Typhoid fever remains a major global health problem. A major impediment to improving outcomes is the lack of appropriate diagnostic tools, which have not significantly improved in low-income settings for 100 years. We evaluated two commercially available rapid diagnostic tests (Tubex and TyphiDot), a prototype (TyphiRapid TR-02), and the commonly used single-serum Widal test in a previously reported high-burden area of Papua New Guinea. Samples were collected from 530 outpatients with axillary temperatures of $\geq 37.5^\circ C$, and analysis was conducted on all malaria-negative samples ($n = 500$). A composite reference standard of blood culture and PCR was used, by which 47 participants (9.4%) were considered typhoid fever positive. The sensitivity and specificity of the Tubex (51.1% and 88.3%, respectively) and TyphiDot (70.0% and 80.1%, respectively) tests were not high enough to warrant their ongoing use in this setting; however, the sensitivity and specificity for the TR-02 prototype were promising (89.4% and 85.0%, respectively). An axillary temperature of $\geq 38.5^\circ C$ correlated with typhoid fever ($P = 0.014$). With an appropriate diagnostic test, conducting typhoid fever diagnosis only on patients with high-grade fever could dramatically decrease the costs associated with diagnosis while having no detrimental impact on the ability to accurately diagnose the illness.
previously reported high incidence of typhoid in the highlands of PNG, the ongoing transmission of the disease, and the need to determine the best diagnostic strategy in PNG, we evaluated two commercially available typhoid serodiagnostic test kits (TubeXTF, manufactured by IDL Biotech AB, Sweden, and TyphiDot, manufactured by Reszon Diagnostics International Sdn. Bhd, Malaysia); one prototype kit, TR-02 (Reszon Diagnostics International Sdn. Bhd, Malaysia); and the currently used Widal test. The TubeXTF test detects serum anti-\textit{Salmonella} O9 (lipopolysaccharide) IgM and IgG antibodies in an enzyme-linked immunosorbent assay (ELISA)-based assay (16). The TyphiDot assay is a dot enzyme immunoassay which detects serum anti-\textit{Salmonella} IgM and IgG antibodies specific for a 50-kDa outer membrane protein (OMP) antigen (6, 12). The TR-02 prototype is an immunochromatography assay which detects serum IgM (but not IgG) antibodies to the same \textit{Salmonella} OMP antigen used in the TyphiDot assay. We used a composite reference standard of blood culture and real-time PCR to evaluate the diagnostic kits.

**MATERIALS AND METHODS**

**Participant recruitment and specimen collection.** Participant recruitment took place at the Goroka General Hospital outpatient center and the Lopi Urban Clinic in Goroka, Eastern Highlands Province, Papua New Guinea. Febrile patients (axillary temperature, \(\geq 37.5^\circ\text{C}\)) who reported having fever for at least 2 days were invited to participate in the study. In addition, patients with an absence of fever at the time of consultation, or self-reported fever for less than 2 days prior to consultation, were included in the study if there was clinical suspicion of typhoid fever by the study staff or senior clinical staff at the study sites. All patients were informed of the study aims, and following informed consent, a brief questionnaire was administered. The questionnaire sought information regarding clinical symptoms and prior antimicrobial treatment. Up to 25 ml of blood was collected from adults, and 3 to 5 ml was collected from children \(\leq 5\) years old. A blood film was prepared for malaria microscopy. Blood, inoculated blood culture bottles (see below), and the blood smear were transported to the laboratory within 2 h of collection. Upon receipt of samples at the laboratory, serum separator tubes were centrifuged at 3,000 \(\times\) \(g\) for 10 min and aliquots of serum were stored at \(-20^\circ\text{C}\) for later serological testing. Aliquots of whole blood were also stored at \(-20^\circ\text{C}\) for later DNA extraction.

**Malaria diagnosis.** Thick and thin blood films were fixed, Giemsa stained, and read according to standard methods (5) by experienced microscopists. Malaria-positive samples were excluded from further analysis on the assumption that malaria was the primary cause of fever.

**Blood culture.** Immediately following blood collection, 5 ml of blood was added to 45 ml of tryptic soy broth with sodium polyanethol sulfonate, as described by Gratten (10). This methodology has been successfully used in our laboratory to culture a variety of organisms (9). Blood cultures were incubated at 37°C, and after 24 h of incubation, approximately 100 \(\mu\)l of blood culture broth was plated onto blood agar, chocolate agar, MacConkey agar, and xylene-lysin deoxycholate agar. Plates were incubated at 37°C for 24 h and examined for growth. Blood agar and chocolate agar plates were incubated in a 5% carbon dioxide-enriched atmosphere and incubated for 24 to 48 h to detect other causes of bacteraemia. Subculture of the blood culture broth onto solid medium was repeated after 3 days and 7 days of blood culture bottle incubation. Blood cultures were considered negative and discarded if there was no growth on solid medium inoculated on day 7. This is largely consistent with WHO guidelines for \textit{S. Typhi} culture (30).

**Serological tests.** Batches of sera were removed from the freezer, and all serological tests were conducted on sera within 24 h of thawing. The Widal test was conducted using \textit{S. Typhi} O antigen (Remel Europe Ltd.). Using a 6-well plate, patient serum was diluted in \textit{S. Typhi} O antigen to produce serum titers of 40, 80, 160, and 320. The serum and antigen were allowed 1 min to agglutinate before reading. This method is standard procedure in PNG. The TubeXTF, TyphiDot, and prototype TR-02 diagnostic tests were conducted according to the manufacturer’s instructions. Results for all serological tests were double read.

**Molecular detection.** DNA extraction was conducted from 200 \(\mu\)l of whole blood using the Qiagen DNeasy blood and tissue extraction kit, according to the manufacturer’s instructions for extraction from blood samples.

Real-time PCR analysis was conducted on all malaria-negative samples using a previously described hydrolysis probe assay for \textit{S. Typhi} (18). This assay targets a 73-bp region of the \textit{S. Typhi} H1-d flagellin gene using the following primers and probe: forward primer ST5 (5’-CAA CCT GGG CAA TAC CGT AAA TAA-3’), reverse primer ST6A (5’-TTG GTG TGC GTA GTC GGA AT-3’), and dually labeled probe ST7 (5’-β-HEX-TG-TCT TCT GGC CGT AGC GGT ATC G-BHQ1-3’). The real-time PCR was conducted in a 20-\(\mu\)l reaction mix containing 2 \(\mu\)l of the DNA extract, 400 nM (each) ST5 and ST6A primers, 400 nM TaqMan probe ST7, 1× QuantiTect Mastermix (Qiagen), and nuclease-free water. All reactions were conducted on a Bio-Rad CFX-96 real-time system with the following cycling parameters: 50°C for 2 min, followed by 40 cycles of 94°C for 1 min and 60°C for 1 min. Negative and positive controls were included in each run. A positive result was defined as a sample having a cycle threshold \((C_T)\) between 16 and 40 using an autocalculated single-threshold baseline using the Bio-Rad CFX Manager software version 2.0.

The specificity of this real-time PCR assay has been previously described, and it was found to be specific for \textit{S. Typhi}. It does not amplify closely related bacteria, including \textit{Salmonella enterica} serovars Paratyphi, Typhimurium, Choleraesuis, andEnteritidis, or non-\textit{Salmonella} species 

**RESULTS**

**Participants.** A total of 530 patients with axillary temperatures of \(\geq 37.5^\circ\text{C}\) for at least 2 days and/or clinical suspicion of typhoid fever were recruited over a period of 18 months from March 2009 to September 2010. Of the 530 participants, 321 (60%) were en-
TABLE 1  Sensitivity, specificity, PPV, and NPV of typhoid fever diagnostic tests, using blood culture and a composite reference standard (blood culture and real-time PCR) as comparators

| Test                                      | % (95% confidence interval) |
|-------------------------------------------|----------------------------|
| Blood culture                             | Sensitivity | Specificity | PPV       | NPV       |
| Tubex                                     | 77.3 (59.8–94.8) | 87.4 (84.5–90.4) | 0.221 (0.128–0.313) | 0.988 (0.978–0.998) |
| TyphiDot                                  | 95.5 (86.8–104) | 79.1 (75.4–82.7) | 0.174 (0.106–0.241) | 0.997 (0.992–1.000) |
| TR-02                                     | 100          | 81.6 (85.1–78.1) | 0.200 (0.125–0.275) | 1          |
| Widal (titer, 160)                        | 86.4 (72.0–100.7) | 95.0 (93.0–96.9) | 0.442 (0.293–0.590) | 0.993 (0.986–1.001) |

Blood culture plus PCR (composite reference standard)

| Test                                      | % (95% confidence interval) |
|-------------------------------------------|----------------------------|
| Tubex                                     | 51.1 (36.8–65.4) | 88.3 (85.3–91.2) | 0.312 (0.208–0.415) | 0.946 (0.924–0.967) |
| TyphiDot                                  | 70.0 (52.4–79.5) | 80.1 (76.5–83.8) | 0.256 (0.178–0.334) | 0.958 (0.938–0.978) |
| TR-02                                     | 89.4 (80.3–98.2) | 85.0 (81.7–88.3) | 0.382 (0.291–0.473) | 0.987 (0.976–0.998) |
| Widal (titer, 160)                        | 51.1 (36.8–65.4) | 95.8 (94.0–97.7) | 0.358 (0.410–0.707) | 0.950 (0.930–0.970) |

TABLE 2  Sensitivity, specificity, PPV, and NPV of typhoid fever diagnostic tests when used on patients with axillary temperatures of ≥38.5°C (using a composite reference standard of blood culture and real-time PCR as the comparator)

| Test                                      | % (95% confidence interval) |
|-------------------------------------------|----------------------------|
| Blood culture                             | Sensitivity | Specificity | PPV       | NPV       |
| Tubex                                     | 52.0 (32.4–71.6) | 90.6 (86.0–95.1) | 0.464 (0.280–0.649) | 0.923 (0.881–0.965) |
| TyphiDot                                  | 64.0 (45.2–82.8) | 81.8 (75.8–87.8) | 0.356 (0.226–0.495) | 0.935 (0.894–0.976) |
| TR-02                                     | 92.0 (81.4–100.3) | 85.5 (80.1–91.0) | 0.500 (0.356–0.644) | 0.986 (0.966–1.01)  |
| Widal (titer, 160)                        | 40.0 (20.8–59.2) | 97.5 (95.1–99.9) | 0.714 (0.478–0.951) | 0.912 (0.869–0.954) |
garding the true utility of PCR as a diagnostic tool for typhoid fever. The validity of the debate is evident in the current study, where seven of 22 (32%) blood culture-positive samples were PCR negative. Nga and colleagues also demonstrated that blood samples that were culture positive could be PCR negative, where 58% of culture-positive samples were negative using detection by real-time PCR. In contrast to our findings, Nga et al. did not detect S. Typhi (or S. Paratyphi A) in any blood samples with symptoms of enteric (typhoid) fever that were culture negative (23). However, other researchers have detected S. Typhi in culture-negative blood by using regular, nested, and real-time PCR platforms (4, 11, 18, 22). Our data suggest that PCR is useful as a complementary form of diagnosis in the context of diagnostic evaluation and provides the advantage of being able to detect nonviable cells (e.g., in patients who have recently commenced antibiotics). However, for reasons previously outlined PCR is not currently applicable for routine diagnostic use (2).

Typhoid fever diagnostic evaluations have consistently demonstrated less than optimal sensitivity and specificity of rapid tests (26). When using blood culture as the reference standard, we found the sensitivity and specificity of the Tubex and TyphiDot tests to be comparable to those in previous reports (13, 14, 25). However, these assays performed less satisfactorily when evaluated against the composite reference standard. In contrast, the performance of the prototype diagnostic test (TR-02) was promising when evaluated against the composite reference standard, having both a sensitivity and a specificity of ≥85%.

The correlation between typhoid fever and high-grade fever (axillary temperature of ≥38.5°C) may have important implications for typhoid fever diagnosis. When study participants with low-grade fever were excluded from analysis, the sensitivity and specificity of the assay improved, albeit marginally. In our study, 22 patients with confirmed typhoid fever would be excluded from initial testing if such a testing algorithm were adopted. However, this is from a total of 316 excluded patients (i.e., 6.96% positive): of the 184 patients who would be tested for typhoid fever, 25 (13.59%) were positive. In a clinical setting, considerable money could be saved on diagnostic tests if only patients with a current fever of ≥38.5°C (axillary temperature) were tested for typhoid fever. Patients not tested would need to be advised to present in 2 to 3 days if symptoms persisted to minimize the number of true cases not correctly diagnosed. On the basis of our findings, there could be a reduction of 63% in the number of tests required, with no compromise in the ability to accurately diagnose typhoid fever.

It has been speculated that the lack of specificity of some rapid diagnostic tests might be in part due to the inability of blood culture to detect all active infections (13), i.e., the rapid diagnostic kits may be correctly diagnosing an active typhoid case when blood culture is negative. The use of both blood culture and PCR to detect S. Typhi infection is no guarantee that all true positive cases will be detected, but it did increase the rate of detection. However, in our study the specificity of the rapid diagnostic tests evaluated did not improve when the composite reference standard was used, as opposed to blood culture alone. This suggests some inherent specificity issues with the currently available typhoid fever diagnostic assays.

In the primary health care setting, none of the established typhoid rapid diagnostic tests is sufficiently sensitive to warrant their immediate routine use in PNG. Similar findings have been observed in Bangladesh (21) and sub-Saharan Africa (14), although under certain circumstances, it has been suggested that the use of rapid diagnostic tests with known limitations might be of some value (7). On the basis of our findings, the TR-02 prototype warrants further evaluation for potential routine use in PNG and other settings. Moreover, the TR-02 is user-friendly, being a solid-phase immunochromatographic assay (similar to a rapid pregnancy test or a malaria RDT); can be stored at 20 to 25°C; is rapid (~10 min); and is easy to interpret. It detects the same anti-Salmonella antibodies as does the TyphiDot assay but has better sensitivity and specificity. This is presumably a function of the different immune platforms on which the tests are based. Another difference is that the TR-02 detects only IgM, whereas the TyphiDot and Tubex detect both IgM and IgG. The TR-02 prototype and the TyphiDot assay have recently become available through Reszon Diagnostics International Sdn. Bhd (Malaysia); the prototype TR-02 is marketed under the name TyphiDot Rapid (or Typhi Rapid).

Typhoid fever diagnostic evaluations conducted on hospitalized patients (13, 25) provide little insight into the application of the diagnostic tests in the community health care setting. However, it is the primary health care level where sensitive, specific, rapid, cheap, and user-friendly typhoid diagnostic kits are most required. The clinical differentiation of typhoid fever from other causes of febrile illness present in regions where typhoid is endemic (e.g., malaria, dengue and other arbovirus infections, rickettsial infections, and leptospirosis) is difficult, but the treatments differ markedly (20, 26). Moreover, with the rollout of malaria rapid diagnostic tests through the Global Fund Initiative, there may be an increased need for diagnosis of other causes of febrile illness if the guidelines are adhered to and no malaria treatment is given to malaria RDT-negative patients. It is in this context that high-grade fever is important: in areas of malaria and typhoid endemicity where a malaria RDT yields a negative result, there may be clinical signs and symptoms such as severity of fever that can help determine the value of conducting a typhoid diagnostic test without negatively impacting patient outcome.

It is likely that the Widal test will remain in use in PNG and many other low-income settings for the foreseeable future, despite its known limitations in such settings (17). We have demonstrated that using the locally recommended cutoff titer of 160 gives rise to a test with poor sensitivity, albeit high specificity. Lowering the cutoff titer may improve sensitivity but decrease specificity. If use of the Widal test is continued in PNG, a multicenter study should be considered to determine background titers in asymptomatic patients and to further investigate the cutoff titer which is optimized for both sensitivity and specificity.

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