Ultra-Fast and Sensitive Detection of Non-Typhoidal Salmonella Using Microwave-Accelerated Metal-Enhanced Fluorescence (‘‘MAMEF’’)

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Abstract

Certain serovars of Salmonella enterica subsp. enterica cause invasive disease (e.g., enteric fever, bacteremia, septicemia, meningitis, etc.) in humans and constitute a global public health problem. A rapid, sensitive diagnostic test is needed to allow prompt initiation of therapy in individual patients and for measuring disease burden at the population level. An innovative and promising new rapid diagnostic technique is microwave-accelerated metal-enhanced fluorescence (MAMEF).

We have adapted this assay platform to detect the chromosomal oriC locus common to all Salmonella enterica subsp. enterica serovars. We have shown efficient lysis of biologically relevant concentrations of Salmonella spp., suspended in bacteriological media using microwave-induced lysis. Following lysis and DNA release, as little as 1 CFU of Salmonella in 1 ml of medium can be detected in <30 seconds. Furthermore the assay is sensitive and specific: it can detect oriC from Salmonella serovars Typhi, Paratyphi A, Paratyphi B, Paratyphi C, Typhimurium, Enteritidis and Choleraesuis but does not detect Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae or Acinetobacter baumanii. We have also performed preliminary experiments using a synthetic Salmonella oriC oligonucleotide suspended in whole human blood and observed rapid detection when the sample was diluted 1:1 with PBS. These pre-clinical data encourage progress to the next step to detect Salmonella in blood (and other ordinarily sterile, clinically relevant body fluids).

Introduction

Salmonella, a genus of more than 2500 serological variants (serovars), includes many organisms that can cause human disease. Salmonella enterica subsp. enterica Typhi and S. Paratyphi A and B, the typhoidal serovars, cause, respectively, typhoid and paratyphoid fevers (enteric fevers), febrile illnesses characterized by infection of the gut-associated lymphoid tissue, liver, spleen, bone marrow and gall bladder and accompanied by a low level bacteremia [1]. Non-typhoidal Salmonella (NTS) generally produce a self-limited gastroenteritis (vomiting, fever and diarrhea) in healthy humans [2-4]. By contrast, in young infants, the elderly and immunocompromised hosts, NTS can cause severe, fatal disease in both industrialized [4,5] and developing countries [2,6-12]. Culture-based surveillance for invasive bacterial infections in sub-Saharan Africa have shown that NTS rival Haemophilus influenzae type b (Hib) and Streptococcus pneumoniae infections in their frequency and severity [6,9,10,12-18]. Incidence rates of 200-350 cases of invasive NTS infections/105 infections in infants and toddlers have been recorded with case fatality rates of 20-30% [10,13,14,16].

The most common serovars isolated from blood in the USA are S. Typhimurium (24%), S. Enteritidis (19%) and S. Heidelberg (15%) [19]. S. Typhimurium and S. Enteritidis are also the most commonly NTS serovars isolated from blood and other normally sterile sites from patients in Europe [20,21] and the United Kingdom [22]. These two serovars, S. Typhimurium and S. Enteritidis, are particularly prominent in sub-Saharan Africa, where they account for 80-90% of all invasive NTS [6,9,10,12-17].

Invasive Salmonella spp. are routinely detected by standard blood culture techniques. Culturing blood specimens has become much faster and easier, since the advent of continuously monitoring blood culture instruments such as the BACTEC 9000 systems (Becton Dickinson, Cockeysville, MD, USA) and BacTAlert (BioMerieux, Durham, NC, USA). However, it still takes several days to detect and identify Salmonella [23,24]. Due to the time required for blood culture identification and the fact that many diagnostic laboratories are unable to serotype Salmonella spp. themselves, alternative methods of identification of Salmonella are being sought [25]. In particular, DNA detection methods such as the polymerase chain reaction (PCR) have been investigated. The food industry routinely uses PCR to detect Salmonella in food [26,27]. There are many reports of PCR primers designed to detect S. Typhi from the blood of enteric fever patients [28-36]. Furthermore, the sensitivity of PCR has often been found to be higher than that of blood culture [30,31,33,34]. However, PCR...
has not yet become an established method for diagnosis of typhoid fever [25]. One reason for this may be that although some reports claim high sensitivity, with detection of as few as 10 CFU/ml of blood [35], Wain et al.’s prospective study of the concentration of S. Typhi in blood of typhoid fever patients showed a median value of 0.3 (range of 0.1 to 399) CFU/ml, well below current PCR-based detection limits [37]. Interestingly, in another study, Wain et al. [38] showed that 65% of the S. Typhi cells were located in the buffy coat layer (presumably in monocytes and polymorphonuclear leukocytes) and the mean number of bacteria per infected leukocyte was 1.3 CFU/cell. These quantitative studies of Wain et al. corroborate a classic early study of Watson [39], who showed a median of 6 CFU/ml of S. Typhi in 15 patients with typhoid fever. Gordon et al. [40] have shown that NTS in bacteremic patients are present at a similarly low concentration (1 CFU/ml).

A promising new rapid diagnostic technique is microwave-accelerated metal-enhanced fluorescence (MAMEF) [41,42], which integrates metal-enhanced fluorescence using surface deposited silver nanoparticles (to amplify fluorescence signatures) with low power microwave heating (to accelerate biomolecular recognition events kinetically). MAMEF has detected DNA from Bacillus anthracis spores and vegetative cells within 1 minute, which included a ~30 second spore lysing and sample preparation time [43,44]. The target was a highly conserved region within the gene encoding protective antigen (PA) [45]. The process was accelerated by using low-power microwave heating. MAMEF has also detected DNA from less than 100 CFU/ml of Chlamydia trachomatis in 40 seconds [45]. MAMEF is an ultra-fast, sensitive, and specific assay, using relatively simple but cost-effective technology that can be performed in a 96-well plate [46] and that can be multiplexed [47].

There is a pressing need for a sensitive and specific rapid diagnostic test to detect NTS bacteremia. In this paper, we describe adaption of the ultra-fast, highly sensitive MAMEF technology to detect the oriC locus, that we currently use as a Salmonella-specific target in PCRs [11], of Salmonella spp. which have been rapidly lysed by focused microwave radiation. This work establishes the proof of concept that MAMEF can be used to detect Salmonella directly from blood.

**Methods**

**Bacterial strains, blood and genomic DNA**

We constructed an attenuated Salmonella Enteritidis strain CVD 1940 (pGEN206) carrying a deletion in the chromosomal guaB1 operon of wild-type strain R11 and also carrying plasmid pGEN206 for developing and optimizing microwave lysis and the MAMEF assay (unpublished results). CVD 1940 is unable to synthesize guanine nucleotides and is highly attenuated, therefore the MAMEF assay (unpublished results). CVD 1940 (pGEN206) carrying a deletion in the chromosomal oriC locus, that we currently use as a Salmonella-specific target in PCRs [11], of Salmonella spp. which have been rapidly lysed by focused microwave radiation. This work establishes the proof of concept that MAMEF can be used to detect Salmonella directly from blood.

**Detection of Non-Typhoidal Salmonella**

Glass microwave slides were covered with a mask (12.5 mm in size with a 1 mm gap between two triangles), leaving a bowtie region exposed. Equilateral gold triangles of 12.5 mm were subsequently deposited onto glass microscope slides through the mask using a BOC Edwards 306 vacuum deposition with vacuum 3.0 x 10^-5 Torr, with a deposition rate of ~1 A/s. Four layers of self-adhesive silicon isolators (D 2.5 mm) were placed over the gold bowtie region to create a sample well (see Figure 1A).

**Measurement of temperature over the gold bowtie**

Since sapphire transmits infrared radiation, it is an ideal substrate for thermal imaging experiments. A slide coated with gold triangles and containing water in the sample well was covered by a sapphire plate. Using this sapphire and metal sandwich configuration, we determined the average temperature increase of the water in proximity to the metal. The optical configuration consisted of a microwave cavity with a 1 inch diameter opening at the base, a gold mirror, and a thermal imaging camera (Silver 420 M; Electrophysics Corp, Fairfield, NJ, USA) that was equipped with a lens that provided a resolution of approximately 300 μm (Figure S1). The clear sapphire plate of the sandwich geometry was fixed to the base of the microwave cavity opening. A gold mirror was positioned such that the image of the opening was reflected onto the thermal camera. Thermal imaging data was recorded at 100 frames/second before, during and after the application of microwave pulses.

**Lysis by microwave radiation**

Bacteria were lysed using gold bowtie deposits on a glass slide and heating in a GE microwave Model No. JE2160BF01, kW:1.65 (M/W), for 15 seconds on maximum power. 2 ml bacterial suspensions were placed into wells sterilized by mixing with 70% ethanol and air-
Detection of Non-Typhoidal Salmonella

Figure 1. Lysis of S. Enteritidis. (A) Gold lysing triangles with bowtie configuration. (B) Lysis of biologically relevant concentrations of CVD 1920 (pGEN206). 2 CFU/ml to 1.3 x 10^4 CFU/ml were lysed using gold lysing triangles and heated for 13 s on high power in a GE microwave. (C) Agarose gel of DNA released from lysed CVD 1920 (pGEN206). An overnight culture of bacteria (1.9 x 10^10 CFU/ml) was lysed using gold lysing triangles and microwave radiation. Lane 1, 1 kb Plus DNA ladder (Invitrogen); 2, lysed bacteria; 3, unlysed bacteria. (D) Transmission electron micrographs of lysed and unlysed CVD 1920 (pGEN206). Bar = 500 nm.

drying. Overnight cultures of bacteria were diluted to the required concentration in APF-LB and lysed. Viable counts of unlysed samples were performed by spread-plating up to 200 µl of bacterial suspension on agar plates or by using the pour plate method (5 ml of the bacterial suspension was added to 20 ml of molten agar and poured into a petri dish). Viable counts of lysed samples were performed by spread-plating 100-200 µl on APF-LB and incubating the remaining suspension at 37°C overnight. A sample was considered to be fully lysed if the broth showed no turbidity.

Transmission Electron Microscopy (TEM)

TEM images of lysed bacteria were taken using an Electron Microscope Tecnai T12 microscope. Samples were drop cast onto Formvar carbon films on copper grids (400 mesh) by placing a droplet of a 10-µl aqueous sample solution on a grid. The grid was air-dried for 24 h.

Formation of silver island films (SiFs) on glass substrates

SiFs, which provide the metal component of ‘metal-enhanced fluorescence’, were prepared as previously published [48]. In a typical SiFs preparation, a solution of silver nitrate (0.5 g in 60 ml of deionized water) was put in a clean 100 ml glass beaker. 200 µl of freshly prepared 5% (w/v) sodium hydroxide solution and 2 ml of ammonium hydroxide were added to a continuously stirred silver nitrate solution at room temperature. Subsequently, the solution was cooled to 5°C by placing the beaker in an ice bath, followed by soaking the Silane-pretP™ glass slides in the solution and adding a fresh solution of D-glucose (0.72 g in 15 ml of water). The temperature of the mixture was then allowed to warm to 40°C. As the color of the mixture turned from yellow-green to yellowish brown, the slides were removed from the mixture, washed with water, and sonicated for 1 min at room temperature.

Anchor and fluorescent probes

We have successfully used previously published primers to detect oriC of Salmonella in several PCR-based assays. Probes specific for the oriC locus of Salmonella spp. were designed using the oriC sequence from S. Typhimurium LT2 (GenBank accession no. AE006460). Anchor probe (5’ GTTTT-TCAACCTGTGTTTGCGCC 3’), fluorescent probe (5’ CTTTC-AGTTCCGCTTGTAT 3’) and a synthetic target oligonucleotide (5′ATAGAAGCGGAAGAAAGCCTGGGGGCAAAACAGTGGTTG 3′) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 16 nucleotides of the anchor probe bind to the oriC target. The remaining nucleotides consist of a guanine to which a thiol group is added and 5 T’s for flexibility of the probe following binding to the glass slide. The fluorescent probe possesses a TAMRA dye attached to the first nucleotide at the 5′ end.

Preparation of MAMEF assay platform for detection of Salmonella DNA

SiFs-deposited glass slides were coated with self-adhesive silicon isolators, containing oval wells (2.0 mm D x 32 mm L x 19 mm W) prior to the assay fabrication and subsequent fluorescence experiments. 1 µM of thiolated anchor probe was incubated overnight at 4°C on the surface of SiFs-deposited glass slides in 1 x TE Buffer. The thiolated anchor probe was covalently linked to SiFs via well-established self-assembled monolayer chemistry [50].

MAMEF-based Salmonella DNA assays

The MAMEF-based DNA capture assay was performed using 1 ml lysed Salmonella mixed with 1 ml TAMRA-labeled fluorescent probe overlayed onto the SiFs containing bound anchor probe and incubated for 30 seconds in a microwave cavity (a 0.7 cu ft, GE Compact Microwave Model: JES735BF, max power 700 W). The power setting of the microwave cavity was set to 2 (which corresponded to 140 W over the entire cavity).

Fluorescence Spectroscopy

Fluorescence emitted by the MAMEF-based DNA capture assay was measured using a 532 nm diode laser and a Fiber Optic Spectrometer (HD2000) from Ocean Optics, Inc. by collecting the emission intensity through a notch filter (532 nm).

Results

Lysis of Salmonella

We tested a variety of configurations of gold deposited on glass slides and found that the gold bowtie configuration was the best in terms of its ability to effectively lyse Salmonella when heated in a microwave (Figure 1A). Figure S1 shows that the temperature of liquid at the point where the two triangles apex is raised during
microwave heating. Overnight cultures of S. Enteritidis CVD 1920 (pGEN206) were diluted to biologically relevant concentrations (up to 10^4 CFU/ml) in APF-LB and 2 ml were lysed by microwave radiation. As can be seen in Figure 1B, a range of 2 CFU/ml to 1.3×10^5 CFU/ml could be efficiently lysed (90%±5% lysis; mean ± standard deviation) using gold lysing triangles and heating in a microwave. DNA released from an overnight culture (1.9×10^10 CFU/ml) of CVD 1920 (pGEN206) lysed using gold lysing triangles and a microwave was fragmented into a range of sizes with most of the fragments around 100 bp, as shown in Figure 1C. Examination of lysed bacteria by electron microscopy showed bacteria with blurred edges surrounded by clumps of lysed debris; bacteria from unlysed samples showed distinct edges against a clear background (Figure 1D).

**Detection of a synthetic oriC oligonucleotide by MAMEF**

As mentioned earlier, oriC is used as a target in several *Salmonella* PCRs [11,51,52]. Figure S2 shows the primer binding sites in *S. Typhimurium* LT2 (GenBank Accession No. AE006468) of two different oriC primer pairs, as well as the location in bold of the segment of DNA targeted by our fluorescent and anchor probes. This oriC target DNA is conserved in all serovars of *Salmonella enterica* subsp. *enterica* except for *S. Agona*, *S. Gallinarum* and *S. Dublin* (Figure S2). We therefore designed anchor and fluorescent probes so that they can detect all *Salmonella enterica* subsp. *enterica* serovars including *S. Agona*, *S. Gallinarum* and *S. Dublin* (Figure S2). We therefore designed anchor and fluorescent probes so that they can detect all *Salmonella enterica* subsp. *enterica* serovars including *S. Agona*, *S. Gallinarum* and *S. Dublin*. Figure 2 shows a schematic diagram of the anchor and fluorescent probes binding to the target DNA. We first tested the ability of the probes to detect a synthetic oriC at concentrations ranging from 500 nM all the way down to 0.5 nM.

**Detection of DNA released from lysed Salmonella**

Once lysis and detection were optimized, we performed the two methods sequentially to detect DNA using MAMEF from *Salmonella* that had been lysed by microwave radiation. Overnight cultures of CVD 1920 (pGEN206) were diluted to biologically relevant concentrations (up to 10^5 CFU/ml) in APF-LB and 2 ml were lysed by microwave radiation. 1 ml of the lysed bacteria was tested by MAMEF. We tested serial dilutions of two independent samples, with each dilution lysed separately. The first consisted of 10-fold serial dilutions of a 10^3 CFU/ml suspension and lysis results are shown in Figure 4A. The second dilution series consisted of 2-fold serial dilutions of a 12 CFU/ml suspension, with results shown in Figure 4B. Once again, the intensity of the fluorescence signal was concentration dependent and we were able to detect *S. Enteritidis* at concentrations down to 1 CFU/ml (Figure 4B).

**Specificity of the Salmonella MAMEF assay**

To demonstrate that the assay is specific for detecting only *Salmonella* DNA, we tested DNA from a variety of *Salmonella* serovars and other bacteria commonly isolated from blood. Sixteen *Salmonella* strains of various serovars were suspended in APF-LB media at 10^3–10^4 CFU/ml and 2 ml of each suspension were lysed using microwave radiation; every strain was lysed (93%±16%, mean ± standard deviation). 1 ml of the lysed sample was then assayed by MAMEF and a fluorescent signal was observed for every strain tested (Table 1). When 2 ml of 10^3 CFU/ml suspensions of *E. coli*, *P. aeruginosa* and *K. pneumoniae* were lysed by microwave lysis and tested by MAMEF, no fluorescence signal was observed. We also tested genomic DNA (diluted to 100 pg/ml) from these 3 species as well as DNA from *S. pneumoniae*, *H. influenzae* and *A. baumanii* and did not observe any detection (Table 1).

**Detection of Salmonella DNA in blood**

The overall goal of our research efforts is to detect *Salmonella* directly from blood. We have performed some preliminary experiments using blood spiked with the synthetic oriC oligonucleotide and were not able to observe fluorescence (Figure 5). However, when the spiked blood sample was diluted with an equal volume of PBS, the synthetic oriC target was readily detected by MAMEF.

**Discussion**

We have developed a MAMEF-based *Salmonella* assay capable of lysing and detecting 1 CFU suspended in 1 ml of bacteriological medium. The time to detection (excluding processing time) was only 30 seconds. This level of speed and detection limit greatly surpasses all currently available assays. For example, Nga et al. [53] have recently described a multiplex real-time PCR assay that targets *S. Typhi* and *S. Paratyphi* A. The sensitivity of the assay on blood samples was low with only 42% sensitivity for *S. Typhi* and 39% sensitivity for *S. Paratyphi* A. This poor sensitivity is most likely due to the poor detection limit of the assay. The authors prepared serial dilutions of *S. Typhi* in blood and observed detection limits of 250 CFU/ml for their real-time assay and 25 CFU/ml for detection by blood culture. The authors concede that identification of invasive *Salmonella* by PCR is not a practical approach.

Sensitivity of nucleic acid-based detection can be markedly increased by introducing an incubation step but only at the cost of greatly increasing the duration of the assay and its adaptation to becoming a point of care diagnostic test. For example, Zhou and Pollard [54] overcame low sensitivity due to small sample volumes by including a 3-hour incubation step in tryptone soya broth containing 2.4% ox bile, prior to detection of *S. Typhi* by PCR. They were able to achieve a detection limit of 0.75 CFU per ml of blood. However, the entire protocol takes almost 8 hours to complete. Moreover, the need for an incubation step in culture media means that this is no longer amenable to being a point of care diagnostic test.

![Figure 2. Schematic representation of anchor and fluorescent probes binding to Salmonella oriC target DNA.](https://example.com/figure2.png)

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Similarly, by incorporating an overnight enrichment step, a fluorescence in situ hybridization (FISH) method for the detection of Salmonella spp. using a novel peptide nucleic acid (PNA) probe has achieved 100% sensitivity and 100% specificity and can detect 1 CFU per 10 ml of blood [55]. Because of the lengthy (overnight) enrichment step, this assay is also neither rapid nor adaptable to become the much needed point of care diagnostic for invasive Salmonella disease.

One molecular assay that does not require an enrichment step is the Lightcycler SeptiFast Test MGRADE kit (Roche Diagnostics, Germany), which is a commercial real-time PCR assay. This kit detects and identifies the 25 most common pathogens known to cause bloodstream infections directly from whole blood in <6 hours but does not target Salmonella [56]. In serial experiments performed on EDTA-blood samples spiked with different concentrations of bacterial and fungal reference organisms, hit rates of 70–100% were achieved for 23 out of 25 organisms at 30 CFU/ml, but for only 15 out of 25 organisms at 3 CFU/ml. These results suggest that the assay may not be as sensitive as blood culture which has a theoretical sensitivity of 1 CFU.

However, two studies indicate that the Lightcycler SeptiFast is more sensitive than blood culture, as it was able to detect target DNA in several samples which were negative by blood culture [57,58]. Disadvantages of this method are that it includes a sample preparation step that requires the use of a centrifuge and the time taken to detection.

Recently, a MAMEF method showing detection of biotinylated BSA (b-BSA) was used to establish that MAMEF can be used to detect targets in complex biological samples such as blood [59]. In this study, phosphate buffer containing b-BSA was mixed with whole blood in a 1:1 ratio, and the target protein was detected using fluorophore-labeled avidin in 1 min. These findings and our own results show that detection of protein and DNA targets in blood is possible though presently the blood needs to be diluted to enable detection. We are currently investigating various methods to lyse red and white blood cells to reduce some of the viscosity of the liquid which we believe is impeding mass transport of the biological components to the surface during microwave-acceleration, and therefore overall fluorescence detection, and to release bacteria within the white blood cells.

Figure 3. Detection of various concentrations of a synthetic oriC oligonucleotide by MAMEF. (A) The graph shows the increase in intensity as the concentration of oligonucleotide increases. (B) The actual fluorescent signal produced at each concentration. Excitation = 532 nm, Emission = 575 nm.
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Figure 4. Detection of DNA released from microwave-lysed CVD 1920 (pGEN206) suspended in bacteriological media. Serial dilutions from two independent samples were examined: A) 10-fold serial dilutions of a 10^3 CFU/ml suspension and B) 2-fold serial dilutions of a 12 CFU/ml suspension.
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The key points of the MAMEF technology that make it attractive for detection of pathogens in blood include: 1) it is a rapid and highly sensitive method; 2) the technology has been multiplexed, so that in one sample well, three DNA or protein targets can presently be identified within 20–40 seconds; 3) detection of well fluorescence can be accomplished by a variety of standard inexpensive sample well-reader technologies; 4) the assay platform requires no washing steps whatsoever to remove excess fluorescent probe or labeled DNA/antibody; 5) chambers that are disposable to minimize cross-contamination; 6) absence of centrifugation steps; and 7) the assay can be made quantitative by comparing levels of fluorescence to a standard curve. These attributes make it possible for the assay to be developed ultimately into a point-of-care device that can be used by people with minimal training.

The novel findings of this study are: 1) we have developed a sensitive and specific MAMEF-based *Salmonella* assay; 2) we have shown detection in 1 ml sample volumes which is greater than previously reported (and which provides the proof-in-principle that large volumes can be tested by MAMEF); 3) we can lyse and detect *Salmonella* without any centrifugation or washing steps; and 4) detection in blood is possible. We anticipate that we will be able to develop a multiplex MAMEF-based *Salmonella* assay that can efficiently detect the chromosomal oriC from blood-borne *Salmonella* and further determine whether the serovar is *S*. Typhimurium or *S*. Enteritidis, the two most commonly isolated NTS from invasive sites.

### Supporting Information

#### Figure S1 Thermal imaging of gold bowties.

A) Optical scheme for thermal imaging of gold bowties and sapphire sample geometries. B) Temperature of water at the apex of the gold bowtie triangles over time. The graph shows the mean intensity temperature over a 100x100 pixel region.

#### Figure S2 Location of oriC primer binding sites and MAMEF target DNA in the *S*. Typhimurium genome.

Bold, segment of *S*. Typhimurium LT2 DNA (5’ to 3’) targeted by *Salmonella* MAMEF assay; underlined, primer binding sites of oriC primers described by Woods et al. [52]; and italicized, Table 1. Detection of *Salmonella* and non-*Salmonella* strains that are commonly found in blood.

| Species/serovar | Number of strains tested | Method of DNA extraction | Detection |
|-----------------|--------------------------|--------------------------|-----------|
| *Salmonella* enterica subsp. enterica serovars |
| Typhi           | 2                        | Microwave lysis          | +         |
| Paratyphi A     | 2                        | Microwave lysis          | +         |
| Paratyphi B     | 2                        | Microwave lysis          | +         |
| Paratyphi C     | 1                        | Microwave lysis          | +         |
| Typhimurium     | 2                        | Microwave lysis          | +         |
| Enteritidis     | 2                        | Microwave lysis          |           |
| Dublin          | 2                        | Microwave lysis          | +         |
| Choleraesuis (sensu stricto) | 1 | Microwave lysis | +         |
| Choleraesuis var. Kunzendorf | 1 | Microwave lysis | +         |
| Newport         | 1                        | Microwave lysis          | +         |
| Non-Salmonella  |
| E. coli         | 2                        | Microwave lysis and genomic DNA isolation | --* |
| P. aeruginosa   | 1                        | Microwave lysis and genomic DNA isolation | --* |
| K. pneumoniae   | 1                        | Microwave lysis and genomic DNA isolation | --* |
| S. pneumoniae   | 4                        | Genomic DNA isolation    | --* |
| H. influenzae   | 2                        | Genomic DNA isolation    | --* |
| A. baumannii    | 2                        | Genomic DNA isolation    | --* |

*Genomic DNA was assayed in 100 µl wells.

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*Figure 5. MAMEF-based detection of a synthetic oriC oligonucleotide suspended in whole human blood. Whole human blood was spiked with the oriC oligonucleotide (250 nM) and tested by MAMEF or diluted 1:1 with PBS and then tested by MAMEF.*

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References

1. Leverin MM (2004) Typhoid Fever Vaccines. In Plotkin SA, Orenstein WA, eds. Vaccines. Philadelphia: Saunders. pp 1057–1093.

2. Adak GK, Long SM, O'Brien SJ (2002) Trends in indigenous foodborne disease and deaths, England and Wales. 1992 to 2000. GMS 51: 832–841.

3. Voetsch AC, Van Gelder TJ, Angulo FJ, Farley MM, Shaffer S, et al. (2004) FoodNet estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States. Clin Infect Dis 38 Suppl 1: S127–S134.

4. Vugia DJ, Samuel M, Farley MM, Marcus R, Shiferaw B, et al. (2004) Invasive Salmonella infections in the United States. FoodNet, 1996–1999: incidence, serotype distribution, and outcome. Clin Infect Dis 38 Suppl 1: S149–S156.

5. Kennedy M, Villar R, Vugia DJ, Rabahsky-Ehr T, Farley MM, et al. (2004) Hospitalizations and deaths due to Salmonella infections, FoodNet, 1996–1999. Clin Infect Dis 38 Suppl 3: S142–S148.

6. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, et al. (2005) Bacteremia among children admitted to a rural hospital in Kenya. N Eng J Med 352: 391–397.

7. Graham SM, Molyneux EM, Walsh AL, Cheesborough JS, Molyneux ME, et al. (2006) Nontyphoidal Salmonella infections of children in tropical Africa. Pediatr Infect Dis J 19: 1189–1196.

8. Hill PC, Onyewu CO, Iminniola AO, Ojefuru UC, Adediran AO, et al. (2013) Molecular epidemiology of community-acquired invasive non-typhoidal Salmonella infections among children aged 2–29 months in rural Ghana and discovery of a new serovar, Salmonella enterica Meningitis. J Med Microbiol 56: 1479–1489.

9. Kariuki S, Revathi G, Kariuki N, Kiuru J, Mwiti S, et al. (2006) Characterisation of community acquired non-typhoidal Salmonella from bacteremia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. BMC Microbiol 6: 101.

10. Levy H, Dallo S, Tennant SM, Livio S, Sos SO, et al. (2008) PCR method to identify Salmonella enterica serovars Typhi, Paratyphi A, and Paratyphi B among Salmonella isolates from the blood of patients with clinical enteric fever. J Clin Microbiol 46: 1860–1866.

11. Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME. (2000) Bacteremia in febrile Malawian children: clinical and microbiologic features. Pediatr Infect Dis J 19: 512–518.

12. Brent AJ, Oundo JO, Mwangi I, Williams T, Bauni E, et al. (2006) Salmonella bacteremia in Kenyan children. Pediatr Infect Dis J 25: 230–236.

13. Graham SM, Walsh AL, Molyneux EM, Phiri AJ, Molyneux ME. (2000) Clinical presentation of non-typhoidal Salmonella bacteremia in Malawian children. Trans R Soc Trop Med Hyg 94: 310–314.

14. Lepage P, Borgaerts J, Van Goethem C, Nahornuta M, Nseungnumuyem F, et al. (1987) Community-acquired bacteremia in African children. Lancet 1: 1458–1461.

15. Mandomando I, Maitere E, Sigaqwe B, Morais I, Quinto L, et al. (2006) Invasive non-typhoidal Salmonella in Mozambican children. Trop Med Int Health 14: 1467–1474.

16. O'Dempsey TJ, McARDLE TF, Lloyd-Evans N, Baldeh I, Laurence ET, et al. (1994) Importance of enteric bacteria as a cause of pneumonia, meningitis and septicaemia among children in a rural community in The Gambia, West Africa. Pediatr Infect Dis J 13: 122–129.

17. Sigaqwe B, Roca A, Mandomando I, Morais I, Quinto L, et al. (2009) Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J 28: 108–113.

18. Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, et al. (2004) Salmonellosis outcomes differ substantially by serotype. J Infect Dis 190: 109–114.

19. Grant KO, Schuurhuyzen HC, Pedersen L, Thommen RW, Noergaard M, et al. (2006) Incidence and prognosis of non-typhoid Salmonella bacteremia in Denmark: a 10-year county-based follow-up study. Eur J Clin Microbiol Infect Dis 25: 151–158.

20. Papavangeliou V, Syroupolou V, Carassiaidou A, Pangalis A, Mostrous G, et al. (2007) Salmonella bacteremia in a tertiary children's hospital. Scand J Infect Dis 39: 547–551.

21. Threlfall EJ, Hall ML, Rowe B (1992) Salmonella bacteremia in England and Wales, 1981–1990. J Clin Pathol 45: 34–39.

22. Kresier BS, Wood GS, GL (1999) Times to detection of bacteria and yeasts in BACTEC 9240 blood culture bottles. J Clin Microbiol 37: 2024–2026.

23. Durum G, Us T, Aydini A, Kiremenii A, Kiraz N, et al. (2005) Optimum detection times for bacteria and yeast species with the BACTEC 9240 aerobic blood culture system: evaluation for a 5-year period in a Turkish university hospital. J Clin Microbiol 41: 819–821.

24. Wain J, Horsgul S (2008) The laboratory diagnosis of enteric fever. J Infect Developing Countries 2: 421–425.
the highly virulent serovars Choleraesuis and Paratyphi C. J Clin Microbiol 46: 4018–4022.

53. Nga TV, Karkey A, Dongol S, Thuy HN, Dunstan S, et al. (2010) The sensitivity of real-time PCR amplification targeting invasive Salmonella serovars in biological specimens. BMC Infect Dis 10: 125.

54. Zhou L, Pollard AJ (2010) A fast and highly sensitive blood culture PCR method for clinical detection of Salmonella enterica serovar Typhi. Ann Clin Microbiol Antimicrob 9: 14.

55. Almeida C, Azevedo NF, Fernandes RM, Keevil CW, Vieira MJ (2010) Fluorescence in situ hybridization method using a peptide nucleic acid probe for identification of Salmonella spp. in a broad spectrum of samples. Appl Environ Microbiol 76: 4476–4483.

56. Lehmann LE, Humfeld KP, Emrich T, Haberhausen G, Wissing H, et al. (2008) A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. Med Microbiol Immunol 197: 313–324.

57. Mancini N, Clerici D, Diotti R, Perotti M, Ghidoli N, et al. (2008) Molecular diagnosis of sepsis in neutropenic patients with haematological malignancies. J Med Microbiol 57: 601–604.

58. Paolucci M, Capretti MG, Dal MP, Corvaglia L, Landini MP, et al. (2009) Laboratory diagnosis of late-onset sepsis in newborns by multiplex real-time PCR. J Med Microbiol 58: 533–534.

59. Aslan K (2010) Rapid whole blood bioassays using Microwave-Accelerated Metal-Enhanced Fluorescence. Nano Biomed Eng 2: 1–9.