Evaluation of a Simple Blood Culture Amplification and Antigen Detection Method for Diagnosis of *Salmonella enterica* Serovar Typhi Bacteremia

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In most areas where typhoid is endemic, laboratory diagnosis is not possible due to the lack of appropriate facilities. We investigated whether the combination of blood culture amplification of *Salmonella enterica* serovar Typhi with an *S. Typhi* antigen rapid diagnostic test (RDT) could be an accurate and inexpensive tool for the accelerated diagnosis of patients with acute typhoid in Laos. For a panel of 23 Gram-negative reference pathogens, the Standard Diagnostics (catalog no. 15FK20; Kyonggi-do, South Korea) RDT gave positive results for *S. Typhi* NCTC 8385, *S. Typhi* NCTC 786 (Vi negative), *Salmonella enterica* serovar Enteritidis (ATCC 13076), and *Salmonella enterica* serovar Ndolo NCTC 8700 (all group D). In a prospective study of 6,456 blood culture bottles from 3,028 patients over 15 months, 392 blood culture bottles (6.1%) from 221 (7.3%) patients had Gram-negative rods (GNRs) seen in the blood culture fluid. The sensitivity, negative predictive value, specificity, and positive predictive value were 96.7%, 99.5%, 97.9%, and 87.9%, respectively, for patients with proven *S. Typhi* bacteremia and 91.2%, 98.4%, 98.9%, and 93.9% for patients with group D *Salmonella*. The median (range) number of days between diagnosis by RDT and reference assays was 1 (–1 to +2) day for those with confirmed *S. Typhi*. The use of antigen-based pathogen detection in blood culture fluid may be a useful, relatively rapid, inexpensive, and accurate technique for the identification of important causes of bacteremia in the tropics.

The estimated global annual incidence of typhoid in 2000 was 21 million patients, with 217,000 deaths (1). Although the causative bacterium was first described 132 years ago, most typhoid patients do not have access to reliable laboratory diagnosis, since the appropriate facilities and techniques usually still do not exist in economically poor areas of endemicity (2, 3). There remains an urgent need for inexpensive, rapid, portable, and simple techniques for diagnosing typhoid in locations away from sophisticated hospital settings, where the burden of disease is greatest.

Diagnosis based on clinical manifestations alone is inaccurate, while laboratory diagnosis relies on the growth of the organism, usually from blood (although bone marrow culture has higher sensitivity), followed by serological and biochemical identification of the cultured bacteria (2). These techniques take a minimum of 3 days; are expensive; and require special training, facilities, equipment, quality assurance, and disposables. Serological tests for *Salmonella enterica* serovar Typhi antibodies, such as the Widal test, enzyme immunoassays, and immunochromatographic rapid diagnostic tests (RDTs), are simple and relatively inexpensive. However, those that have been developed have not been shown to be accurate, field appropriate, and rapid. They suffer from antibody persistence after cure or immunization, lack of determination of locally appropriate cutoffs, and cross-reactivity (2, 4–7). Sensitivity is also an issue with PCR-based tests, due to the low venous blood bacterial concentration (8–10). Although fast and specific (10–15), they cannot be used in areas where the disease is most common because of severe problems of human and financial capacity.

Lateral-flow RDTs are widely used for the diagnosis of malaria by the detection of *Plasmodium* antigens in blood. Their use does

not require extensive technical training; they are relatively inexpensive and have revolutionized malaria diagnostics in remote areas where facilities, such as quality-assured microscopy, are limited (16). Several RDTs have been developed to detect *S. Typhi* antigen in feces (below). Although the detection of *S. Typhi* in stool may be useful for detecting chronic carriers, <30% of patients with acute *S. Typhi* bacteremia are stool culture positive, and such RDTs are therefore not optimum for diagnosis of acute typhoid fever (3, 17, 18). Typhoid diagnosis in the tropics could be enhanced by making the identification of *S. Typhi* in blood cultures simpler, less expensive, and faster. Methods for the detection of *S. Typhi* or group D antigens in blood culture fluid have been developed using coagglutination and slide latex agglutination, but they may be difficult to read and have not, as far as we are aware, either been evaluated prospectively or entered routine clinical practice (19–22).

Typhoid is an important disease in the Lao People's Democratic Republic (Laos); it was responsible for 51% of all identified causes of community-acquired septicemia in Vientiane between 2000 and 2005 (23, 24). There is no typhoid vaccination program,

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TABLE 1 Reference bacterial strains used for the spiked-blood bottle assay^a and RDT results

Species/strain ^b	Organism reference no.	Group O and H antigens	RDT			
			Standard Diagnostics (S. Typhi)	Science with a Mission (S. Typhi)	Labcare Diagnostics	
					S. Typhi	S. Paratyphi
<i>Salmonella bongori</i>	NCTC 12419		Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi A	NCTC 8388	A, 1, 2, 12	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi B	NCTC 3176	B, 4, 5, 12; b, 1, 2	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium	NCTC 74	B, 4, 5, 12; i, 1, 2	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi C	NCTC 96	C ₁ , 6, 7, (Vi); c, 1, 5	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi C	NCTC 5734	C ₁ , 6, 7; c, 1, 5	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Choleraesuis	NCTC 5735	C ₁ , 6, 7; c, 1, 5	Negative	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Ndolo	NCTC 8700	D ₁ , 9, 12; d, 1, 5	Positive	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi	NCTC 786	D ₁ , 9, 12 (Vi ⁻); d	Positive	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi	NCTC 8385	D ₁ , 9, 12 (Vi ⁺); d	Positive	Negative	Negative	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis	ATCC 13076	1, 9, 12; g, m	Positive	Negative	Negative	Negative
<i>Acinetobacter baumannii</i>	NCTC 12156		Negative	Negative	Negative	Negative
<i>Aeromonas hydrophila</i>	NCTC 8049		Negative	Negative	Negative	Negative
<i>Burkholderia cepacia</i>	NCTC 10743		Negative	Negative	Negative	Negative
<i>Burkholderia pseudomallei</i>	UI 8976 ^c		Negative	Negative	Negative	Negative
<i>Citrobacter freundii</i>	NCTC 9737		Negative	Negative	Negative	Negative
<i>Edwardsiella tarda</i>	NCTC 10396		Negative	Negative	Negative	Negative
<i>Enterobacter aerogenes</i>	NCTC 10006		Negative	Negative	Negative	Negative
<i>Enterobacter cloacae</i>	NCTC 11580		Negative	Negative	Negative	Negative
<i>Klebsiella oxytoca</i>	NCTC 8167		Negative	Negative	Negative	Negative
<i>Klebsiella pneumoniae</i>	NCTC 9633		Negative	Negative	Negative	Negative
<i>Ochrobactrum anthropi</i>	NCTC 12168		Negative	Negative	Negative	Negative
<i>Yersinia enterocolitica</i>	NCTC 11175		Negative	Negative	Negative	Negative

^a The blood culture bottles contained (per 100 ml of fluid): tryptic hydrolysate casein, 1.7 g; soy peptone, 0.3 g; sodium chloride, 0.5 g; potassium phosphate, 0.25 g; dextrose, 0.25 g; and sodium polyanetholsulfonate, 0.025 g, made up to 100 ml with water for injection.

^b All *Burkholderia*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Klebsiella*, *Salmonella*, and *Yersinia* species were grown on MacConkey agar plates in a 7% CO₂ incubator at 37°C. *Ochrobactrum anthropi* was kept under the same conditions but plated on 7% goat blood agar. *Aeromonas* and *Acinetobacter* species were grown on 7% goat blood agar at 37°C in air.

^c A clinical isolate from a Lao patient; its identity was confirmed by API NE (bioMérieux; France), colonial morphology, latex agglutination test, resistance to colistin, and susceptibility to coamoxiclav (25).

and the only laboratories able to culture and identify *S. Typhi* are in the capital. We therefore tested whether blood culture bottles could be used to amplify *S. Typhi* in blood sufficiently to allow the detection of *S. Typhi* antigens in blood culture fluid by RDTs.

MATERIALS AND METHODS

We initially evaluated three *S. Typhi* antigen detection RDTs, designed for detecting *S. Typhi* antigen in stools, with 23 reference Gram-negative rods (GNRs) likely to be grown in blood cultures in Laos. We then prospectively evaluated the accuracy of the best-performing RDT in blood cultures from Lao patients that grew GNRs. This study was part of the studies of community-acquired bacteremia granted ethical approval by the National Ethics Committee for Health Research, Vientiane, Laos, and the Oxford Tropical Research Ethics Committee, University of Oxford, Oxford, United Kingdom.

RDTs with reference strains. After 24 h of incubation, colonies of the reference organisms (Table 1) were emulsified in sterile 0.85% saline, and

the turbidity was adjusted to 0.5 MacFarland unit. One milliliter of each bacterial suspension was used to spike patient blood culture bottles that showed no growth after 7 days. Coinoculation of fresh blood was not performed because of the local difficulties of obtaining sufficient volumes of pathogen-free fresh blood. The spiked bottles were incubated for 72 h at 37°C before RDTs were performed on the supernatants. All experiments were performed in duplicate.

Prospective study patients. Blood cultures received at Mahosot Hospital, Vientiane, Laos, between September 2010 and December 2011 as part of a prospective study examining the causes of community-acquired bacteremia (23) were included. Consenting febrile patients for whom the responsible physician suspected community-acquired bacteremia were recruited (23). Patients whose blood cultures grew GNRs were included in the evaluation of the RDTs.

Blood cultures and bacterial identification. Two aerobic bottles, injected with ~5 ml of blood/bottle for adults, ~2 ml for children <15 years old, and ~1 ml for those <1 year old were taken from each patient who

TABLE 2 Clinical features of patients included in the prospective evaluation of the SD *S. Typhi* rapid diagnostic test

Variable	Value	
	All patients growing GNRs thought to be clinically significant (n = 196)	Patients <i>S. Typhi</i> blood culture positive by reference tests (n = 30)
Age (yr) [median (range)]	46 (0–97)	22 (1–50)
Male [no. (%)]	100 (51.0)	19 (63.3)
Admitted at another hospital in Vientiane city ^a [no. (%)]	46 (23.5)	5 (16.7)
Admitted outside of Vientiane city ^b [no. (%)]	7 (3.6)	10 (33.3)
Days of fever before admission [median (range)]	5 (0–120)	7 (2–23)
Days of fever before blood culture taken [median (range)]	7 (0–120)	8.5 (3–24)
Days from blood culture draw to RDT performed [median (range)]	2 (0–13)	2 (0–9)
Days from blood culture draw to identity confirmed by reference tests [median (range)]	3 (1–12)	3 (2–9)

^a Blood cultures were sent from patients admitted to the following hospitals: Sethathirat (n = 33), Military (n = 1), Friendship (n = 45), and Mother and Child (n = 10).

^b Blood cultures were sent from patients admitted to the following hospitals: Luang Nam Tha (n = 13), Salavan (n = 12), Udomxay (n = 1), Xayabuly (n = 2), and Vientiane Province (n = 2).

gave written informed consent. Blood culture bottles (Pharmaceutical Factory No. 2, Vientiane, Laos) contained tryptic hydrolysate of casein and soy peptone with sodium polyanethol sulfonate (Table 1). The bottles were vented and cultured aerobically at 37°C for 7 days. The bottles were checked daily and subcultured on blood, chocolate, and MacConkey agars if they were turbid and Gram stain demonstrated GNRs. Blind subculture was performed at ~24 h and on day 7 postinoculation. Organisms were identified by conventional gallery tests and API determination (23). *S. Typhi* was identified by API 20E (bioMérieux, Marcy l'Étoile, France), and with polyvalent O, O9, Vi, and Hd antisera (Bio-Rad, Marnes-la-Coquette, France) (26). Eight non-*S. Typhi* *Salmonella* isolates were identified at the Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, by multilocus sequence typing using standard alleles and amplification methods, as described previously (27).

RDT methods. Three different RDTs were evaluated for detection of *S. Typhi* antigen in blood culture fluid using the reference strains: the Accucare *S. Typhi*-S. Paratyphi Direct Antigen Detection kit (catalog no. STYC 25; Labcare Diagnostics, Sarigam, India [28]), the One Step *Salmonella* Typhi Ag Rapid Detection Kit (catalog no. 15FK20; Standard Diagnostics [SD], Kyonggi-do, South Korea), and the *Salmonella* Typhi Antigen Strip (Science with a Mission [SMI], Sharon, MA [29]). The package inserts stated that the tests were developed to detect *S. Typhi* antigens in stool, as well as in serum (Labcare [28]) and plasma (SMI [29]). The manufacturers' instructions were therefore modified as follows: 1 ml of blood culture fluid was removed from the blood culture bottle growing GNRs and centrifuged for 1 min at 500 × g in a 1.5-ml tube, facilitating the reading of the RDT by using the clearer supernatant. For the SD assay, 4 drops were added to the cassette with the disposable dropper provided, and the results were read at 20 min; for the Labcare assay, 100 µl was added to the test, and the results were read at 20 min; for the SMI assay, 60 µl was added to the cassette, and the results were read at 15 min. The RDTs were read by laboratory technicians blinded to the clinical and microbiological features of the patient and his/her sample (except for knowing that the patient had GNRs in one or more blood culture bottles). Technicians performing the formal blood culture bottle GNR identification were blinded to the RDT results. We have attempted to report these results according to the STARD guidelines (30).

RESULTS

RDTs with reference strains. All reference bacteria grew in the blood culture bottles within 72 h, and all RDTs expressed the control lines correctly. Neither the Labcare nor the SMI RDT was positive for any of the reference bacteria. However, with the SD RDT, *S. Typhi* NCTC 8385, *S. Typhi* NCTC 786 (Vi negative), *Salmonella enterica* serovar Enteritidis (ATCC 13076), and *Salmo-*

nella enterica serovar Ndolo NCTC 8700 (all group D) were positive (Table 1). There was 100% concordance for all duplicates, and no result was uncertain. We therefore proceeded with a prospective evaluation of the SD RDT.

Prospective evaluation. A total of 6,456 blood culture bottles from 3,028 patients were received at Mahosot Hospital during the study period. Of these, 392 blood culture bottles (6.1%) from 221 (7.3%) patients had GNRs seen in the blood culture fluid, and 196 patients (6.5%) grew clinically significant GNRs. The majority (137/196; 70%) of those with GNRs were admitted to Mahosot Hospital (Table 2). The median (range) age of patients with clinically significant GNRs was 46 (0 to 97) years (all hospitals included). Three patients were infants with blood cultures taken on the day of birth, and 34 (15%) were children <15 years old. The most frequent clinically significant GNRs identified were *Escherichia coli* (73 patients; 37.2%), *Burkholderia pseudomallei* (34; 17.3%), *S. Typhi* (30; 15.3%), and *Klebsiella pneumoniae* (28; 14.3%) (Table 3). Bottles from an additional 10 (5.1%) patients grew nontyphoidal *Salmonella enterica*, of which 4/196 (2.0%) were nontyphoidal group D (all *S. Enteritidis*), three were *Salmonella enterica* serovar Paratyphi A, and three were *Salmonella enterica* serovar Typhimurium (Table 3). Of 196 patients whose blood grew clinically significant GNRs, 151 (77%) grew GNRs in both bottles, and there was 100% concordance between the identities of GNRs in bottle pairs. Blood cultures from 23 (10.4%) patients grew GNRs that were thought to be contaminants, and 2 patients grew Gram-positive cocci (*Aerococcus viridans*), despite GNRs having been seen in blood culture fluid (Table 3).

The SD RDT was performed on the same day that GNRs were detected by microscopy for 189/221 (86%) patients; the blood culture fluid from 32 patients was assayed later, but always within 7 days. The SD RDT control line was expressed correctly every time.

When first performed, the SD RDTs correctly identified *S. Typhi* antigen in blood culture bottles from 29/30 (96.7%) patients in comparison to *S. Typhi* reference assays. Of the non-*S. Typhi* patients, four gave positive RDT results: two *S. Enteritidis* (a group D *Salmonella*) and two *E. coli*. The sensitivity, negative predictive value, specificity, and positive predictive value were therefore 96.7%, 99.5%, 97.9%, and 87.9%, respectively, for patients with proven *S. Typhi* bacteremia and 91.2%, 98.4%, 98.9%, and

TABLE 3 RDT results in a prospective evaluation of the SD S. Typhi RDT

Bacteria cultured	No. of patients (%)	Results ^a	
		No. SD RDT positive (%)	No. SD RDT negative (%)
Clinically significant GNRs	196	33 (16.8)	163 (83.2)
<i>Escherichia coli</i>	73 (37.2)	2 (2.7)	71 ^b (97.3)
<i>Burkholderia pseudomallei</i>	34 (17.3)	0	34 (100)
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi	30 (15.3)	29 ^c (96.7)	1 ^c (3.3)
<i>Klebsiella pneumoniae</i> ^e	28 (14.2)	0	28 (100)
<i>Acinetobacter baumannii</i>	5 (2.6)	0	5 (100)
<i>Aeromonas hydrophila</i> ^e	5 (2.6)	0	5 (100)
<i>Enterobacter cloacae</i>	5 (2.6)	0	5 (100)
<i>Salmonella enterica</i> subsp. <i>enterica</i> group D (excluding S. Typhi)	4 (2.0)	2 (50)	2 ^f (50)
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi A (<i>n</i> = 3) and <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium (<i>n</i> = 3)	6 (3.1)	0	6 (100)
Other GNRs (<i>Edwardsiella tarda</i> [<i>n</i> = 1], <i>Enterobacter aerogenes</i> [<i>n</i> = 1], <i>Hafnia</i> sp. [<i>n</i> = 1], <i>Serratia</i> sp. [<i>n</i> = 1], <i>Pseudomonas aeruginosa</i> [<i>n</i> = 1], and <i>Vibrio vulnificus</i> [<i>n</i> = 1])	6 (3.1)	0	6 (100)
<i>Salmonella enterica</i> subsp. <i>enterica</i> group D (including S. Typhi)	34 (17.3)	31 (91.2)	3 ^d (8.8)
Probable GNR contaminants	23 ^g	0	23 (100)
<i>Acinetobacter baumannii</i>	5 (21.7)	0	5 (100)
<i>Burkholderia cepacia</i>	3 (13.0)	0	3 (100)
<i>Escherichia hermannii</i>	2 (8.7)	0	2 (100)
<i>Stenotrophomonas maltophilia</i>	2 (8.7)	0	2 (100)
Other species	11 (47.8)	0	11 (100)

^a Results for initial evaluation only.^b On repeat testing, all 73 *E. coli* isolates were RDT negative.^c On repeat testing, all 30 *S. Typhi* isolates were RDT positive.^d On repeat testing, all *Salmonella enterica* serovar *enterica* group D were RDT positive.^e One patient had *Aeromonas hydrophila* and *K. pneumoniae* cultured from within the same 2 bottles.^f On repeat testing, all *Salmonella enterica* subsp. *enterica* group D isolates (excluding *S. Typhi*) were RDT positive.^g Two additional patients grew Gram-positive cocci (*Aerococcus viridans*).

93.9% for patients with group D *Salmonella*. For blood culture bottles, the sensitivity, negative predictive value, specificity, and positive predictive value were 94.1%, 99.1%, 97.7%, and 85.7%, respectively, for patients with proven *S. Typhi* bacteremia and 89.7%, 98.2%, 98.8%, and 92.9% for patients with group D *Salmonella* (Table 4).

We further investigated the results from the five patients who gave discordant results (Table 3). On regrowing their pathogens from Protec Bacterial Preservation Cryovials (Fisher Scientific, United Kingdom), seeding in blood culture bottles (as described

above), and repeating the SD RDTs (with the blinding of investigators, as described above), different results were obtained (Table 3). With these repeats, the SD RDTs identified the *S. Typhi* antigen in all 51 of the bottles from all 30 *S. Typhi* patients, as well as in 7 bottles from all 4 patients that grew *S. Enteritidis*. The two positive RDTs for *E. coli* were negative on repeat. After these repeats, the sensitivity, negative predictive value, specificity, and positive predictive value (with 95% confidence interval [CI]) were 100% (88.3 to 100%), 100% (98.0 to 100%), 97.9% (94.7 to 99.4%), and 88.2% (72.5 to 96.6%), respectively, for patients diagnosed with *S.*

TABLE 4 Concordance between *S. Typhi* and *Salmonella* group D reference determination of blood culture growth and *S. Typhi* and *Salmonella* group D diagnosis using the SD RDT^a

RDT result	No. of patients (<i>n</i> = 221) with diagnosis				No. of bottles (<i>n</i> = 392) with diagnosis			
	<i>S. Typhi</i>	Non- <i>S. Typhi</i>	<i>Salmonella</i> group D	Non- <i>Salmonella</i> group D	<i>S. Typhi</i>	Non- <i>S. Typhi</i>	<i>Salmonella</i> group D	Non- <i>Salmonella</i> group D
Positive	29	4	31	2	48	8	52	4
Negative	1	187	3	185	3	333	6	330
Sensitivity (%) (95% CI)	96.7 (82.7–99.4)		91.2 (76.3–98.0)		94.1 (83.7–98.7)		89.7 (78.8–96.1)	
Specificity (%) (95% CI)	97.9 (94.7–99.4)		98.9 (96.2–99.8)		97.7 (95.4–98.9)		98.8 (97.0–99.7)	
Negative predictive value (%) (95% CI)	99.5 (97.1–99.9)		98.4 (95.4–99.7)		99.1 (97.4–99.8)		98.2 (96.2–99.3)	
Positive predictive value (%) (95% CI)	87.9 (71.8–96.5)		93.9 (79.7–99.1)		85.7 (73.8–93.6)		92.9 (82.7–98.0)	

^a For initial results of prospective study only.

Typhi. For patients with a diagnosis of group D *Salmonella*, the sensitivity, negative predictive value, specificity, and positive predictive value (with 95% CI) of the SD RDT were 100% (89.6 to 100%), 100% (98.0 to 100%), 100% (98.0 to 100%), and 100% (89.6 to 100%), respectively. Presumably, these differences between initial and later results were due to observer error, and the initial diagnostic accuracy indices reflect a real-life situation in a busy clinical laboratory.

The median (range) delays between venipuncture and RDT result and between venipuncture and formal confirmation of bacterial identity were 2 days (0 to 13 days) and 3 days (0 to 12 days), respectively, for patients for whom we grew clinically significant GNRs (Table 2). For patients with confirmed *S. Typhi* bacteremia, the delays were 2 days (0 to 9 days) and 3 days (1 to 9 days), respectively. The median (range) number of days between diagnosis by RDT and reference assays between the two techniques were 2 (−4 to +10) days for all community-acquired isolates and 1 (−1 to +2) days for those with confirmed *S. Typhi*.

DISCUSSION

These results suggest that the combination of blood culture amplification of *S. Typhi* with an SD *S. Typhi* antigen RDT is promising as an accurate and inexpensive tool for the accelerated diagnosis of typhoid. This is suggested both by work with reference strains and in a real-life prospective evaluation of clinical samples of various bacterial concentrations in a routine diagnostic laboratory. The only consumables required are those for taking blood—a blood culture bottle, a venting needle, and gloves to safely remove blood culture fluid—as well as an RDT and a safe method for disposal of contaminated items. The combined cost of these items, as bought in or imported into Laos, is approximately \$4 for the examination of one blood culture bottle. In contrast, the laboratory consumables required to diagnose *S. Typhi* from blood culture using reference biochemical and serological techniques is approximately \$6/bottle. The latter estimate does not take into account the necessity for additional refrigerators and incubators, longer work hours, and more highly trained technicians. Whether a centrifuge to remove red blood cells is required for SD *S. Typhi* antigen rapid diagnostic testing remains uncertain. However, inexpensive manual centrifuges or fans adapted as centrifuges are available (<http://www.sciencewithamission.org/centrifuge.htm>). *S. Typhi* appears to be relatively tolerant of growth in tropical climates outside an incubator (Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit [LOMWRU], unpublished data). The disposal of contaminated consumables, especially the blood culture bottles, could be performed in the field with innovative solar-powered autoclaves (31). SD recommends that the SD *S. Typhi* antigen RDTs be stored at <30°C. In the field, the low-cost cool boxes developed for storage of malaria RDTs in Cambodia could be used (32). There were 5/221 (2.3%) apparent RDT reading errors in the prospective study. Regular training and a quality assurance system are required.

Tam et al. (22) adapted the Tubex test to detect *S. enterica* group D O9 antigen in blood culture bottles, correctly identifying 13/15 isolates. A PCR has recently been developed to detect *S. Typhi* in blood culture bottles after 3 h of incubation (10). A similar investigation of the accuracy of the SD RDT for diagnosis of *S. Typhi* from blood culture bottles in the Democratic Republic of the Congo has been described; the sensitivity and specificity were only 82.1% and 75.2%, respectively, but the low specificity

was mostly due to nontyphoidal *Salmonella* (O. Lunguya, B. Miwanda, E. Bonebe, M. F. Phoba, P. Gillet, S. Bertrand, J. J. Muyembe, J. Verhaegen, and J. Jacobs, presented at the 5ième Congrès International de Pathologie Infectieuse et Parasitaire, Kinshasa, Democratic Republic of the Congo, 4 to 6 November 2009).

Limitations of this study include the fact that only fluid from bottles showing evidence of GNRs was evaluated. Whether there is any reactivity of the RDTs with Gram-positive organisms or Gram-negative cocci was not determined, but it seems unlikely. If this assumption is correct, the demonstration of GNRs before performing RDTs on turbid blood cultures would not be necessary, obviating the need for a microscope and Gram staining, further facilitating use in the field. Although *S. Typhi* is the most common cause of enteric fever worldwide, the assay will not detect the other causes, *S. Paratyphi* A, B, and C. More importantly, we did not determine whether the antigen tests would detect *S. Typhi* before GNRs were seen by Gram staining of blood culture fluid or whether the RDT could detect *S. Typhi* antigen in blood or urine before culture. It is unclear whether the antigen detected is present predominantly as intact bacteria or from the disintegration of dead bacteria. The lysis of blood cells may assist the early antigen detection of *S. Typhi* (10). We did not assess the lower limit of detection of *S. Typhi* concentration in blood culture fluid, as the question posed was whether RDT antigen testing was a useful identification method when GNRs were first visible. RDTs were not performed on the same day that GNRs were seen for 14% of patients, and for 7 (3.2%) patients, until after reference assays were available. This was a consequence of a busy laboratory and reference assays being relied upon for the diagnosis. We would expect, in the absence of reference assays, that RDTs would be performed on the day GNRs were seen.

The use of RDT detection of *S. Typhi* antigen in blood cultures does not address the important issue that blood cultures detect *S. Typhi* in only ~40 to 80% of patients with typhoid (3, 9). However, if improved media optimized for growth of *S. Typhi* became available (9), without compromising the growth of other important causes of community-acquired septicemia, they, in combination with antigen testing, might enhance the practical diagnosis of typhoid. Also, importantly, antigen detection does not provide information on the antibiotic susceptibility profile of the infecting organisms. RDT-positive blood cultures could be sent to a reference laboratory for (delayed) susceptibility testing. However, with evidence that *P. falciparum* DNA can be extracted from malaria RDTs for detection of molecular markers of resistance (33, 34), it may be possible to send positive *S. Typhi* RDTs to a national center for PCR for *gyr* marker determination of fluoroquinolone resistance (35) using DNA extracted from RDTs. However, as Lao national policy for uncomplicated typhoid is 3 days of ofloxacin and fluoroquinolone-resistant *S. Typhi* remains extremely rare in Laos (23; LOMWRU, unpublished data), antibiotic susceptibility testing is currently much less important than making an etiological diagnosis. The fact that the SD antigen test was positive for other group D *Salmonella* strains is not important for individual patient management, as fluoroquinolones remain the recommended therapy.

The SMI test detects Vi antigen (SMI, personal communication). The fact that the SD RDT was positive with Vi-negative and -positive *S. Typhi*, *S. Enteritidis*, and *S. Ndolo* suggests that it detects the O9 antigen of group D *Salmonella*, as did the tech-

niques of Tam et al. (22) and Lim and Fok (36). Two of the three RDTs designed for detecting fecal *S. Typhi* did not detect *S. Typhi* in blood culture fluid. Vi-negative *S. Typhi* has been described (37), but all Lao *S. Typhi* isolates used here agglutinated with Vi antiserum. It is possible that Vi agglutination was reduced in the blood culture microenvironment, resulting in false-negative *S. Typhi* identification in the RDTs. Vi agglutination is reduced in media with high sodium chloride concentrations (>0.3 M NaCl) (37, 38), which may have a beneficial effect on the sensitivity of O9-based RDTs, as there may be fewer Vi-bearing, envelope-masking O antigens (20). However, the sodium chloride concentration of the blood culture medium used here was 0.5 g/100 ml (~ 0.09 M NaCl/liter), inconsistent with the hypothesis that Vi agglutination is reduced by high salt concentrations in these blood culture bottles. Jesudason et al. (20) found that a Vi-based latex agglutination test had 98.4% sensitivity and 100% specificity for *S. Typhi* in brain heart infusion broth, but the formulation was not given.

These data suggest that hospitals without fully equipped microbiology laboratories in areas where typhoid is endemic could use the combination of blood culture amplification and *S. Typhi* antigen RDTs for diagnosis. The technique could assist with the *in situ* diagnosis of outbreaks in rural communities far from microbiology laboratories. The evaluation of blood culture *S. Typhi* Ag assays should be repeated with different blood culture systems in different areas where typhoid is endemic and evaluated in the practical investigation of remote outbreaks.

Microbiology laboratory services have usually arisen *ad hoc* without evidence-based analysis of the most locally appropriate, cost-effective, and accurate techniques or evaluation of their impact. There is a great need for discussion as to the most locally appropriate techniques for the vast populations without access to infectious disease diagnostics (39–41). Antigen-detecting RDTs/latex tests have been shown to detect *Streptococcus pneumoniae* (42, 43), *Staphylococcus aureus* (44) and *B. pseudomallei* (45) in blood culture fluid. A panel of region-appropriate pathogen antigen-detecting RDTs, combined with simple staining and biochemical and serological algorithms (46) with inexpensive blood culture bottles, could be a potential accurate and accelerated but inexpensive diagnostic strategy for clinical laboratories in resource-poor countries.

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