

The diagnostic accuracy of three rapid diagnostic tests for typhoid fever at Chittagong Medical College Hospital, Chittagong, Bangladesh

Rapeephan R. Maude¹, Hanna K. de Jong^{2,3}, Lalith Wijedoru¹, Masako Fukushima⁴, Aniruddha Ghose⁵, Rasheda Samad⁵, Mohammed Amir Hossain⁵, Mohammed Rezaul Karim⁵, Mohammed Abul Faiz^{1,6} and Christopher M. Parry^{1,4,7,8} on behalf of CMCH Typhoid Study Group*

1 Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

2 Department of Internal Medicine, Division of Infectious Diseases and Center for Infection and Immunity Amsterdam (CINIMA), Amsterdam, The Netherlands

3 Center for Experimental Molecular Medicine (CEMM), Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

4 Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

5 Chittagong Medical College Hospital, Chittagong, Bangladesh

6 Centre for Specialized Care and Research, Chittagong, Bangladesh

7 London School of Hygiene and Tropical Medicine, London, UK

8 School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan

Abstract

OBJECTIVE To determine the diagnostic accuracy of three rapid diagnostic tests (RDTs) for typhoid fever in febrile hospitalised patients in Bangladesh.

METHODS Febrile adults and children admitted to Chittagong Medical College Hospital, Bangladesh, were investigated with Bact/Alert[®] blood cultures and real-time PCR to detect *Salmonella enterica* Typhi and Paratyphi A and assays for *Rickettsia*, leptospirosis and dengue fever. Acute serum samples were examined with the LifeAssay (LA) Test-it[™] Typhoid IgM lateral flow assay detecting IgM antibodies against *S. Typhi* O antigen, CTKBiotech Onsite Typhoid IgG/IgM Combo Rapid-test cassette lateral flow assay detecting IgG and IgM antibodies against *S. Typhi* O and H antigens and SD Bioline line assay for IgG and IgM antibodies against *S. Typhi* proteins.

RESULTS In 300 malaria smear-negative febrile patients [median (IQR) age of 13.5 (5–31) years], 34 (11.3%) had confirmed typhoid fever: 19 positive by blood culture for *S. Typhi* (three blood PCR positive) and 15 blood culture negative but PCR positive for *S. Typhi* in blood. The respective sensitivity and specificity of the three RDTs in patients using a composite reference standard of blood culture and/or PCR-confirmed typhoid fever were 59% and 61% for LifeAssay, 59% and 74% for the CTK IgM and/or IgG, and 24% and 96% for the SD Bioline RDT IgM and/or IgG. The LifeAssay RDT had a sensitivity of 63% and a specificity of 91% when modified with a positive cut-off of $\geq 2+$ and analysed using a Bayesian latent class model.

CONCLUSIONS These typhoid RDTs demonstrated moderate diagnostic accuracies, and better tests are needed.

keywords typhoid fever, *Salmonella enterica* serovar Typhi, blood culture, real-time PCR, rapid diagnostic tests, diagnostic accuracy

Background

Typhoid (enteric) fever is a common infection in adults and children in Bangladesh [1–3]. Antimicrobial resistance to standard treatments for *Salmonella enterica* serovars Typhi and Paratyphi A infections is widespread

Dr R. R. Maude and Dr H. K. de Jong contributed equally to this work.

*Members of CMCH Typhoid Study Group are provided in Appendix 1.

[4, 5]. In routine practice, a diagnosis of typhoid fever is rarely confirmed as diagnostic tests are unavailable or have limited diagnostic accuracy. Blood culture is the commonest reference standard test but has an estimated sensitivity of between 40% and 80% [6]. Blood culture sensitivity is limited by low number of bacteria circulating in the blood, small volumes of blood taken for culture and prior antimicrobial therapy [7]. Bone marrow culture is more sensitive than blood culture, because of the higher number of bacteria present, and can remain positive several days after effective antimicrobial treatment has started, yet is rarely used except in a research context [8, 9]. Nucleic acid amplification methods are available but demonstrate variable sensitivities and specificities [6, 10]. The Widal test is still used but as it is based on cross-reactive antigens, it lacks sensitivity and specificity [11, 12].

Clinicians often use rapid diagnostic tests (RDTs) to diagnose typhoid. A number of typhoid fever RDTs are commercially available but have variable performance [6, 13]. We have measured the diagnostic accuracy of three commercially available typhoid RDTs as part of a fever study conducted at Chittagong Medical College Hospital (CMCH) in Bangladesh. We used Bayesian latent class models (LCM) to evaluate the results in the absence of an adequate reference standard [14–16].

Patients and methods

Ethics statement

The study was conducted according to the principles expressed in the Declaration of Helsinki. The Bangladesh National Research Ethical Committee (BMRC/NREC/2010-2013/1543), the Chittagong Medical College Hospital Ethical Committee, the Oxford Tropical Research Ethics Committee (Oxtrec 53-09) and the Research Ethics committee of the Liverpool School of Tropical Medicine gave ethical approval for the study. Informed written or thumbprint consent was taken from the subject, their parent or caretaker for all cases and controls.

Study site and patients

Chittagong Medical College Hospital is a 1000-bed teaching hospital serving the population of Chittagong and surrounding province. Adults and children (age > 6 months) consecutively admitted to the adult and paediatric wards at CMCH with a documented axillary temperature of ≥ 38 °C in the period up to 48 h after

admission and history of fever of <2 weeks were eligible for entry to the study. We also recruited 38 healthy adult controls from among the hospital staff to determine background levels of antibodies, as determined by the RDTs, in this population. Study recruitment was between January and June 2012.

Clinical procedures

Demographic and clinical information was recorded on a study case report form at the time of study admission. Blood was collected at the time of study admission for complete blood count, renal function, aspartate transaminase (AST), alanine transaminase (ALT), blood culture, EDTA whole blood for PCR, serum for the RDTs and a malaria smear. The samples for PCR and the RDTs were frozen at -20 °C and tested later at Oxford Clinical Research Unit (OUCRU), Ho Chi Minh City, Vietnam. A diagnosis or differential diagnosis was proposed and recorded by the research team at the time of discharge or death including a judgment, based on the blood culture results and assessment by the research team, as to whether the clinical features, laboratory results and inpatient progress of the patient were consistent with typhoid fever. These features included the following: a febrile illness of >3 days duration, the presence of abdominal symptoms (abdominal pain, diarrhoea or constipation), a documented fever of ≥ 39 °C; hepatomegaly and/or splenomegaly, a low or normal white cell count, elevation of liver enzymes (AST, ALT) 2–3 times above the normal range, a slow defervescence with ceftriaxone treatment (the standard antimicrobial used for hospital admitted febrile patients); no alternative confirmed diagnosis established [17].

Demographic and clinical information from the healthy control subjects was also recorded on the study case report form and blood collected for complete blood count, EDTA whole blood for PCR, and serum for the RDTs.

Laboratory investigations and microbiology

A volume of blood, 5–12 ml in adults and 1–12 ml in children, was inoculated into a Bact/Alert® FA and PF blood culture bottles and incubated in the Bact/Alert® automated system (bioMérieux, Marcy l'Etoile, France) for 5 days. A Gram-stained smear was prepared from the broth of blood bottles that were culture positive and was subcultured onto 5% sheep blood agar and chocolate agar incubated in a candle jar and MacConkey agar incubated in air for 48 h (all media Oxoid, Basingstoke, UK).

Bacterial isolates were identified by standard methods including biochemical test using API test strips (bioMérieux) and agglutination with specific antisera (Bio-Rad, Hertfordshire, UK). All media and tests were subject to regular internal quality assessment. Bacterial isolates were stored on beads in glycerol at -80°C and later transferred to the OUCRU, Vietnam, for reconfirmation of their identification.

DNA extraction was performed at OUCRU, Vietnam, from the stored whole blood in EDTA using a QIAmp[®] DNA mini kit (QIAGEN, UK). The volume of blood used to extract DNA was usually 2 ml from adults and 1 ml from children. The real-time PCR method previously published by Nga *et al.* was followed using 25 μl reactions containing 5 μl of extracted DNA targeting *STY0201* (Putative fimbrial adhesion in *S. Typhi* CT18) or *SSPA2308* (hypothetical protein in SPA AKU-12601) for the detection of *S. Typhi* and *S. Paratyphi A* in blood [10]. Probe-based real-time PCR was also performed on DNA extracted from blood to detect *Leptospira* spp. [18], *Rickettsia typhi* [19] and *Orientia tsutsugamushi* [20]. Low-positive plasmid controls determined adequate detection limits of each assay. Serum was analysed by ELISA for dengue NS1 antigen using a PanBio Kit (PanBio, Australia) according to the manufacturer's instructions.

The Life Assay Test-it[™] Typhoid IgM (Life Assay Diagnostics (Pty), Cape Town, South Africa) is a lateral flow assay detecting IgM antibodies against *S. enterica* Typhi (ST) O antigen and takes 15 min to perform and read. The LifeAssay result for each sample was graded as recommended using the band intensity from 0 to 4+. The SD Biotline test (Standard Diagnostics Inc., Gyeonggi, Korea) uses an immunochromatographic method to visually and qualitatively detect IgM and IgG antibodies to serotype Typhi antigens (unspecified) that are indirectly labelled with colloidal gold (via an antibody). The antigen-bound antibodies are captured by anti-IgM or anti-IgG antibodies. The test takes 30 min to perform and read. The CTKBiotech Onsite Typhoid IgG/IgM Combo Rapid-test cassette (CTK Biotech, Inc., San Diego, CA, USA) is a lateral flow assay that detects IgG and IgM antibodies against recombinant O and H *S. Typhi* antigens. The test can be performed and read in 15 min. The SD Biotline test and CTKBiotech Onsite Typhoid IgG/IgM Combo kits are available and used in Chittagong diagnostic laboratories. The tests were performed on the stored serum at OUCRU, Vietnam, according to the manufacturer's instructions. The real-time PCR and RDTs were conducted blind to the other laboratory results including blood culture results and clinical data.

Analysis

The Standards for Reporting of Diagnostic Accuracy reporting guidelines were followed [21]. The sensitivity, specificity and predictive values with 95% confidence intervals were calculated. Laboratory-confirmed typhoid cases were defined if the patient's blood culture and/or blood PCR was positive for Typhi or Paratyphi A (composite reference standard positive). Cases that were not typhoid were defined if the patient's blood culture and blood PCR was negative for Typhi or Paratyphi A (composite reference standard negative).

Analysis was performed using SPSS version 22 (IBM SPSS Statistics, Chicago, IL, USA). The Bayesian LCM as described by Dendukuri and Joseph [22] was used to approximate the prevalence, sensitivities and specificities of all tests. The Bayesian LCM does not assume that any test is perfect, but considers that each test could be imperfect in diagnosing the true disease status. The true disease status of the patient population is then defined on the basis of overall prevalence (the probability that a patient with suspected typhoid fever is truly infected with *S. Typhi*). LCMs estimate prevalence and accuracy of each test based on the observed frequency of the possible combinations of test results. The model assumed that no other prior information (non-informative priors) about the unknown parameters (prevalence, sensitivities and specificities) was available except the specificity of blood culture was set at 100% and that there was a correlation between the blood culture and PCR result. The median values of all the parameters were reported together with the 95% credible intervals [23]. The Bayesian LCM was run using WinBUGS 1.4 (Medical Research Council, and Imperial College London, UK).

Results

Clinical features

The flow of patients in the study is illustrated in Figure 1. We enrolled 304 eligible febrile patients admitted to CMCH. In one patient, it was not possible to take blood, and in three patients, who were blood culture negative, there was insufficient blood for the PCR assay. These four patients were excluded from this analysis. Of the 300 patients analysed, 156 were children (age ≤ 15 years) and 144 were adults. The median (interquartile range, range) age was 13.5 (5.0–31.0, 0.5–89) years, and the median (IQR, range) duration of illness before admission was 5 (2–8, 1–14) days. A total of 29 (9.7%) patients died during their hospital admission including two with

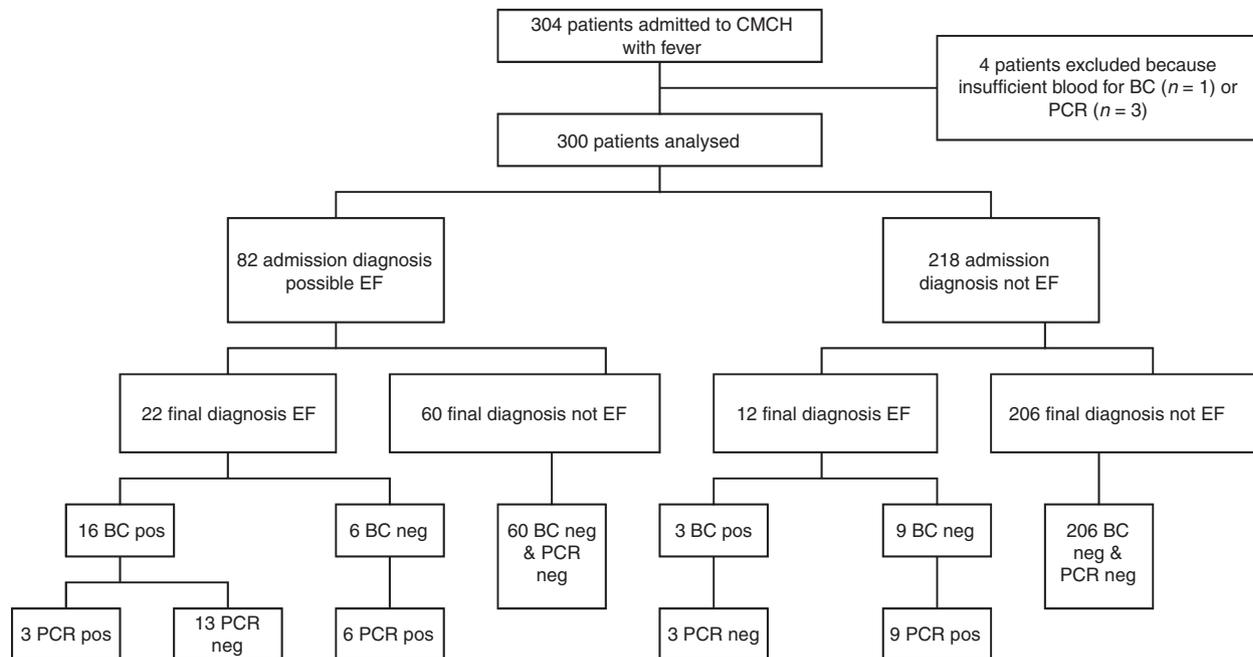


Figure 1 Flowchart of the 304 febrile patients admitted to the study and the analysis results.

typhoid fever (one blood culture and one PCR confirmed).

Typhoid fever was confirmed in 34 patients (11.3%, 95% CI; 8.2–15.5). Nineteen (6.3%) had a positive blood culture for *S. Typhi* (three of which were also blood PCR positive) and 15 (5.0%) had a negative blood culture but tested PCR positive for *S. Typhi* in blood. Eighteen (6.0%) additional patients had a clinical syndrome suggestive of typhoid fever but tested negative by blood culture and PCR. No patient was positive for *S. Paratyphi A* by blood culture or by blood PCR. Diagnoses in the 248 other patients were a non-specific febrile illness in 48 (16.0%), lower respiratory tract infection in 48 (16.0%), CNS infections in 37 (12.3%), urinary sepsis in 23 (7.7%), upper respiratory tract infection in 21 (7.0%), diarrhoea or dysentery in 21 (7.0%), malaria suspected in seven (2.3%) (none were microscopy proven probably because of prior treatment), liver conditions in seven (2.3%), skin or soft tissue infection in four (1.3%), dengue in 3 (1.0%), severe sepsis in two (0.7%), septic arthritis in two (0.7%), nephrotic syndrome in two (0.7%), murine typhus in two (0.7%), scrub typhus in one (0.3%), leptospirosis in one (0.3%), dental abscess in one (0.3%) and non-infection conditions in 18 (6.0%). Pathogens detected by blood culture and/or by blood PCR were *Staphylococcus aureus* (2), *Streptococcus pneumoniae* (1), *Streptococcus acidominimus* (1),

Enterococcus gallinarum (1), *Escherichia coli* (2), *Klebsiella pneumoniae* (1), *Enterobacter cloacae* (2), *Acinetobacter* spp. (1), *Burkholderia cepacia* (3), dengue (2), *R. typhi* (2) and *O. tsutsugamushi* (1).

The patients were stratified into two groups for subsequent analysis: blood culture positive and/or PCR positive for *S. Typhi* ($n = 34$) and blood culture and PCR negative for *S. Typhi* ($n = 266$). The clinical features and laboratory results from these patient groups are in Table 1. Patients with typhoid were more commonly adults, with a longer duration of illness prior to admission, more likely to have recently taken an antimicrobial, more likely to have a headache and diarrhoea but less likely to have had a seizure. The white cell count and platelet count were lower in those with typhoid than those without typhoid and the liver enzymes. ALT and AST were more likely to be elevated. In adults, the median volumes of blood taken for culture were comparable between the patient groups, whereas larger blood volumes were taken for culture in those with typhoid compared with those without typhoid in the children. Among patients with confirmed typhoid, 7 of 19 (37%) with a positive blood culture had taken an antimicrobial active against local strains of *S. Typhi* before admission compared with 8 of 15 (53%) with a negative blood culture but positive by blood PCR ($P = 0.489$).

R. R. Maude *et al.* **Rapid diagnostic tests for typhoid fever****Table 1** Demographic, clinical and laboratory features of studied patients (proportions are expressed as number (%) and continuous variables as median (interquartile range))

Variable	All patients	Typhoid fever [BC positive and/or PCR positive]	Not typhoid fever [BC negative and PCR negative]	P-value*
Number	300	34	266	
Age (years)	13.5 (5–31)	25 (20–32)	10 (4–30)	0.002
Sex (male)	173 (58)	20 (59)	153 (58)	1.00
Duration of illness (days)	5 (2–8)	7 (5–13)	4 (2–7)	<0.001
Recent antimicrobial	151 (50)	26 (77)	125 (47)	0.002
Recent antimicrobial active against <i>S.Typhi</i> †	73 (24)	15 (46)	58 (22)	0.005
Headache	106 (35)	21 (62)	85 (32)	0.001
Abdominal pain	96 (32)	15 (44)	81 (31)	0.108
Diarrhoea	58 (19)	11 (32)	47 (18)	0.041
Constipation	41 (14)	6 (18)	35 (13)	0.435
Vomiting	126 (42)	19 (56)	107 (40)	0.082
Cough	122 (41)	12 (35)	110 (41)	0.498
Shortness of breath	14 (5)	2 (6)	12 (5)	0.721
Seizures	53 (18)	1 (3)	52 (20)	0.015
Temperature ≥ 39 °C	56 (33)	8 (30)	48 (33)	0.707
Jaundice	22 (7)	4 (12)	18 (7)	0.292
Hepatomegaly	37 (12)	6 (18)	31 (12)	0.402
Splenomegaly	15 (5)	0 (0)	15 (16)	0.233
Altered consciousness	59 (20)	2 (6)	57 (21)	0.037
Haemoglobin (g/dl)	11.4 (10.2–12.8)	11.6 (10.4–12.7)	11.4 (10.2–12.8)	0.798
White cell count ($\times 10^9/l$)	12.0 (8.0–16.0)	7.1 (5.0–11.1)	12.0 (8.5–16.0)	<0.001
Neutrophils ($\times 10^9/l$)	8.5 (5.0–12.2)	4.9 (3.1–7.5)	8.9 (5.6–12.6)	<0.001
Lymphocytes ($\times 10^9/l$)	2.2 (1.4–3.2)	1.5 (1.0–2.4)	2.2 (1.5–3.3)	<0.001
Monocytes ($\times 10^9/l$)	0.3 (0.2–0.4)	0.2 (0.1–0.3)	0.3 (0.2–0.4)	<0.001
Eosinophils ($\times 10^9/l$)	0.14 (0.08–0.24)	0.08 (0.04–0.15)	0.16 (0.09–0.24)	<0.001
Platelet count ($\times 10^6/l$)	240 (180–338)	190 (158–233)	240 (180–346)	<0.001
AST (IU/l)	37 (25–68)	62 (33–125)	35 (23–60)	<0.001
ALT (IU/l)	30 (25–55)	55 (30–100)	30 (19–49)	<0.001
Volume of blood taken for culture (age < 5 years) (ml)	2.2 (1.7–2.9)	–	2.2 (1.7–2.9)	–
Volume of blood taken for culture (age 5–9 years) (ml)	5.2 (4.2–5.8)	8.7 (8.2–8.7)	5.1 (4.2–5.8)	0.049
Volume of blood taken for culture (age 10–15 years) (ml)	6.6 (5.5–9.9)	7.6 (5.9–9.9)	6.5 (5.4–10.2)	0.837
Volume of blood taken for culture (age ≥ 16 years) (ml)	9.2 (8.4–10.3)	9.3 (8.4–10.7)	9.2 (8.5–10.2)	0.580
Death in hospital	29 (9.7)	2 (5.9)	27 (10.2)	0.552
Duration of admission (days)	4 (2–7)	5 (3–8)	3 (2–6)	0.013

ALT, alanine transaminase; AST, aspartate transaminase; BC, blood culture; PCR, real-time PCR.

*Comparison of patients with confirmed typhoid fever and those without typhoid fever.

†Recent antimicrobial reliably active against locally circulating *S.Typhi* strains (ceftriaxone or azithromycin).

Rapid diagnostic tests for typhoid fever

A total of 124 of the 300 febrile patients had a positive result with the LifeAssay test with a reading of 1+ in 76 patients and $\geq 2+$ in 48 patients. The CTK RDT was positive in 90/300 patients, positive $\geq 1+$ for IgG in 89 patients and positive for IgM in one patient. The SD Bioline RDT was positive in 20/300 patients, positive for IgM only in 11 patients, positive for IgM and IgG in

three patients and positive for IgG only in six patients. The LifeAssay RDT was positive in 30 of the 38 healthy control subjects (29 at a score of 1+ and one at score of 3+). The CTK and SD Bioline RDTs were negative in all the healthy control subjects.

The sensitivity, specificity, and the positive and negative predictive values of the three typhoid fever RDTs are shown in Table 2 using the 34 patients with blood

culture and/or PCR-confirmed diagnosis of typhoid fever as the composite reference standard. The calculated blood culture sensitivity was 55.9%, and the PCR sensitivity was 52.9%. The sensitivity of all RDTs was low. The highest sensitivities were 58.8% with the LifeAssay (IgM) test with the threshold set at $\geq 1+$ and the CTK test (IgM and/or IgG positive), but the specificities were only 60.9% and 73.7%, respectively. When the LifeAssay RDT threshold was set at $\geq 2+$, the sensitivity was 41.2% and specificity was 87.2%. The highest specificities were observed with the SD Bioline RDT ($>95\%$), but the test lacked sensitivity ($<25\%$).

Evaluation using a Bayesian latent class model

We applied Bayesian LCMs to explore the sensitivity and specificity of blood culture, real-time PCR and the various RDTs for typhoid fever diagnosis. We ran the model with the LifeAssay RDT threshold set at $\geq 1+$ and then again with the threshold set at $\geq 2+$ (Table 3). The specificity of blood culture was set at 100%. The sensitivities and specificities of all tests apart from the LifeAssay RDT were consistent between the two runs as expected. The sensitivity of blood culture was 55.6% and 47.5% in the two model runs, and the sensitivity of the real-time PCR was only 14.2%. The best combination of sensitivity and specificity was observed with the LifeAssay RDT with the threshold set at $\geq 2+$ at 63.0% and 91.2%, respectively. The SD Bioline specificity was higher than the other RDTs ($>95\%$) but with a sensitivity of $<40\%$. The calculated prevalence in the two model runs was 11.8% and 13.6%

consistent with the observed prevalence of 11.3% based on laboratory-confirmed cases.

If the proportion of positive samples for each test at different durations of illnesses prior to admission is considered, PCR was more frequently positive in the initial three days of illness, whereas blood cultures were only positive in patients with four or more days of fever.

Discussion

The primary finding of this study was that the diagnostic accuracy of three RDTs for typhoid fever in febrile hospitalised patients in Chittagong, Bangladesh, was only moderate. The sensitivity results of the three tests ranged from 24% to 59% with specificities of 61–96%. In the Bayesian LCM, the sensitivity of the Life Assay RDT was higher at 87% but with a specificity of only 65% and the sensitivity and specificity of the other two RDTs broadly similar. The model estimates for prevalence of typhoid were close to the observed prevalence of 11.3%, based on laboratory-confirmed cases. The three typhoid RDTs were also tested in healthy adult controls subjects. The CTK and SD Bioline RDTs gave negative results, but with the LifeAssay RDT 30 of the 38 controls, subjects gave a positive result (29 at $1+$). In the light of this, the LifeAssay RDT results were re-analysed with a threshold of $\geq 2+$ rather than $\geq 1+$ to indicate a positive result and the sensitivity fell from 59% to 41% but the specificity rose from 61% to 87%. When the Bayesian LCM was rerun using this threshold, the sensitivity of 63% and specificity of 91% was the best result combination of all the three RDTs.

Table 2 Conventional sensitivity, specificity, positive and negative predictive values for each laboratory method (blood culture, PCR and each rapid diagnostic test). Laboratory-confirmed typhoid (blood culture and/or PCR positive; $n = 34$) was the composite reference standard and was compared with cases that were not typhoid based on laboratory confirmation ($n = 266$) (values are means with 95% confidence intervals)

Diagnostic test	Test positive in typhoid cases ($n = 34$)	Test positive in not typhoid cases ($n = 266$)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Blood culture	19	0	55.9 (39.4–71.1)	100 (98.3–100)	100 (80.2–100)	94.7 (91.3–96.8)
PCR	18	0	52.9 (36.7–68.6)	100 (98.3–100)	100 (79.3–100)	94.3 (90.9–96.5)
LA (IgM) $\geq 1+$	20	104	58.8 (42.2–73.7)	60.9 (54.9–66.6)	16.1 (10.6–23.7)	92.1 (87.0–95.3)
LA (IgM) $\geq 2+$	14	34	41.2 (26.3–57.8)	87.2 (82.6–90.7)	29.2 (18.2–43.3)	92.1 (88.0–94.9)
CTK (IgM)	0	1	0 (0–12.1)	99.6 (97.7–100)	0 (0–83.3)	88.6 (84.5–91.8)
CTK (IgG)	20	69	58.8 (42.2–73.7)	74.1 (68.5–79.0)	22.5 (15.0–32.3)	93.4 (89.1–96.1)
CTK (IgM &/or IgG)	20	70	58.8 (42.2–73.7)	73.7 (68.1–78.6)	22.2 (14.8–31.9)	92.3 (89.0–96.1)
SD Bioline (IgM)	7	7	20.6 (10.1–37.1)	97.4 (94.6–98.8)	50.0 (26.8–73.2)	90.1 (86.6–93.5)
SD Bioline (IgG)	3	6	8.8 (2.3–23.8)	97.7 (95.1–99.1)	33.3 (11.7–64.9)	89.4 (85.2–92.4)
SD Bioline (IgM &/or IgG)	8	12	23.5 (12.2–40.2)	95.5 (92.2–97.5)	40.0 (21.8–61.4)	90.7 (86.7–93.6)

Table 3 Bayesian LCM model prevalence sensitivity and specificity for the results of blood culture, real-time PCR and each individual rapid diagnostic test. The specificity of blood culture was set at 100%. Results are medians with 95% credible intervals. The model was run with the Life Assay (IgM) threshold set at $\geq 1+$ and again with the Life Assay (IgM) threshold set at $\geq 2+$

Diagnostic test	Parameter	Model run using Life Assay (IgM) $\geq 1+$ as positive cut-off	Model run using Life Assay (IgM) $\geq 2+$ as positive cut-off
Blood Culture	Prevalence	11.8 (6.7–19.5)	13.6 (8.1–22.1)
	Sensitivity	55.6 (27.6–87.7)	47.5 (23.2–76.9)
	Specificity	100	100
PCR	Sensitivity	14.2 (2.8–32.8)	14.2 (3.0–32.0)
	Specificity	95.0 (91.8–97.3)	95.3 (92.1–97.6)
Life Assay (IgM)	Sensitivity	86.7 (68.6–96.8)	63.0 (43.3–80.8)
	Specificity	64.8 (58.4–71.3)	91.2 (86.5–96.0)
CTK (IgM &/or IgG)	Sensitivity	60.5 (40.3–79.1)	60.8 (41.8–78.9)
	Specificity	73.9 (68.2–79.4)	74.7 (68.8–80.3)
SD Bioline (IgM &/or IgG)	Sensitivity	37.4 (20.0–57.2)	36.0 (20.3–55.8)
	Specificity	97.1 (94.0–99.4)	97.6 (94.7–99.6)

The LifeAssay RDT format has been studied in 209 febrile patients presenting to hospital in Makassar, Indonesia, in whom 116 (55.5%) were diagnosed with typhoid fever, 54 (25.8%) confirmed by blood culture [24]. The sensitivity and specificity (with an intensity of $\geq 1+$ as positive) was 62.1% and 97.8%, respectively. A further study, conducted in Cambodia, of 500 hospitalised febrile children included 44 (8.8%) cases of typhoid fever, of which 32 (6.4%) were confirmed by blood culture and/or real-time PCR [15]. The sensitivity and specificity (with a threshold of $\geq 2+$ considered positive) was 55% and 98%, respectively. When assessed using LCM analysis, the sensitivity of the LifeAssay RDT increased to 77% and the specificity was 98%. A cut-off threshold of $\geq 2+$ could be reliably determined with high inter- and intra-observer kappa scores [15].

The SD Bioline RDT was studied in 177 febrile hospitalised children and adults in the Philippines of which 75 (39.5%) were blood culture positive for *S. Typhi* [25]. The sensitivity and specificity was 69% and 79%, respectively, for IgM and 71% and 76%, respectively, for IgG. These sensitivities are higher than the results presented here. We are unaware of any published evaluations of the CTK RDT in febrile patients.

The sensitivity of blood culture in this study was relatively low at 56% of laboratory-confirmed cases and 48–56% in the LCMs. This is comparable to studies where bone marrow culture was additionally performed [26–29]. Considerable efforts were made to

take large and reproducible blood volumes for culture in this study. The recent consumption of antimicrobials was commonly reported in all groups although we cannot be sure that the drugs taken were as described or of the required quality to be effective. In the study in Cambodian children using LCMs to evaluate diagnostic tests for typhoid, blood culture sensitivity approached 80%, despite lower volumes of blood, although a lower number of patients reported recent antimicrobial use [15].

The proportion of positive cases positive by both blood culture and PCR was low. The lack of PCR-positive samples from patients with a positive blood culture may relate to the smaller volume of blood used to extract the DNA for the PCR compared with that used for the blood culture and also the potential presence of PCR inhibitors. The PCR-positive/blood culture-negative patients may be the result of prior antimicrobial therapy. The proportion of blood culture negative/PCR-positive patients who had taken an antimicrobial active against local strains of *S. Typhi* was higher than those with a positive blood culture, but the numbers were small and not significant. The PCR was often positive in the first few days of fever, a similar observation to the study in Cambodia [15]. There may be an early bacteraemic period in which the organism is difficult to culture and this is an area that warrants further study.

The inclusion of consecutive patients in this endemic setting makes the findings more generalisable for hospitalised patients with fever although may not apply to patients in ambulatory settings. All diagnostic tests were performed on samples collected at the time, or within 48 h, of admission, and the reference and index tests were performed blind. The small number of laboratory-confirmed typhoid cases is a limitation resulting in large confidence intervals for the diagnostic accuracy estimates. All patients received the same diagnostic composite reference standard although this reference standard is not perfect. Some of the patients with clinically suspected typhoid fever who were blood culture and PCR negative may have been true positives. Analysis with Bayesian LCMs attempts to address this issue. There were few patients with bacteremia caused by alternative organisms and therefore limited opportunity to assess potential cross-reactivity of these RDTs with other causes of bacteremia.

Conclusions

There is no consensus as to the required target product profile for a RDT to diagnose acute typhoid fever. In our opinion, cut-offs of $>80\%$ for sensitivity and $>98\%$

for specificity would be reasonable targets. None of the RDTs tested reach these levels of sensitivity and specificity. Similar observations apply to other widely used commercially available typhoid RDTs [13]. This does not necessarily mean that tests with these reported sensitivity and specificity values are not useful as long as the positive and negative predictive values of the test are clearly understood by the user. Although larger evaluations of typhoid RDTs in other settings are needed to substantiate these findings, it is clear that new RDTs using more specific antigens against *S. Typhi* and *S. Paratyphi A* are needed [30]. The lack of a satisfactory reference standard remains an important limitation of such studies and the place of real-time PCR in the laboratory confirmation of typhoid fever needs to be further improved and evaluated.

Acknowledgements

We thank Sujat Pal, ASM Zahed, Wahid Rahman, Rifat Kranain and Rezina Islam who recruited patients during the study period; Suman, Sanjeeb and staff from Chevron Laboratories Ltd., and James Campbell for laboratory support; Shafiqul Chowdhury for administrative support. We thank the nurses and doctors at Chittagong Medical College Hospital, Chittagong, who were responsible for providing all care for the patients in the study. Standard Diagnostics, Korea, provided the SD Bioline test kits without charge. This work was presented in part at the ASTMH 62nd annual meeting, November 2013, Washington, United States. The study was supported by the Wellcome Trust of Great Britain.

References

- Brooks WA, Hossain A, Goswami D *et al.* Bacteremic typhoid fever in children in an urban slum, Bangladesh. *Emerg Infect Dis* 2005; **11**: 326–329.
- Naheed A, Ram PK, Brooks WA *et al.* Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. *Int J Infect Dis* 2010; **14**(Suppl 3): e93–e99.
- Saha SK, Baqui AH, Hanif M *et al.* Typhoid fever in Bangladesh: implications for vaccination policy. *Pediatr Infect Dis J* 2001; **20**: 521–524.
- Ahmed D, D'Costa LT, Alam K, Nair GB, Hossain MA. Multidrug-resistant *Salmonella enteric* serovar *Typhi* isolates with high-level resistance to ciprofloxacin in Dhaka, Bangladesh. *Antimicrob Agents Chemother* 2006; **50**: 3516–3517.
- Saha SK, Darmstadt GL, Baqui AH *et al.* Molecular basis of resistance displayed by highly ciprofloxacin resistant *Salmonella enterica* serovar *Typhi* in Bangladesh. *J Clin Microbiol* 2006; **44**: 3811–3813.
- Parry CM, Wijedoru L, Arjal A, Baker S. The utility of diagnostic tests for enteric fever in endemic locations. *Expert Rev Anti Infect Ther* 2011; **9**: 711–725.
- Wain J, Diep TS, Ho VA *et al.* Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility and antimicrobial resistance. *J Clin Microbiol* 1998; **36**: 1683–1687.
- Gasem MH, Keuter M, Dolmans WM *et al.* Persistence of *Salmonellae* in blood and bone marrow: randomised controlled trial comparing ciprofloxacin and chloramphenicol treatments against enteric fever. *Antimicrob Agents Chemother* 2003; **47**: 1727–1731.
- Wain J, Pham VB, Ha V *et al.* Quantitation of bacteria in bone marrow from patients with typhoid fever: relationship between counts and clinical features. *J Clin Microbiol* 2001; **39**: 1571–1576.
- Nga TV, Karkey A, Dongol S *et al.* The sensitivity of real-time PCR amplification targeting invasive salmonella serovars in biological specimens. *BMC Infect Dis* 2010; **10**: 125.
- Dutta S, Sur D, Manna B *et al.* Evaluation of new-generation serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. *Diagn Microbiol Infect Dis* 2006; **56**: 359–365.
- Keddy KH, Sooka A, Letsoalo ME, Hoyland G, Chaignat CL. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. *Bull World Health Organ* 2011; **89**: 640–647.
- Thriemer K, Ley B, Menten J, Jacobs J, van den Ende J. A systematic review and meta-analysis of the performance of two point of care typhoid fever tests, Tubex TF and Typhidot, in endemic countries. *PLoS One* 2013; **8**: e81263.
- Limmathurotsakul D, Jamsen K, Arayawichanon A *et al.* Defining the true sensitivity of culture for the diagnosis of melioidosis using Bayesian latent class models. *PLoS One* 2010; **5**: e12485.
- Moore EC, Pan-Ngum W, Wijedoru LPM *et al.* Evaluation of the diagnostic accuracy of a typhoid IgM flow assay for the diagnosis of typhoid fever in Cambodian children using a Bayesian latent class model assuming an imperfect gold standard. *Am J Trop Med Hyg* 2014; **90**: 114–120.
- Pan-Ngum W, Blacksell SD, Lubell Y *et al.* Estimating the true accuracy of diagnostic tests for dengue infection using Bayesian latent class models. *PLoS One* 2013; **8**: e50765.
- Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. *N Engl J Med* 2002; **347**: 1770–1782.
- Thaipadunpanit J, Chierakul W, Wuthiekanun V *et al.* Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and *lipI32* genes for human leptospirosis in Thailand: a case-control study. *PLoS One* 2011; **6**: e16236.
- Henry KM, Jiang J, Rozmajzl PJ *et al.* Development of quantitative real-time PCR assays to detect *Rickettsia typhi* and *Rickettsia felis*, the causative agents of murine typhus and flea-borne spotted fever. *Mol Cell Probes* 2007; **21**: 17–23.
- Jiang J, Chan TC, Temenak JJ *et al.* Development of a quantitative real-time polymerase chain reaction assay

R. R. Maude *et al.* **Rapid diagnostic tests for typhoid fever**

- specific for *Orientia tsutsugamushi*. *Am J Trop Med Hyg* 2004; **70**: 351–356.
21. Bossuyt PM, Reitsma JB, Bruns DE *et al.* The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Ann Intern Med* 2003; **138**: W1–W12.
 22. Dendukuri N & Joseph L. Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics* 2001; **57**: 158–167.
 23. Lunn D, Spiegelhalter D, Thomas A, Best N. The BUGS project: evolution, critique and future directions. *Stat Med* 2009; **28**: 3049–3067.
 24. Pastoor R, Hatta M, Abdoel T, Smits H. Simple, rapid, and affordable point-of-care test for the serodiagnosis of typhoid fever. *Diagn Microbiol Infect Dis* 2008; **61**: 129–134.
 25. Kawano RL, Leano SA, Agdamag DMA. Comparison of serological test kits for the diagnosis of typhoid fever in the Philippines. *J Clin Microbiol* 2007; **45**: 246–247.
 26. Akoh JA. Relative sensitivity of blood and bone marrow cultures in typhoid fever. *Trop Doct* 1991; **21**: 174–176.
 27. Farooqui BJ, Khurshid M, Ashfaq MK, Khan MA. Comparative yield of *Salmonella typhi* from blood and bone marrow cultures in patients with fever of unknown origin. *J Clin Pathol* 1991; **44**: 258–259.
 28. Gasem MH, Dolmans WM, Isbandrio BB *et al.* Culture of *Salmonella typhi* and *Salmonella paratyphi* from blood and bone marrow in suspected typhoid fever. *Trop Geogr Med* 1995; **47**: 164–167.
 29. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet* 1975; **1**: 1211–1213.
 30. Baker S, Favarov M, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis* 2010; **10**: 45.

Appendix I CMCH Typhoid Study Group

Abdullah Abu Sayeed, Mahtab Uddin Hasan, Wirichada Pan-Ngum, Thomas W. van der Vaart, Asok Kumar Dutta, Nasir Uddin Mahmud, Murad Hero, Nafiz Iqbal, Zabeen Chaudhury, Tran Vu Thieu Nga, Pham Thanh Duy, Voong Vinh Phat, Richard J. Maude, Stephen Baker, W. Joost Wiersinga, Tom van der Poll, Nicholas P. Day, Arjen M. Dondorp.

Corresponding Author Christopher M. Parry, Clinical Research Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. Tel.: +44 (0)20 7927 2256; Fax +44 (0)20 7637 4314; E-mail: chris.parry@lshtm.ac.uk