the correlation coefficient (R^2) was 0.999, when tested on a standard curve of 1 ng/ μ L, 100 pg/ μ L and 10 pg/ μ L *Salmonella* Typhimurium SL1344 in triplicate (Additional file 1: Figure S1). When testing the 16 bacterial species included in the study (Table 1) at 1 ng/ μ L, the assay detected all *Salmonella* serovars and did not detect any non-*Salmonella* bacterial species (Additional file 2: Table S1 and Figure 1). Mean Cp values ranged between 16,09 and 19,22 (Additional file 2: Table S1). In ten-fold serial dilutions of purified *Salmonella* Typhimurium RNA, the LOD was 10 pg/ μ L corresponding to 40 pg RNA per RT-PCR reaction (Figure 2). Considering that one bacterial cell contains 16 fg rRNA [18], the observed analytical sensitivity corresponded to ~2500 CFU per reaction.

Limit of detection in human blood

The LOD of the 16S rRNA assay was assessed in human blood spiked with known numbers of *Salmonella* Typhimurium SL1344 and compared with 16S rDNA detection. We observed an LOD of 10^5 CFU/ml blood for 16S rRNA detection (Cp value μ =32.69, s=0.55) and 10^7 CFU/ml blood for 16S rDNA detection (Cp value μ =31.91, s=1.12) (Additional file 2: Table S1 and Figure 3). We observed a difference of 15 cycles between 16S rRNA (Cp value μ =16.95, s=0.42) and 16S rDNA (Cp value μ =31.91, s=1.12) detections in the blood samples spiked with 10^7 *Salmonella* cells per ml blood. There was no amplification detection in negative control samples (Additional file 2: Table S1). The RNA samples did not show DNA contamination when run with PCR without reverse transcription.

Limit and speed of detection in spiked blood cultures

The LOD of the 16S rRNA assay was compared with 16S rDNA detection in time-course measurements of blood culture bottles spiked with 5 CFU/ml blood in triplicate. *Salmonella* 16S rRNA was detected after 10 hours of incubation (Cp value $\mu=31.5$, $s=0.9$) and 16S rDNA after 12 hours (Cp value $\mu=33.52$, $s=0.6$). There was no amplification in the non-spiked blood culture bottle (Additional file 2: Table S1). In none of the blood culture bottles growth was detected by BacT/ALERT at 12 hours of incubation. Our experimental setting did not allow a continuous monitoring of blood culture growth by BacT/ALERT after 12 hours incubation.

Discussion

We report a novel TaqMan real-time reverse transcription PCR (RT-PCR) assay for detection of *Salmonella* 16S rRNA in biological samples. The assay shows high specificity by detecting all *Salmonella* serovars included in the study and remaining negative for non-*Salmonella* bacteria. Despite the high analytical specificity and repeatability of the 16S rRNA assay, the sensitivity of 10^5 *Salmonella* cells per ml of blood is too low for diagnostic use without prior *in vitro* blood culture. Bacterial concentrations in blood of a patient with *Salmonella* blood infection can be as low as 1 to 10 CFU per ml of blood. We assessed the required time to detection of blood culture prior to RT-PCR testing for a 10 ml blood sample with 5 CFU per ml blood. While 16S rDNA testing required 12 hours blood culture incubation, the 16S rRNA assay turned positive after 10 hours. BacT/ALERT detected growth in the spiked blood culture bottles >12 hours incubation, but the exact time could not be monitored due to the experimental setting. Our comparative analysis in grown blood cultures confirm our hypothesis that detection of 16S rRNA is more sensitive than detection of 16S rDNA.

Previous PCRs for *Salmonella* detection in blood have been published. They mainly target *Salmonella* serovar specific genes and show various values of LOD. For instance, three different studies have applied different single genes (flagellin, *clyA*, flagellar genes *fliC-a* and *fliC-d*) targeted PCR respectively to detect typhoidal serovars in blood (20-23). While the 16S rRNA gene has been previously used as a potential molecular target in *Salmonella* PCRs (24-26), this is the first assay that targets the highly abundant 16S rRNA molecules. 16S rRNA and its gene has, in addition to highly conserved regions, nine hypervariable regions across bacterial species and its function has likely not changed over a long period of time through the evolution [27]. In 1996, Lin and Tsen developed PCR primers for identification of *Salmonella* based on the 16S rRNA gene [24], which were later improved by the same authors [28] and Trkov and Avguštin [25]. Both improved assays were based on conventional PCR followed by agarose gel electrophoresis. We here present the design of a new set of primers with a corresponding TaqMan MGB for real-time RT-PCR of *Salmonella* 16S rRNA. MGB-based TaqMan probes allow stable interactions with the amplified DNA with relatively high melting temperature providing high specificity to its target sequence [29].

The low sensitivity of our designed assay is probably due to the high annealing temperature (T_a) of the primers used in the RT-PCR assay to obtain the required specificity given that *Salmonella* specific detection is based on single nucleotide polymorphisms (SNPs) in the 16S rRNA sequences. The forward

primer shows only 1 SNP with *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Pluralibacter gergoviae* while the reverse primer target sequence has no SNPs in those 3 species. The use of locked nucleic acids (LNA) or peptide nucleic acids (PNA) may be considered to lower the Ta while maintaining the primers' melting temperatures and thus specificity [30, 31]. RT-PCR-inhibitors in blood, such as immunoglobulins, haemoglobin and lactoferrin as well as the anticoagulants EDTA and heparin, are known to inhibit reverse transcription and PCR amplification. In addition, commercial blood culture bottles contain media, color indicators, and sometimes antimicrobial removal resins or beads, and may thus also contain RT-PCR inhibitory components. To our knowledge, we applied for the first time the Polaris enrichment technique to bacterial RNA extraction from blood samples and presence of RT-PCR inhibitors in the RNA samples cannot be excluded. Different methods for bacterial RNA extraction from blood and blood cultures should be compared for optimal yield and purity in order to select the most efficient method for 16S rRNA RT-PCR.

Despite the low sample size specially with spiked blood cultures, with our study we deliver the proof of concept of 16S rRNA testing for *Salmonella* detection in blood and confirm our hypothesis that 16S rRNA detection is more sensitive than 16S rDNA testing. Hereto, further evaluation of the developed 16S rRNA RT-PCR test on larger size of clinical blood samples is needed. The potential value of the 16S rRNA RT-PCR test in clinical practice is that it can detect bacteria in blood samples earlier than conventional blood culture by BacT/ALERT and allows direct identification of *Salmonella enterica*, omitting biochemical testing for species identification.

Patients frequently use antibiotics prior to blood culture sampling and a major advantage of molecular diagnostics is that cell-free bacterial DNA can be detected as proxy for a bacterial bloodstream infection. Given the relatively long half-life of rRNA after bacterial death [32, 33], we expect that rRNA is an equal marker for bacterial infection as DNA. The same RT-PCR platform could allow development of multiplex 16S rRNA assays detecting in parallel multiple bacterial species responsible of bacterial bloodstream infections. However, targeting 16S rRNA in multiplexed PCRs will require a 16S rRNA based resolution to the species level and this is not always possible for some bacterial species such as *E. coli* and *Shigella*. On the other hand, 16S metagenomics can simultaneously detect all bacteria in a given blood sample based on PCR amplification of the 16S ribosomal RNA gene followed by deep sequencing of the PCR amplicons and taxonomic labelling of the sequence reads at genus or species level. However, the technique currently presents various technical limitations such as limited taxonomic resolution and high background signals jeopardizing its implementation in routine patients' diagnosis [11]. Based on the proof-of-concept presented here, 16S rRNA amplicon deep sequencing may be more sensitive than conventional 16S metagenomics at DNA level. Therefore, direct sequencing of 16S rRNA could complement the 16S metagenomics tool set for bacterial identification and decrease false positive hits as RNA represents a marker of viable organisms [33, 34].

Conclusions

We developed a 16S rRNA TaqMan real-time RT-PCR assay for specific detection of *Salmonella* applicable on grown blood cultures. The assay detects all *Salmonella* serovars tested in this study and does not cross-react with non-*Salmonella* bacterial species but requires prior *in vitro* blood culture incubation to obtain the required sensitivity for clinical diagnosis.

Methods

Bacterial strains

Salmonella Typhimurium reference strain ATCC SL1344 was used in this study for assay optimization and 15 additional *Salmonella* and non-*Salmonella* bacterial strains were selected for *in vitro* evaluation of the specificity of the designed primers and probe (Table 1). All strains were grown in 5 ml BBL™ culture medium broth (Becton, Dickinson and Company, Sparks, Maryland, USA) at 37°C aerated at 200 rpm. Bacteria were harvested at the logarithmic phase (OD600 = 0.5) by centrifugation at 4000 rpm, 4°C, for 20 minutes.

In silico primer and probe design

AlleleID software v7.01 (PREMIER Biosoft International, Palo Alto CA, USA) was used to design oligonucleotide primers and TaqMan minor groove binding (MGB) probes. The 16S rRNA gene sequences of a list of 44 bacterial strains including 8 *Salmonella enterica* serovars and 36 non-*Salmonella* bacterial species was generated and their 16S rRNA gene sequences retrieved from the SILVA database [35] (Additional file 3: Table S2). 16S rRNA sequences of ≥ 1450 bp and sequence alignment quality of $\geq 93\%$ (ranked by SILVA database) were retained (Additional file 4: Supplemental information S1). The 16S rRNA sequences were imported into AlleleID and subjected to sequence alignment using ClustalW [36] for the design of *Salmonella enterica* primers and TaqMan MGB probe sets. The specificity of the top-five ranked sets of primers and probes was further evaluated *in silico* using Geneious v11.1 (Auckland, New Zealand) and BLAST against 16S rRNA gene sequences of *Salmonella enterica* and the most closely related *Enterobacteriaceae* (Additional file 3: Table S2). The primers and probe set with the highest *in silico* specificity for *Salmonella enterica* was selected and the sense primer was manually curated to avoid cross-amplification of *Pluralibacter gergoviae* (Additional file 5: Figure S2). The sequences of the selected and curated sense and anti-sense primers with the 5' FAM reporter and 3' Eclipse quencher labelled probe are presented in Table 2 and the locations in the 16S rRNA gene alignment of 8 *Salmonella* serovars and 35 non-*Salmonella* bacterial species in the additional file 5: Figure S2. Oligonucleotides were purchased from Integrated DNA Technologies (Leuven, Belgium).

Salmonella 16S rRNA TaqMan real-time reverse transcription PCR

The TaqMan real-time reverse transcription PCR (RT-PCR) assay was performed using the SensiFAST Probe No-ROX One-Step Kit (Bioline, London, UK). A total RT-PCR reaction volume of 20 μ l contained 3.6 μ l of RNase-free water, 0.4 μ l of Ribosafe RNase inhibitor, 0.2 ml of reverse transcriptase, 1x SensiFast Probe No-ROX one-step mix, 0.4 μ M of each primer, 0.1 μ M TaqMan probe, and 4 μ l of RNA

template. The One-step RT-PCR cycling conditions were 15 minutes at 45°C, 2 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 65°C and 30 seconds at 72°C. For DNA detection, the same assay was applied but with replacement of the volume of reverse transcriptase and Ribosafe RNase inhibitor with RNase-free water and omitting the reverse transcription step (15 minutes at 45°C) from the PCR cycling. All RT-PCR reactions were performed on a Lightcycler 480 instrument (Roche Applied Science, Penzberg, Germany) and fluorescence data were acquisitioned during the extension step. A positive signal of detection was determined if amplification intersected with the threshold within 35 cycles (threshold cutoff criterium); and results are reported in Cp (cross points) values. Samples with Cp ³ 35 cycles or no Cp values recorded were considered as negative. For all RT-PCR assays, the negative control consisted of sterile RNase-free water. Genomic DNA contamination in the RNA samples was verified using PCR without reverse transcription.

RNA and DNA extraction

Total RNA from *in vitro* cultured bacteria was extracted using the RNeasy Protect Bacteria Mini Kit (Qiagen, Hilden, Germany). An additional step of bacterial lysis was included after the RNA Protect Bacteria treatment by adding 100 µl of 100 mg/ml lysozyme dissolved in 30 mM Tris buffer. Before extracting bacterial RNA and DNA from spiked blood and grown blood cultures, the samples were first enriched using the Polaris buffers (Biocartis, Mechelen, Belgium) which has been previously applied for enrichment of bacterial DNA from human whole blood samples [37]. One ml of human whole blood was mixed with an equal volume of selective lysis buffer (SLB), vortexed and mixed for exactly 3 minutes to lyse blood cells and fragment the released human DNA, and 5 ml neutralization buffer (NB) was added to stop the reaction. Suspensions were subsequently centrifuged for 15 minutes at 2800 x g and pellets were resuspended in 1 ml washing buffer (WB) and centrifuged for 10 minutes at maximum speed (>16000 x g). Supernatants were removed and pellets were subjected to total RNA extraction with the RNeasy Protect Bacteria Mini Kit, and to total DNA extraction using the Gentra Pure Gene Yeast/Bacteria Kit (Qiagen, Hilden, Germany). RNA and DNA were eluted in 30 µl RNase-free water and DNA hydration solution, respectively. RNA and DNA concentrations were measured with the Nanodrop ND-100 Spectrophotometer (Isogen Life Science, Utrecht) and samples were stored at -80°C until further analysis. All RNA samples were treated with DNase I (Invitrogen, USA) prior to RT-PCR testing.

RT-PCR assay performance

The efficiency and limit of detection (LOD) were determined as performance characteristics of the assay. *Salmonella* Typhimurium SL1344 RNA at 1 ng/µl, 100 pg/µl and 10 pg/µl were prepared and tested in RT-PCR to determine the efficiency of the primers and probe set. The correlation coefficient (R^2) and efficiency were calculated by linear regression of the mean Cp scored against log₁₀ of its concentration in pg/µl. To determine the LOD of the assay, 10-fold serial dilutions of *Salmonella* Typhimurium SL1344 RNA ranging from 1ng/µl to 1fg/µl were prepared in triplicate and assayed. The LOD was defined as the lowest concentrations of RNA for which all replicates are positive. Throughout the study all experiments were conducted in triplicate to assess repeatability of the assay.

Spiked human blood samples

Salmonella Typhimurium SL1344 cells grown in 5 ml BBL™ culture medium broth to logarithmic phase (OD₆₀₀=0.5) were pulled through a 0.45x13 mm needle (Terumo, Tokyo, Japan) and the OD was measured from which the corresponding bacterial cells/ml was calculated. 10-fold serial dilutions of bacterial cells were prepared in sterile 1xPBS ranging from 10⁸ to 10 cells per ml. A 100 µl of each *Salmonella* suspension was then spiked in triplicates in 1 ml of healthy human whole blood with EDTA as anticoagulant, resulting in triplicated spiked 1 ml blood samples ranging from 10⁷ to 1 bacterial cell(s) per ml blood. The exact number of spiked *Salmonella* in 1 ml blood was retrospectively determined by colony counting. The *Salmonella* suspensions at 10³, 10² and 10 cells per ml 1XPBS buffer were plated by spreading their respective 100 µl on Luria-Bertani (LB) agar plates and incubated overnight at 37°C, followed by colony counting on the next day.

Spiked and grown blood culture samples

Salmonella Typhimurium SL1344 cells grown on an LB agar plate were diluted in PBS to prepare a suspension at OD₆₀₀=0.5, corresponding approximately to 10⁸ CFU/ml, and diluted to 250 CFU/ml in 10 ml sterile physiological saline solution. From this suspension, 800 µl was added to 40 ml defibrinated horse whole blood (International Medical Products, Oudergem, Belgium) to obtain approximately 5 CFU/ml. Ten ml was added to BacT/ALERT adult blood culture bottle (bioMérieux, Marcy-l'Étoile, France) in triplicate and 10 ml horse blood with no *Salmonella* spiked was used as negative control. The BacT/ALERT bottles were incubated at 35°C for growth in 3D BacT/ALERT automated culture machine (bioMérieux) and at each hour, starting from the 2nd till the 12th hour of incubation, two times 1 ml blood culture broth were collected for RNA and DNA extraction.

Abbreviations

CFU: Colony forming unit; Cp: Cross point; iNTS: Invasive non-typhoidal *Salmonella*; LB: Luria-Bertani; LOD: Limit of detection; LNA: Locked nucleic acid; NB: Neutralization buffer; PCR: Polymerase chain reaction; PNA: Peptide nucleic acids; rRNA: Ribosomal RNA; RT-PCR: Real-time reverse transcription.

Declarations

Acknowledgements

We are grateful to Ms Marjan Peeters and Ms Ellen Corsmit from the Institute of Tropical Medicine Antwerp for their technical assistance.

Authors' contributions

JPR, SVP, CMM, JJ and SD conceived the study. JPR, TdB, KV, SVP, LH, JJ and SD designed the experiments. JPR, TdB, KV and SD designed the assay primers and probe. JPR, TdB and JC conducted

the experiments. JPR, TdB, JJ and SD wrote the original and final versions of the manuscript. KV, SVP, JC, LH and CMM reviewed the original version of the manuscript. All authors read and approved the final version of the manuscript.

Funding

This work was financially supported by the Belgian Directorate General for development (DGD), Belgium and the Baillet Latour Fund for the Bacterial Infections in the Tropics (BIT) research cluster at the Institute of Tropical Medicine (ITM), Antwerp, Belgium.

Availability of data and materials

All data generated in this study are presented in the main content of this article and its supplementary files.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Bacterial species with serovar and strain identification code used for *in vitro* evaluation of the *Salmonella* 16S rRNA RT-PCR.

Bacterial species	Serovar	Strain ID
<i>Salmonella enterica</i>	Typhimurium	ATCC SL1344
<i>Salmonella enterica</i>	Typhi	2427
<i>Salmonella enterica</i>	Paratyphi A	8121108
<i>Salmonella enterica</i>	Enteritidis	ATCC13076
<i>Salmonella enterica</i>	Heidelberg	854
<i>Salmonella enterica</i>	Weltevreden	MY063SEA5
<i>Escherichia coli</i>	NA	ATCC25922
<i>Enterobacter cloacae complex</i>	NA	NACI10
<i>Pluralibacter gergoviae</i>	NA	M/5383
<i>Shigella dysenteriae</i>	NA	A18b1-05
<i>Staphylococcus aureus</i>	NA	ATCC25923
<i>Pseudomonas aeruginosa</i>	NA	ATCC27823
<i>Klebsiella pneumoniae</i>	NA	ATCC700603
<i>Enterococcus faecium</i>	NA	M/5652
<i>Enterococcus faecalis</i>	NA	ATCC29212
<i>Acinetobacter baumannii</i>	NA	M/4371

Note. NA = not applicable; ID = Identification code.

Table 2. Sequences and characteristics of the designed *Salmonella* 16S rRNA RT-PCR primers and probe.

Oligonucleotides	Sequences (5' to 3') and fluorophore/quencher	T _m (°C)	GC%	Hairpin ΔG (kcal/mol)	Self-dimer ΔG (kcal/mol)
Forward (F) primer	GCAGGCTTGAGTCTT	57.9	50	-0.6	-0.6
Reverse (R) primer	CCCGTCAATTCATTTGAG	57.3	30.4	-0.2	-2
TaqMan MGB Probe	FAM-CCAGGCGGTCTACTTAAC-MGB Eclipse	68	55.6	0	-0.8

Note. MGB = Minor groove binding; T_m = Melting temperature; ΔG = Gibbs free energy.

Supplemental Information Note

Supplementary information

Additional file 1: Figure S1. Efficiency of the developed *Salmonella* 16S rRNA TaqMan real-time reverse transcription PCR.

Additional file 2: Table S1. The developed assay results (C_p values) for specific *Salmonella* detection, LOD in 10-fold serial dilutions of *Salmonella* Typhimurium RNA and comparison of sensitivity between 16S rRNA and 16S rDNA detection in spiked blood samples and grown blood cultures.

Additional file 3: Table S2. Bacterial species and bacterial serovars, with 16S rRNA gene SILVA accession numbers, used in the *in silico* design and evaluation of the *Salmonella* 16S rRNA primers and probe.

Additional file 4: Supplemental information S1. 16S rRNA gene sequences for *Salmonella enterica* serovars and non-*Salmonella* bacterial species used in this study, retrieved from the SILVA database.

Additional file 5: Figure S2. Location of the forward primer (Fprimer), probe (TaqMan) and reverse primer (Rprimer) in the alignment of 16S rRNA gene sequences of *Salmonella enterica* serovars and non-*Salmonella* bacterial genera and species.

Figures

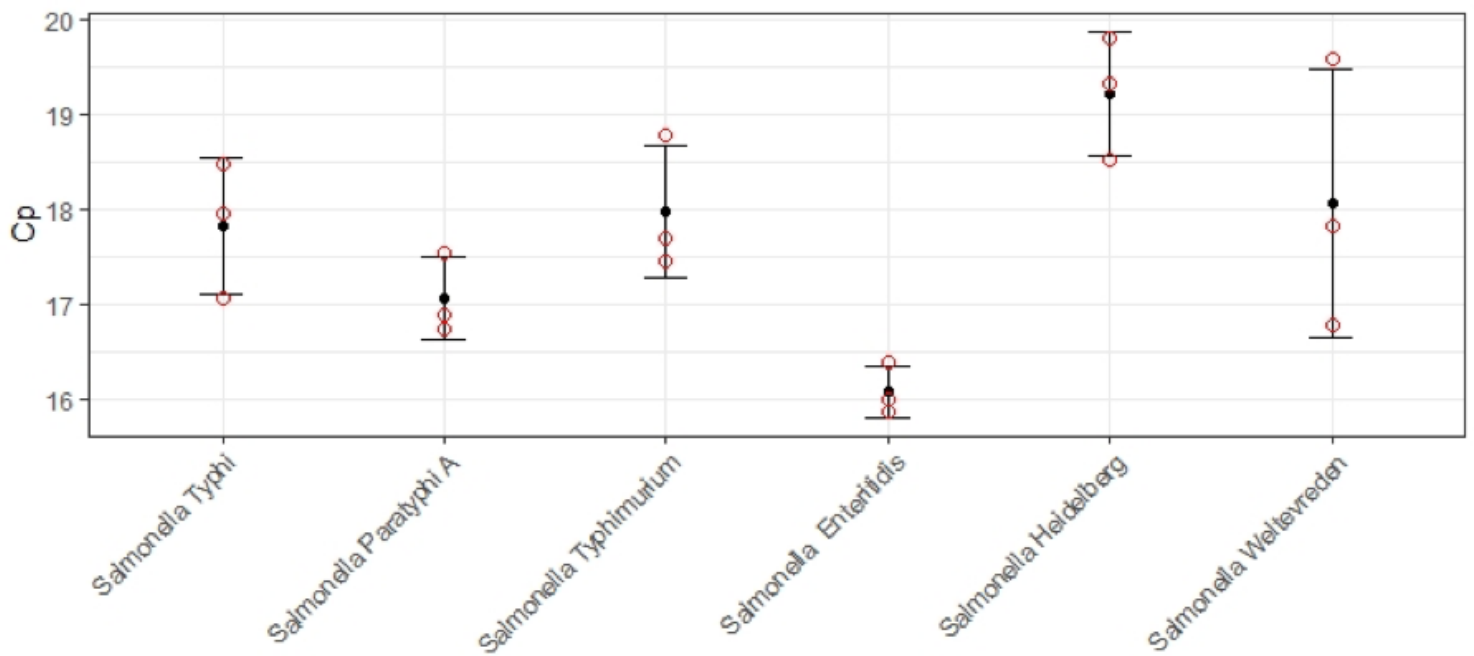


Figure 1

Specificity of the Salmonella 16S rRNA TaqMan RT-PCR assay on 6 Salmonella serovars and 10 non-Salmonella bacterial species (Table 1) at 1ng/μl RNA concentrations in triplicate. Mean and standard deviation of the Cp values are represented by a closed black dot and a vertical error bars, respectively; overlaid by Cp values displayed as open red dots. The assay detected all 6 tested Salmonella serovars and did not detect any of the 10 tested non-Salmonella bacterial species.

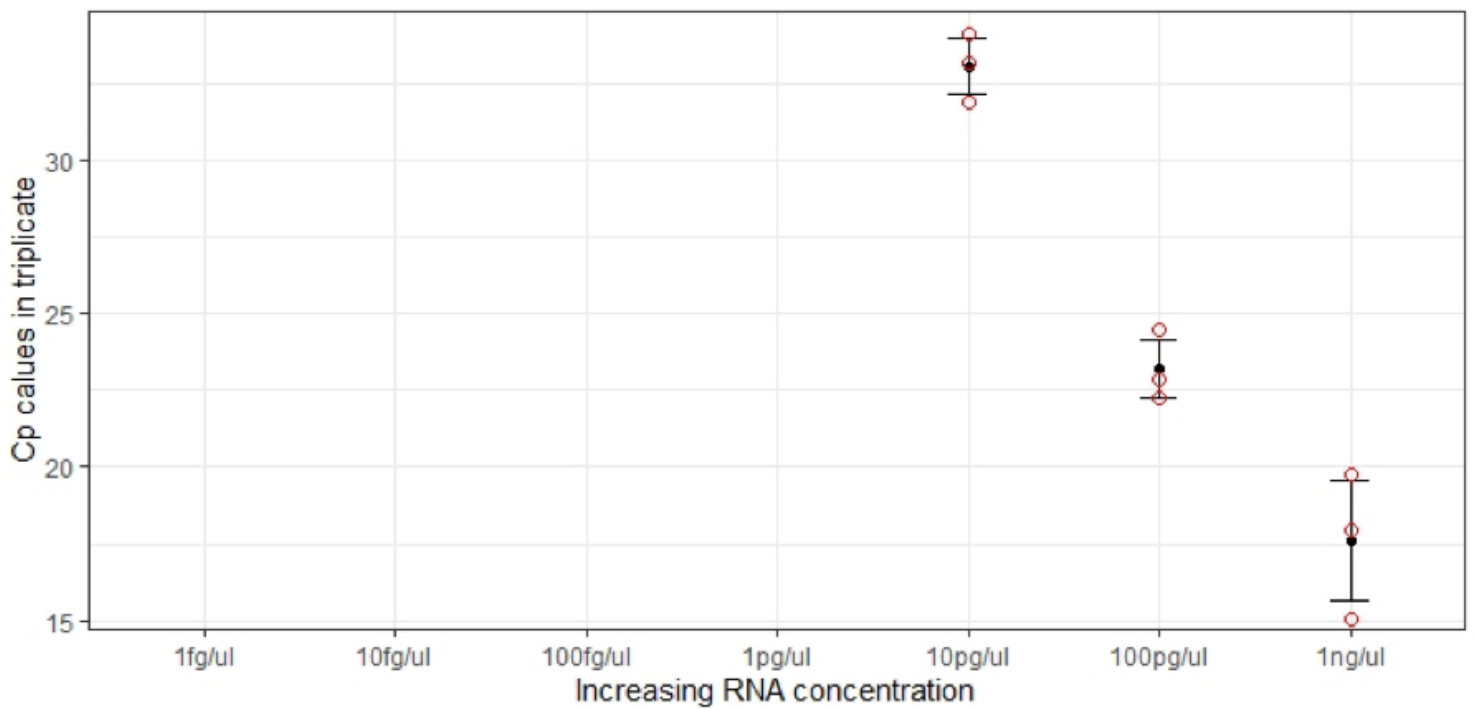


Figure 2

Limit of detection of the Salmonella 16S rRNA TaqMan real-time RT-PCR assay in increasing concentrations of Salmonella Typhimurium SL1344 RNA from 1fg/μl to 1ng/μl in triplicate. Mean and standard deviation of the Cp values are represented by a closed black dot and a vertical error bar, respectively; overlaid by Cp values displayed as open red dots.

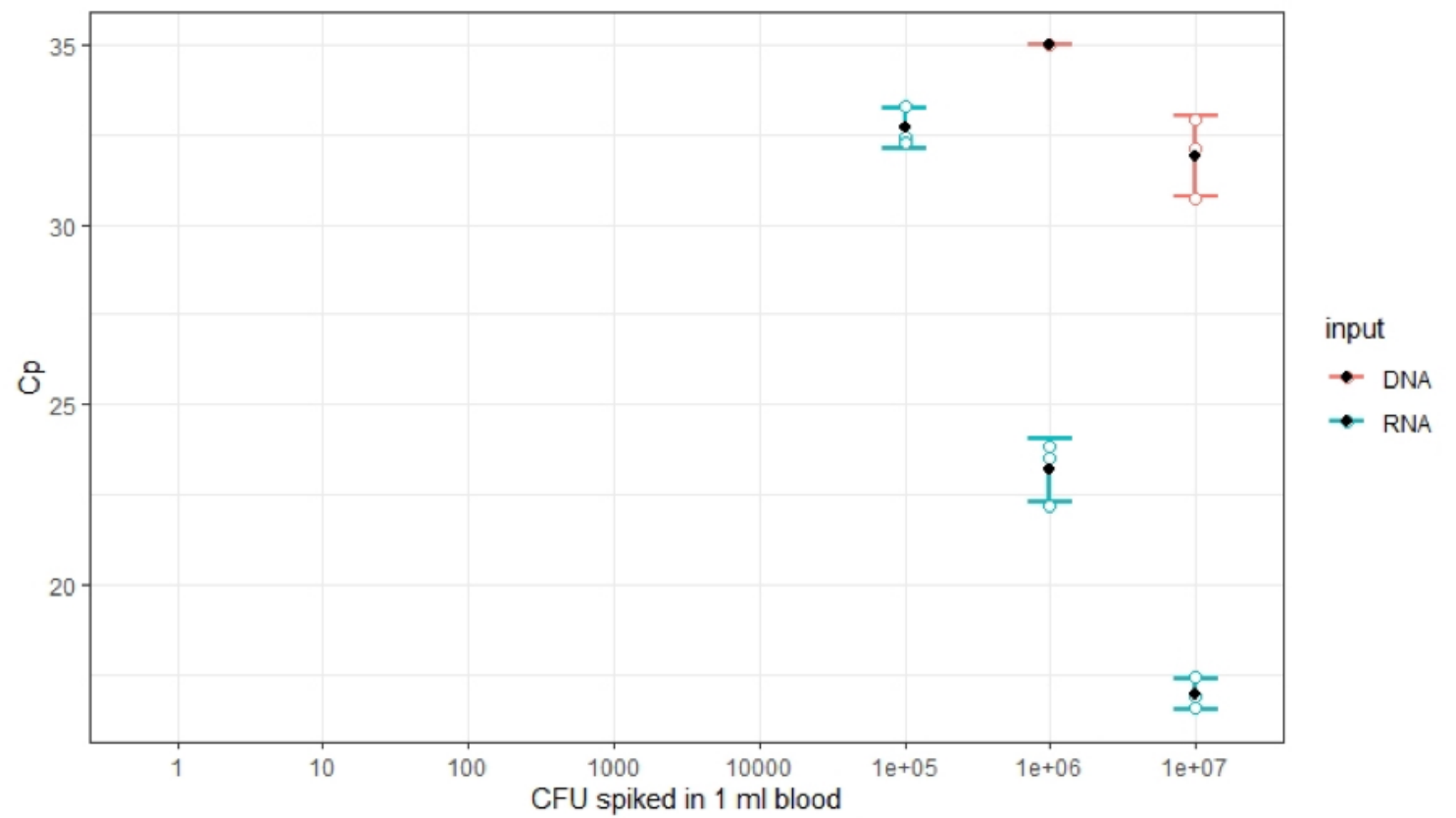


Figure 3

Limit of detection of 16S rRNA and 16S rDNA in 10-fold serial dilutions of Salmonella Typhimurium SL1344 in 1 ml human blood. Mean and standard deviation of the Cp values are represented by a closed black dot and a vertical error bar, respectively; overlaid by Cp values displayed as open red dots.

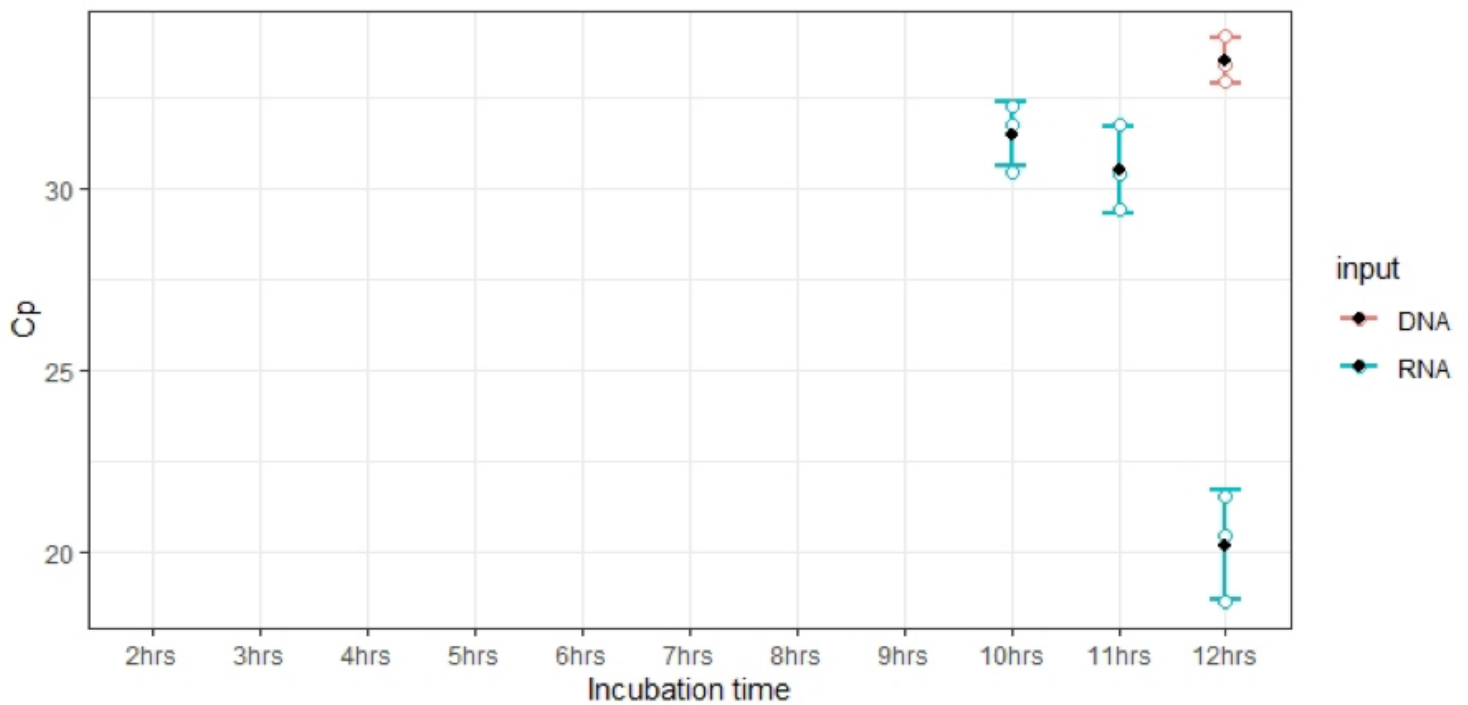


Figure 4

Limit of detection of 16S rRNA and 16S rDNA detection in time course measurements of blood cultures of 10 ml blood spiked with 5 CFU Salmonella Typhimurium SL1344 per ml blood. Mean and standard deviation of the Cp values are represented by a closed black dot and vertical error bar, respectively; overlaid by Cp values displayed as open dots.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile5.FigureS2.docx](#)
- [Additionalfile2.TableS1.xlsx](#)
- [Additionalfile3.TableS2.docx](#)
- [Additionalfile4.SupplementalinformationS1.rtf](#)
- [Additionalfile1.FigureS1.docx](#)