Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites

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Objective To evaluate three commercial typhoid rapid antibody tests for *Salmonella* Typhi antibodies in patients suspected of having typhoid fever in Mpumalanga, South Africa, and Moshi, United Republic of Tanzania.
Methods The diagnostic accuracy of Cromotest® (semiquantitative slide agglutination and single tube Widal test), TUBEX® and Typhidot® was assessed against that of blood culture. Performance was modelled for scenarios with pretest probabilities of 5% and 50%.
Findings In total 92 patients enrolled: 53 (57.6%) from South Africa and 39 (42.4%) from the United Republic of Tanzania. *Salmonella* Typhi was isolated from the blood of 28 (30.4%) patients. The semiquantitative slide agglutination and single-tube Widal tests had positive predictive values (PPVs) of 25.0% (95% confidence interval, CI: 0.6–80.6) and 20.0% (95% CI: 2.5–55.6), respectively. The newer typhoid rapid antibody tests had comparable PPVs: TUBEX®, 54.1% (95% CI: 36.9–70.5); Typhidot® IgM, 56.7% (95% CI: 37.4–74.5); and Typhidot® IgG, 54.3% (95% CI: 36.6–71.2). For a pretest probability of 5%, PPVs were: TUBEX®, 11.0% (95% CI: 6.6–17.9); Typhidot® IgM, 9.1% (95% CI: 5.8–14.0); and Typhidot® IgG, 11.0% (6.3–18.4). For a pretest probability of 50%, PPVs were: TUBEX®, 70.2% (95% CI: 57.3–80.5); Typhidot® IgM, 65.6% (95% CI: 54.0–75.6); and Typhidot® IgG, 70.0% (95% CI: 56.0–81.1).
Conclusion Semiquantitative slide agglutination and single-tube Widal tests performed poorly. TUBEX® and Typhidot® may be suitable when pretest probability is high and blood cultures are unavailable, but their performance does not justify deployment in routine care settings in sub-Saharan Africa.

Abstracts in عربي, 中文, Français, Русский and Español at the end of each article.

Introduction

Typhoid fever remains an important cause of disease in developing countries. In 2002, it caused an estimated 408 837 episodes of illness in Africa.[1] *Salmonella* Typhi, the causative agent, is most frequently isolated from blood during the first week of illness but can also be isolated during the second or third week of illness, during the first week of antimicrobial therapy and during clinical relapse.[2] Isolation of *Salmonella* Typhi from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this requires equipment, supplies and trained laboratory personnel seldom found in primary health-care facilities in the developing world.[3,4] Blood culture is a more practical albeit less sensitive alternative to bone marrow culture. However, it is not always available and, when it is, it takes 2 to 3 days. As a result, diagnosis may be delayed or overlooked and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment. For this reason, in developing countries typhoid rapid antibody tests can facilitate diagnosis and disease management.

New commercially available typhoid rapid antibody tests have been evaluated in Asia, where typhoid fever is known to be highly endemic.[1,5] In Asian studies the tests have shown variable performance. While the TUBEX® test was the most sensitive and specific in the Philippines,[6] neither TUBEX® nor Typhidot® was both sensitive and specific in two evaluations undertaken in Viet Nam[7,8] and performance was poor in a trial conducted in a community clinic in Bangladesh[9] and in a study in Egypt in which it was compared with a new ELISA not yet commercially available.[10] Rapid typhoid tests have not been evaluated in sub-Saharan Africa, where the typhoid fever burden may be smaller than in Asia.[11–13] The World Health Organization (WHO) has issued no recommendations on the use of typhoid rapid antibody tests.[14] Accurate diagnostics for typhoid fever could provide valuable diagnostic information for patient management and make it possible to estimate the incidence of typhoid fever in low-resource settings.

We evaluated three diagnostic kits that are commercially available internationally using four rapid methods for detecting antibodies to *Salmonella* Typhi (typhoid rapid antibody tests) and used blood culture as the standard for comparison.

Methods

Participants

Patients were recruited from two sub-Saharan African sites: Mpumalanga province, South Africa, and Moshi, United Republic of Tanzania. They were selected to represent patients from southern and eastern Africa, respectively.

The recruitment method differed between sites. In South Africa, we enrolled subjects suspected of having typhoid fever; in the United Republic of Tanzania, we enrolled patients who were participants in a study on the etiology of febrile illness.

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(*Submitted: 23 February 2011 – Revised version received: 25 May 2011 – Accepted: 26 May 2011 – Published online: 13 June 2011*)

Patients were recruited at both sites between 2007 and 2009. In the South African site we obtained blood from suspected typhoid fever cases reporting no current use of antimicrobials who presented to Rob Ferreira Hospital (RFH), in Nelspruit, Mpumalanga province, or to hospitals referring patients to RFH. In the United Republic of Tanzania site, we obtained blood from consecutive febrile inpatients admitted to Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Hospital.[15,16] At both sites we incubated the blood in a continuously monitored blood culture system (Bac-T Alert, bioMérieux, Marcy L'Étoile, France). Bottles flagged as positive by the instrument were removed for subculture and identification by standard techniques.[17]

In both study sites, we enrolled patients who presented with a febrile illness suspected of being typhoid fever. We collected data on those patients who fulfilled the clinical criteria for suspected typhoid fever (a history of fever or demonstrated pyrexia [body temperature > 38 °C.]) before performing the index test and blood culture.

Test methods

In both study sites a typhoid fever case was defined as being a patient whose blood culture was positive for *Salmonella* Typhi. Patients whose blood cultures were negative or yielded pathogens other than *Salmonella* Typhi were used as controls. We drew additional blood and separated the serum, which was stored at −20 or −80 °C in cryotubes and shipped on dry ice to the Enteric Diseases Reference Unit, National Institute of Communicable Diseases (Sandringham, South Africa), for evaluation with typhoid rapid antibody tests. We screened the serum using the semiquantitative slide agglutination and tube Widal tests, TUBEX® and the typhidot test. Laboratory staff were blinded to the blood culture results, which were reviewed only after testing was completed.

Typhoid rapid antibody tests were carried out according to manufacturers' instructions. Test characteristics are summarized in Table 1. Laboratory personnel, trained in the use of all tests, recorded information about the ease of use and non-kit consumables and equipment required for each test. Because the cost of consumables, equipment and personnel differed between the two study sites, we did not calculate the cost of the tests.

Linear Cromotest® (Linear Chemicals, Barcelona, Spain)

This test, derived from *Salmonella* Typhi O and H antigens, was performed in two ways: (i) as a semiquantitative slide agglutination test with visual examination as per the package insert; (ii) as a Widal test[18] performed with a single tube, as described by Parry et al.[19] The presence or absence of visible agglutination indicates the presence or absence of the corresponding antibody to the O and H antigens of *Salmonella* Typhi. We defined the positivity cut-off point for the slide and tube agglutination reactions for both O and H antigens as antibody titres ≥ 1:80.

IDL TUBEX® TF (IDL Biotech AB, Bromma, Sweden)

This semiquantitative colorimetric test detects anti-O:9 antibody titres in patient specimens on visual examination.[20] A positive TUBEX® result was defined as a reading of ≥ 4, as per manufacturer's instructions. The manufacturers warn that the test may have to be repeated after 48 hours if indeterminate results are obtained.

Typhidot® (Malaysian Biodiagnostic Research, Bangi, Malaysia)

This qualitative antibody detects the presence of IgM and IgG antibodies to a 50kDa outer membrane protein.[21] A positive Typhidot® result (IgG and IgM) was defined as a visible reaction of an intensity equal to or greater than that of the control reaction on the commercially prepared filter paper. The manufacturers warn that if indeterminate results are obtained, the test may have to be repeated after 48 hours.

Statistical methods

Data were captured into Excel 2003 (Microsoft Corporation, Redmond, United States of America) and converted to STATA version 11 (StataCorp. LP, College Station, USA), in which analysis was performed by Stat/Transfer version 10 (Circle Systems, Seattle, USA). Stata's *diagt* command was used to determine each test's sensitivity, specificity and positive and negative predictive values (PPV and NPV, respectively),[22] which are presented with the understanding that exposure to antimicrobials could have affected the final results. Analysis was performed at a two-sided significance level of 5%. Pretest probabilities of background typhoid fever rates were also calculated at 5% and 50% to ensure that the results were applicable even in conditions of few typhoid fever outbreaks – since incidence would be higher during outbreaks – and of lower endemicity, given that study patients were selected on the probability of having typhoid fever.

Research ethics

The NICD has blanket ethics clearance in relationship to its surveillance duties (M06–04–49), but further approval was obtained from the Committee for Research on Human Subjects (CRHS) at the University of the Witwatersrand to update CRHS on this aspect of typhoid fever surveillance.

The part of the study conducted in the United Republic of Tanzania was approved by the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, the United Republic of Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an institutional review board of Duke University Medical Center.

Results

Participants

Ninety-two patients were enrolled: 53 (58%) in South Africa (between 25 May 2007 and 10 November 2009) and 39 (42%) in the United Republic of Tanzania (between 17 September 2007 and 25 August 2008). Participants had a median age of 24 years (range: < 1 to 96). Twenty-five (27%) patients (23 South African and 2 Tanzanian) were under the age of 15 years; the ages of two participants (2.2%) were unknown. Forty-two (46%) patients were female; the sex of two (2%) was not available. Thirty-six (39%) blood cultures grew a pathogen; 28 (78%) of these cultures grew *Salmonella* Typhi. Other pathogens isolated included *Salmonella* Typhimurium, *Streptococcus pneumoniae, Staphylococcus aureus* and *Mycobacterium tuberculosis* (one culture each) and *Cryptococcus neoformans* (four cultures). Of the 92 blood cultures, 52 (57%) were negative and 4 (4%) grew organisms considered to be contaminants.

In compliance with eligibility criteria, no South African patient was taking antimicrobials at the time of the blood culture. Of the 20 Tanzanian patients

Table 1. **Comparative characteristics of three rapid tests for the detection of *Salmonella* Typhi antibodies**

Characteristic	Cromotest® – semi-quantitative slide agglutination	Cromotest® – single tube Widal	TUBEX®	Typhidot®
Antibody	IgM and IgG	IgM and IgG	IgM	IgM or IgG
Antigen	O and H	O and H	O9	OMP
Turnaround time per test	2 minutes at room temperature	O: 4 hours at 50 °C H: 2 hours at 50 °C	3 minutes at room temperature	60 minutes at room temperature
Storage temperature (°C)	2–8	2–8	2–8	2–8
Equipment supplied by manufacturer	Febrile antigen Positive control Negative control	Febrile antigen Positive control Negative control	Colour scale Blue and brown reagent Negative control Positive control Reaction well strip Sealing tape Coloured sticker Timer	Predotted antigen strips Sample diluent Washing buffer Prediluted anti-human IgM and IgG Substrate A and B Positive control Negative control Worksheet
Equipment supplied by laboratory	Disposable slides Saline solution	Thermostatic waterbath (30–50 °C) Disposable sterile glass tubes (12 × 100 mm) Disposable stirrers Saline solution Mechanical stirrer	Precision pipette Vortex	Measuring cylinder Micropipettes and tips Conical flask Forceps, wash bottle Filter paper, distilled water Rocker platform Aspirator Aluminium foil Dark reagent bottle/flask covered with aluminium foil
User comments	Particles present before adding the antisera, rendering false-positive results. Simple to use and inexpensive.	Particles present before adding the antisera, rendering false-positive results. Requires costly additional laboratory equipment.	Subjective interpretation of colour reactions. Haemolysis may result in difficulty in interpretation. Simple to use and limited need for additional laboratory equipment.	More complex assay requiring additional steps and preparation of consumables. Interpretation may be affected, as IgG can persist for more than 2 years after typhoid infection. Detection of specific IgG cannot differentiate between acute and convalescent cases. Requires costly additional laboratory equipment.

OMP, outer membrane protein.

whose blood cultures were negative for *Salmonella* Typhi, 2 (10%) had received trimethroprim–sulfamethoxazole and 8 (40%) had received quinine or sulfadoxine-pyrimethamine or had an unknown history of antimicrobial exposure. Of 19 Tanzanian patients with blood cultures positive for *Salmonella* Typhi, 17 (89%) had received antibacterials or antimalarials or had an unknown history of antimicrobial exposure.

Test results

Blood cultures were done as soon as patients were admitted to hospital. Serological tests were performed within the subsequent 6 months at the South African site and within 2 years at the Tanzanian site. Table 1 shows the characteristics of the three assays, the equipment required to perform each test and the results of the technologists' assessments regarding ease of use and perceived laboratory costs. None of the sera gave indeterminate results.

Estimates

Sensitivity, specificity and predictive values are shown in Table 2. Of 28 patients with a blood culture positive for *Salmonella* Typhi, 1 (4%) was positive on the Cromotest® semiquantitative slide O test; 14 (50%) were positive on the Cromotest® semiquantitative slide H test; 2 (7%) were positive on the Cromotest® Widal O agglutination test; 4 (14%) were positive on the Cromotest® Widal H agglutination test; and 19 (68%) were positive on the TUBEX® test. Of 27 patients with a blood culture positive for *Salmonella* Typhi with sufficient serum available for testing, 19 (70%) were positive on the Typhidot® IgG test and 17 (63%) on the Typhidot® IgM test. The positive and negative predictive values for each of the pretest probability calculations are presented in Table 3.

Discussion

All four typhoid rapid antibody tests performed poorly compared with blood culture. Some tests performed better than others, but none stood out in all respects. In sub-Saharan Africa, cost and ease of use are important considerations in addition to diagnostic accuracy.

The single-tube Widal and Typhidot® tests were found to require the most non-kit laboratory supplies, consumables

Table 2. **Sensitivity, specificity and predictive values of four rapid diagnostic tests for typhoid fever as determined by comparison with blood culture results**

Kit	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Cromotest® O : semiquantitative slide agglutination	95.2 (86.5–99.0)	3.6 (0.1–18.3)	25.0 (0.6–80.6)	68.6 (57.7–78.2)
Cromotest® H : semiquantitative slide agglutination	80.3 (68.2–89.4)	50.0 (30.6–69.4)	53.8 (33.4–73.4)	77.8 (65.5–87.3)
Cromotest® O: single tube Widal	87.3 (76.5–94.4)	6.9 (0.8–22.8)	20.0 (2.5–55.6)	67.1 (55.8–77.1)
Cromotest® H: single tube Widal	95.2 (86.5–99.0)	13.8 (3.9–31.7)	57.1 (18.4–90.1)	70.2 (59.3–79.7)
TUBEX®	73.0 (60.3–83.4)	69.0 (49.2–84.7)	54.1 (36.9–70.5)	83.6 (71.2–92.2)
Typhidot® IgM	75.0 (61.1–86.0)	60.7 (40.6–78.5)	56.7 (37.4–74.5)	78.0 (64.0–88.5)
Typhidot® IgG	69.2 (54.9–81.3)	70.4 (49.8–86.2)	54.3 (36.6–71.2)	81.8 (67.3–91.8)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

and equipment, and this increased the overall cost of the test. The semiquantitative slide agglutination and TUBEX® tests had shorter turnaround times than the Widal tube and Typhidot® tests. However, the results of all the tests were available the same day the specimen was received in the laboratory.

Of the four tests evaluated, the semiquantitative slide agglutination test performed the worst. It had very poor specificity and low PPV and NPV, even though it was performed under optimal conditions in a national reference laboratory. This poor performance was further compounded by substantial inter-test variability, which suggests that in a field situation results would not be comparable between sites.[23] Hence, the slide agglutination test should not be used as a diagnostic tool. Although the sensitivity and specificity of the H slide agglutination test appeared to be greater, this was offset by the inconsistent results obtained with the O slide agglutination. Others have noted this disparity between the sensitivity and specificity of the Widal test containing O and H antigens.[19]

The single-tube Widal agglutination test also performed poorly. The original Widal agglutination test was described using paired sera obtained 10 days to 2 weeks apart and examined for a twofold or greater change in titre.[18] It is possible that the Widal test would have performed better in our study had we used paired sera, but we chose to apply the test under the conditions normally found in clinical practice. In our experience, patients rarely return for outpatient follow-up once treated, so that obtaining paired sera in a routine clinical setting is unlikely. Recently, the use of paired sera has been re-examined and has been shown to improve both the sensitivity and specificity of serological tests for typhoid fever.[24]

Both the TUBEX® and Typhidot® tests had lower sensitivity than the semiquantitative slide agglutination and the Widal tests, but they had considerably greater specificity. In our setting, TUBEX® had marginally less sensitivity but more specificity than the Typhidot® IgM test and it had a slightly better PPV. Typhidot® IgG was comparable to TUBEX® with respect to sensitivity, specificity and PPV, but none of these tests performed as well as the blood culture comparator assay.

This study had several limitations. Typhoid fever was confirmed by blood culture in almost one third of the study participants, a much larger proportion than expected under field conditions in sub-Saharan Africa, where typhoid fever is relatively uncommon.[12,13] Lowering the pretest probability for typhoid fever to 5% further degraded the performance characteristics of the typhoid rapid antibody tests (Table 3), which suggests that these tests would not be useful in routine diagnostic situations. At a pretest probability of 50%, higher than the actual fraction of blood-culture-positive cases used in this evaluation, the performance of the new rapid antibody tests improved. Hence, these tests can perhaps be judiciously used during outbreaks.

The time elapsed between the onset of symptoms and serum collection can affect the performance of antibody-based tests.[25] We did not analyse this aspect to reflect how the tests would be used under routine health-care conditions in sub-Saharan Africa. Similarly, human immunodeficiency virus (HIV) infection is highly prevalent in sub-Saharan Africa[26] and we enrolled participants without

Table 3. **Predictive values of four rapid diagnostic tests for typhoid fever as determined by comparison with blood culture results under assumed pretest probabilities of 5% and 50%**

Kit	Pretest probability			
	5%		50%	
	PPV % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Cromotest® O : semiquantitative slide agglutination	4.9 (4.5–5.4)	93.3 (60.4–99.2)	49.7 (47.4–51.9)	42.5 (7.4–87.2)
Cromotest® H : semiquantitative slide agglutination	7.8 (5.4–11.1)	98.0 (96.3–98.9)	61.6 (52.1–70.4)	71.8 (57.6–82.6)
Cromotest® O: single tube Widal	4.7 (4.1–5.4)	91.2 (70.0–97.9)	48.4 (45.0–51.8)	35.2 (10.9–70.6)
Cromotest® H: single tube Widal	5.5 (4.7–6.4)	98.2 (92.8–99.6)	52.5 (48.6–56.3)	74.0 (40.5– 2.3)
TUBEX®	11.0 (6.6–17.9)	98.0 (96.8–98.7)	70.2 (57.3–80.5)	71.9 (61.4–80.4)
Typhidot® IgM	9.1 (5.8–14.0)	97.9 (96.4–98.8)	65.6 (54.0–75.6)	70.8 (58.2–80.9)
Typhidot® IgG	11.0 (6.3–18.4)	97.8 (96.4–98.6)	70.0 (56.0–81.1)	69.6 (58.7–78.6)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

reference to their HIV serostatus to reflect field conditions, although many of our patients could have been HIV-infected. HIV infection rates among 1504 adult outpatients tested in Nelspruit (RFH) were reportedly as high as 45% in 2010 (G Hoyland, personal communication). The prevalence of HIV infection among participants in the study on febrile illness at KCMC and Mawenzi Regional Hospital was 39% for adolescents and adults and 12% for infants and children.[15,16] Although recent studies at KCMC have shown that HIV appears to protect against typhoid fever, disease may still occur in HIV-infected individuals.[15] It is possible that HIV-associated immune dysregulation affects the production of antibodies specific to *Salmonella* Typhi outer membrane proteins, present in both the Typhidot® and the older Widal tests. This has been observed in patients infected with invasive non-typhoidal *Salmonella* (NTS).[27] This theoretical effect can also impair antibody binding in the TUBEX® test, which is based on the O9 antigen. The production of antibodies against *Salmonella* lipopolysaccharide (LPS) is increased in patients with invasive NTS infection who are also HIV-infected. If antibody production were also higher in HIV+ typhoid fever patients, TUBEX® would have performed better than the other typhoid rapid antibody tests, but it did not.

The sensitivity of blood culture is known to be less than 100%, even in the absence of antimicrobial exposure, and is further reduced by patient antimicrobial use. Although two Tanzanian patients had been exposed to antibacterials, they represented only 3.8% of the 52 patients whose blood cultures were negative for *Salmonella* Typhi. These patients probably affected our results very little. Furthermore, blood culture sensitivity was optimized in our study because we used modern blood culture techniques.[28]

Our findings on the Widal test and the newer typhoid rapid antibody tests are similar to those from studies conducted in Asia and Egypt;[8–10,25,29,30] none of the rapid tests performed nearly as well as blood culture for the diagnosis of typhoid fever. Some reports suggest that the Typhidot® test may be more useful in Asia.[31,32] However, the true incidence of typhoid fever in the catchment population differed in these studies and in ours. The pretest probability of typhoid fever was artificially elevated in our evaluation because we included South African patients suspected of having typhoid fever and specifically analysed a subset of antisera from the United Republic of Tanzania in which half of the cases were known to have typhoid fever. The earlier studies also focused on paediatric populations and allowed for inclusion of microbiologically unconfirmed typhoid fever.[31,32]

NTS bacteraemia, which is predominantly caused by *Salmonella* serotypes Typhimurium and Enteritidis, is much more common in sub-Saharan Africa than typhoid fever.[12,13] An important limitation of our study is the absence of cases of NTS in the control group; one patient in our study had *Salmonella* Typhimurium bacteraemia and none had *Salmonella* Enteritidis bacteraemia. It has been observed in previous studies that bacteraemia due to *Salmonella* Enteritidis may result in false-positive results with TUBEX® because they have an O9 antigen in common.[33] Although the patient with *Salmonella* Typhimurium had negative typhoid rapid antibody tests, we could not examine the rate of false positives for the TUBEX® test in patients with *Salmonella* Enteritidis bacteraemia.

In conclusion, typhoid rapid antibody tests appear to correlate poorly with blood culture results in sub-Saharan Africa, even in a study with inflated pretest probability. While such tests may be useful for rapidly diagnosing typhoid fever in emergencies – e.g. during outbreaks, when pretest probability would be high, and following blood culture confirmation of initial cases – their performance is unlikely to justify deployment in routine care settings in sub-Saharan Africa. TUBEX® and Typhidot® appeared to have comparable performance and were more specific although less sensitive than the semiquantitative slide agglutination test and the unpaired Widal test. Unpaired Widal and semiquantitative slide agglutination are unreliable, with poor specificity and PPV. It is important to remember that antimicrobial susceptibility testing and molecular epidemiological linkage cannot be elicited on serological diagnosis. Blood culture before initiating antimicrobial therapy remains the diagnostic method of choice. ■

Acknowledgements

We thank EDRU laboratory staff members: Mimmy Ngomane, Florah Mnyameni, Innocent Mtambo and Mzikazi Dickmolo for their assistance. KHK has a dual appointment with Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. JAC has dual appointments with Duke Global Health Institute, Duke University, Durham, United States of America; Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania and Kilimanjaro Christian Medical College, Tumaini University, Moshi, United Republic of Tanzania.

Funding: This work was supported in part by IDL Biotech AB, Bromma, Sweden (TUBEX®) and by Malaysian Biodiagnostic Research, Bangi, Malaysia (Typhidot®), who supplied kits for evaluation and training in the use of these kits. Additional work was funded by WHO, Geneva, Switzerland. Research done in the United Republic of Tanzania was supported by an International Studies on AIDS Associated Co-infections (ISAAC) award, a United States National Institutes of Health (NIH) funded program (U01 AI062563). Authors received support from NIH awards ISAAC (ABM, JAC); AIDS International Training and Research Program D43 PA-03–018 (JAC); the Duke Clinical Trials Unit and Clinical Research Sites U01 AI069484 (JAC), and the Center for HIV/AIDS Vaccine Immunology U01 AI067854 (JAC).

Competing interests: None declared.

ملخص

حساسية ونوعية الاختبارات السريعة لضدّ الحمى التيفيّة للتشخيص المختبري في موقعين في أفريقيا جنوب الصحراء الكبرى

الغرض تقييم ثلاثة اختبارات تجارية سريعة لضِدّ الحمى التيفية للسلمونيلة التيفيّة في المرضى المشتبه في إصابتهم بالحمى التيفية في مبومالانغا في جنوب أفريقيا، وفي موشي في جمهورية تنزانيا المتحدة.

الطريقة أُجْرِى تقييم لدقة تشخيص اختبار كروموتست Cromotest® (الـتراص نصف الكمي على الشريحة، واختبار الأنبوبة الفردية لفيدال Widal)، واختبار تيوبكس TUBEX® واختبار تيفيدوت Typhidot® وذلك مقابل المستنبت الدموي. جرى تشكيل الأداء لسيناريوهات بحيث تكون الاحتمالات السابقة للاختبار 5% و 50%.

النتائج وجد في مجمل المرضى المدرجين في الدراسة وعددهم 92 مريضاً: 53 مريضاً من جنوب أفريقيا (57.6%)، و39 مريضاً من جمهورية تنزانيا المتحدة (42.4%). وقد استفردت السالمونيلة التيفية من دم 28 مريضاً (30.4%). وكان لاختبار التراص نصف الكمي على الشريحة قيمة تكهنية إيجابية قدرها 25.0% (فاصلة الثقة 95%: 0.6-80.6) والقيمة التكهنية الإيجابية لاختبار الأنبوبة الفردية لفيدال 20.0% (فاصلة الثقة 95%: 2.5-55.6). وكانت القيم التكهنية الإيجابية المقارنة للاختبارات السريعة الأكثر حداثة لضدّ الحمى التيفية هي: اختبار تيوبكس 54.1% (فاصلة الثقة 95%: 36.9 - 70.5)، واختبار تيفيدوت للغلوبين المناعي M 56.7% (فاصلة الثقة 95%: 37.4-74.5)؛ واختبار تيفيدوت للغلوبين المناعيG هي 54.3% (فاصلة الثقة 95%: 36.6-71.2). أما الاحتمال السابق للاختبار والبالغ 5% فإن القيم التكهنية الإيجابية كانت: اختبار تيوبكس 11.0% (فاصلة الثقة 95%: 6.6-17.9)؛ اختبار تيفيدوت للغلوبين المناعيM هي 9.1% (فاصلة الثقة 95%: 5.8-14.0)؛ واختبار تيفيدوت للغلوبين المناعي G هي 11.0%(فاصلة الثقة 95%: 6.3-18.4)؛ وبالنسبة للاحتمال السابق للاختبار البالغ 50% فإن القيم التكهنية الإيجابية كانت: لاختبار تيوبكس 70.2% (فاصلة الثقة 95%: 57.3-80.5)؛ واختبار تيفيدوت للغلوبين المناعيM هي 65.6% (فاصلة الثقة 95%: 54.0-75.6)؛ واختبار تيفيدوت للغلوبين المناعي G هي 70.0% (فاصلة الثقة 95%: 56.0-81.1).

الاستنتاج كان أداء اختبار التراص نصف الكمي على الشريحة، واختبار الأنبوبة الفردية لفيدال Widal ضعيفاً. أما اختبار تيوبكس وتيفيدوت فيمكن أن يكونا ملائمين إذا كان الاحتمال السابق للاختبار مرتفعاً ولم تكن المستنبتات الدموية متوفرة، ولكن أداءهما لا يبرر استخدامهما في مواقع الرعاية الروتينية في أفريقيا جنوب الصحراء الكبرى.

摘要

撒哈拉沙漠以南两个非洲地区用于实验室诊断的伤寒热快速抗体检测的敏感性与特异性

目的 旨在评估南非普马兰加和坦桑尼亚联合共和国莫希的伤寒热疑似病例中针对伤寒杆菌抗体的三种商用伤寒快速抗体检测。

方法 Cromotest®（半定量玻片凝集试验和单管维达尔试验），TUBEX®和Typhidot®的诊断准确性均通过与血液培养的诊断准确性进行比较来评估。针对预测试概率为5%和50%的不同情况进行了性能建模。

结果 登记的92名患者中，53名（57.6%）来自南非，39名（42.4%）来自坦桑尼亚联合共和国。伤寒杆菌从28名（30.4%）患者的血液中分离。半定量玻片凝集试验和单管维达尔试验的阳性预测值（PPVs）分别为25.0%（95%可信区间：0.6-80.6）和20.0%（95%可信区间：2.5-55.6）。较新的伤寒快速抗体检测均有可比的阳性预测值：TUBEX®，54.1%（95%可信区间：36.9-70.5）；Typhidot® IgM，56.7%（95%可信区间：37.4-74.5）；Typhidot® IgG，54.3%（95%可信区间：36.6-71.2）。如果预先测试概率为5%，则阳性预测值为：TUBEX®，11.0%（95%可信区间：6.6-17.9）；Typhidot® IgM，9.1%(95%可信区间：5.8-14.0)；Typhidot® IgG，11.0%（6.3-18.4）。如果预先测试概率为50%，则阳性预测值为：TUBEX®，70.2%（95%可信区间：57.3-80.5）；Typhidot® IgM，65.6%（95%可信区间：54.0-75.6）；Typhidot® IgG，70.0%（95%可信区间：56.0-81.1）。

结论 半定量玻片凝集试验和单管维达尔试验效果不佳。当预测试概率高且血液培养不可用时，可以适当选用TUBEX®和Typhidot®，然而其效果并不能证明该等测试方法可以在撒哈拉沙漠以南非洲地区常规医疗机构中使用。

Résumé

Sensibilité et spécificité des tests d'anticorps rapides de la fièvre typhoïde pour le diagnostic biologique de deux sites d'Afrique subsaharienne

Objectif Évaluer trois tests d'anticorps rapides de la typhoïde commercialisés pour rechercher les anticorps de type *Salmonella* typhi chez des patients soupçonnés d'avoir contracté la fièvre typhoïde à Mpumalanga, en Afrique du Sud, et à Moshi, en République-Unie de Tanzanie.

Méthodes La précision du diagnostic de Cromotest® (test d'agglutination sur lame semi-quantitative et test de Widal à tube unique), de TUBEX® et de Typhidot® a été évaluée par rapport à celle de l'hémoculture. Les résultats ont été modélisés pour des scénarios avec des probabilités pré-test de 5% et 50%.

Résultats Un total de 92 patients ont participé: 53 (57,6%) d'Afrique du Sud et 39 (42,4%) de la République-Unie de Tanzanie. Le germe *Salmonella* typhi a été isolé dans le sang de 28 (30,4%) patients. Les tests d'agglutination sur lame semi-quantitative et de Widal à tube unique présentaient des valeurs prédictives positives (VPP) de 25% (intervalle de confiance, IC, de 95%: 0,6-80,6) et de 20% (IC de 95%: 2,5-55,6), respectivement. Les tests d'anticorps rapides de la typhoïde les plus récents présentaient des VPP comparables: TUBEX®, 54,1% (IC de 95%: 36,9–70,5); Typhidot® IgM, 56,7% (IC de 95%: 37,4-74,5); et Typhidot® IgG, 54,3% (IC de 95%: 36,6–71,2). Pour une probabilité pré-test de 5%, les VPP étaient les suivantes: TUBEX®, 11% (IC de 95%: 6,6-17,9); Typhidot® IgM, 9,1% (IC de 95%: 5,8-14,0); et Typhidot® IgG, 11% (6,3–18,4). Pour une probabilité pré-test de 50%, les VPP étaient les suivantes: TUBEX®, 70,2% (IC de 95%: 57,3-80,5); Typhidot® IgM, 65,6% (IC de 95%: 54,0-75,6); et Typhidot® IgG, 70% (IC de 95%: 56,0–81,1).

Conclusion Les tests d'agglutination sur lame semi-quantitative et de Widal à tube unique ont donné des résultats médiocres. TUBEX® et Typhidot® peuvent être adaptés lorsqu'une probabilité pré-test est élevée et que les hémocultures ne sont pas disponibles, mais leurs résultats ne justifient pas le déploiement d'un environnement de soins de routine en Afrique subsaharienne.

Резюме

Чувствительность и специфичность экспресс-диагностики брюшного тифа на антитела для постановки лабораторного диагноза в двух районах Африки к югу от Сахары

Цель Оценить эффективность трех коммерческих экспресс-анализов на антитела для *Salmonella* Typhi для пациентов с подозрением на наличие брюшного тифа в Мпумаланга, ЮАР, и Моши, Объединенная Республика Танзания.

Методы Диагностическая точность Cromotest® (полуколичественный тест Видаля по агглютинации на предметном стекле и в пробирке), TUBEX® и Typhidot® была сопоставлена с точностью анализа гемокультуры. Действие было смоделировано для сценариев с предтестовыми вероятностями от 5 до 50%.

Результаты Было охвачено 92 пациента: 53 (57,6%) из ЮАР и 39 (42,4%) из Объединенной Республики Танзания. *Salmonella* Typhi была выделена из крови 28 (30,4%) пациентов. Полуколичественные тесты Видаля на предметном стекле и в пробирке имели положительное предсказуемое значение (ППЗ) от 25,0% (95%-ный доверительный интервал, CI: 0,6–80,6) и 20,0% (95% CI: 2,5–55,6), соответственно. Новейшие экспресс-тесты на антитела для тифа имели сравнимые ППЗ TUBEX®, 54,1% (95% CI: 36,9–70,5); Typhidot® IgM, 56,7% (95% CI: 37,4–74,5); и Typhidot® IgG, 54,3% (95% CI: 36,6–71,2). Для 5%-ной предтестовой вероятности ППЗ были: TUBEX®, 11,0% (95% CI: 6,6–17,9); Typhidot® IgM, 9,1% (95% CI: 5,8–14,0); и Typhidot® IgG, 11,0% (6,3–18,4). Для 50%-ной предтестовой вероятности ППЗ были: TUBEX®, 70,2% (95% CI: 57,3–80,5); Typhidot® IgM, 65,6% (95% CI: 54,0–75,6); и Typhidot® IgG, 70,0% (95% CI: 56,0–81,1).

Resumen

Sensibilidad y especificidad de las pruebas rápidas de anticuerpos de fiebre tifoidea para el diagnóstico clínico en dos centros del África subsahariana

Objetivo Evaluar tres pruebas comerciales rápidas de anticuerpos tifoideos para detectar anticuerpos de *Salmonella typhi* en pacientes de los que se sospecha que sufren fiebre tifoidea en Mpumalanga, Sudáfrica y Moshi, República Unida de Tanzania.

Métodos Se evaluó la precisión diagnóstica del Cromotest® (pruebas semicuantitativas de aglutinación en laminas y de Widal en tubo único), el TUBEX® y el Typhidot®, en comparación con el hemocultivo. Se elaboraron modelos de funcionamiento de los supuestos, con probabilidades de las pruebas iniciales del 5% y el 50%.

Resultados Se reclutó a un total de 92 pacientes: 53 (57,6%) de Sudáfrica y 39 (42,4%) de la República Unida de Tanzania. La *Salmonella typhi* se aisló en la sangre de 28 pacientes (30,4%). Las pruebas semicuantitativas de aglutinación en lámina y de Widal en tubo único ofrecieron valores predictivos positivos (VPP) del 25,0% (intervalo de confianza [IC] del 95%: 0,6–80,6) y del 20,0% (IC del 95%: 2,5–55,6), respectivamente. Las pruebas rápidas de anticuerpos tifoideos más novedosas presentaron VPP comparables: TUBEX®, 54,1% (IC del 95%: 36,9–70,5); Typhidot® IgM, 56,7% (IC del 95%: 37,4-74,5); Typhidot® IgM, 54,3% (IC del 95%: 36,6–71,2). Para una probabilidad de la prueba inicial del 5%, los VPP fueron: TUBEX®, 11,0% (IC del 95%: 6,6-17,9); Typhidot® IgM, 9,1% (IC del 95%: 5,8-14,0) y Typhidot® IgG, 11,0% (6,3-18,4). Para una probabilidad de la prueba inicial del 50%, los VPP fueron: TUBEX®, 70,2% (IC del 95%: 57,3-80,5); Typhidot® IgM, 65,6% (IC del 95%: 54,0-75,6); y Typhidot® IgM, 70,0% (IC del 95%: 56,0–81,1).

Conclusión Las pruebas semicuantitativas de aglutinación en lámina y de Widal en tubo único no funcionaron bien. TUBEX® y Typhidot® podrían ser aptos cuando la probabilidad de la prueba inicial es alta y los hemocultivos no están disponibles, pero su funcionamiento no justifica su uso en los centros de asistencia sanitaria habituales en el África subsahariana.

References

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ* 2004;82:346–53. PMID:15298225
2. Baker S, Favorov MO, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis* 2010;10:45. doi:10.1186/1471-2334-10-45 PMID:20205702
3. Wain J, Hosoglu S. The laboratory diagnosis of enteric fever. *J Infect Dev Ctries* 2008;2:421–5. PMID:19745517
4. Archibald LK, Reller LB. Clinical microbiology in developing countries. *Emerg Infect Dis* 2001;7:302–5. doi:10.3201/eid0702.010232 PMID:11294729
5. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD et al.; Domi Typhoid Study Group. A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bull World Health Organ* 2008;86:260–8. doi:10.2471/BLT.06.039818 PMID:18438514
6. Kawano RL, Leano SA, Agdamag DM. Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. *J Clin Microbiol* 2007;45:246–7. doi:10.1128/JCM.01403-06 PMID:17065261
7. House D, Ho VA, Diep TS, Chinh NT, Bay PV, Vinh H et al. Antibodies to the Vi capsule of Salmonella Typhi in the serum of typhoid patients and healthy control subjects from a typhoid endemic region. *J Infect Dev Ctries* 2008;2:308–12. PMID:19741294
8. Olsen SJ, Pruckler J, Bibb W, Nguyen TM, Tran MT, Nguyen TM et al. Evaluation of rapid diagnostic tests for typhoid fever. *J Clin Microbiol* 2004;42:1885–9. doi:10.1128/JCM.42.5.1885-1889.2004 PMID:15131144
9. Naheed A, Ram PK, Brooks WA, Mintz ED, Hossain MA, Parsons MM et al. Clinical value of Tubex and Typhidot rapid diagnostic tests for typhoid fever in an urban community clinic in Bangladesh. *Diagn Microbiol Infect Dis* 2008;61:381–6. doi:10.1016/j.diagmicrobio.2008.03.018 PMID:18501549
10. Fadeel MA, House BL, Wasfy MM, Klena JD, Habashy EE, Said MM et al. Evaluation of a newly developed ELISA against Widal, TUBEX-TF and Typhidot for typhoid fever surveillance. *J Infect Dev Ctries* 2011;5:169–75. doi:10.3855/jidc.1339 PMID:21444985
11. Clemens JD. Meeting on establishment of consortium to study invasive salmonelloses in sub-Saharan Africa [conference summary]. *Emerg Infect Dis* 2009;15.
12. Mweu E, English M. Typhoid fever in children in Africa. *Trop Med Int Health* 2008;13:532–40. doi:10.1111/j.1365-3156.2008.02031.x PMID:18312473
13. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;10:417–32. doi:10.1016/S1473-3099(10)70072-4 PMID:20510282

14. *Background document: the diagnosis, treatment and prevention of typhoid fever*. Geneva: World Health Organization; 2003.
15. Crump JA, Ramadhani HO, Morrissey AB, Saganda W, Mwako MS, Yang L-Y et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. *Clin Infect Dis* 2011;52:341–8. doi:10.1093/cid/ciq103 PMID:21217181
16. Crump JA, Ramadhani HO, Morrissey AB, Msuya LJ, Yang L-Y, Chow S-C et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania. *Trop Med Int Health* 2011;16:830–7. doi:10.1111/j.1365-3156.2011.02774.x PMID:21470347
17. Bopp CA, Brenner FW, Fields PI, Wells JG, Strockbine NA. *Escherichia, Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. *Manual of clinical microbiology*. 8th edition. Washington: ASM Press; 2003. pp. 654-71.
18. Widal F. Serodiagnostique de la fievre typhoid. *La Semaine Medicale* 1896;16:259.
19. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H et al. Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. *J Clin Microbiol* 1999;37:2882–6. PMID:10449469
20. Lim PL, Tam FC, Cheong YM, Jegathesan M. One-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes. *J Clin Microbiol* 1998;36:2271–8. PMID:9666004
21. Choo KE, Oppenheimer SJ, Ismail AB, Ong KH. Rapid serodiagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. *Clin Infect Dis* 1994;19:172–6. doi:10.1093/clinids/19.1.172 PMID:7948526
22. Reichenheim ME, Ponce de Leon A. Estimation of sensitivity and specificity arising from validity studies with incomplete design. *Stata J* 2002;2:267–79.
23. Bakr WM, El Attar LA, Ashour MS, El Toukhy AM. The dilemma of widal test - which brand to use? a study of four different widal brands: a cross sectional comparative study. *Ann Clin Microbiol Antimicrob* 2011;10:7. doi:10.1186/1476-0711-10-7 PMID:21303511
24. House D, Chinh NT, Diep TS, Parry CM, Wain J, Dougan G et al. Use of paired serum samples for serodiagnosis of typhoid fever. *J Clin Microbiol* 2005;43:4889–90. doi:10.1128/JCM.43.9.4889-4890.2005 PMID:16145168
25. Dutta S, Sur D, Manna B, Sen B, Deb AK, Deen JL 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;56:359–65. doi:10.1016/j.diagmicrobio.2006.06.024 PMID:16938421
26. *HIV/AIDS epidemiological surveillance report for the WHO African Region – 2007 update*. Geneva: World Health Organization; 2007. Available from: http://www.afro.who.int/en/clusters-a-programmes/atm/acquired-immune-deficiency-syndrome/aids-publications.html [accessed 28 May 2011].
27. MacLennan CA, Gilchrist JJ, Gordon MA, Cunningham AF, Cobbold M, Goodall M et al. Dysregulated humoral immunity to nontyphoidal Salmonella in HIV-infected African adults. *Science* 2010;328:508–12. doi:10.1126/science.1180346 PMID:20413503
28. Mirrett S, Reller LB, Petti CA, Woods CW, Vazirani B, Sivadas R et al. Controlled clinical comparison of BacT/ALERT standard aerobic medium with BACTEC standard aerobic medium for culturing blood. *J Clin Microbiol* 2003;41:2391–4. doi:10.1128/JCM.41.6.2391-2394.2003 PMID:12791854
29. Rahman M, Siddique AK, Tam FC, Sharmin S, Rashid H, Iqbal A et al. Rapid detection of early typhoid fever in endemic community children by the TUBEX O9-antibody test. *Diagn Microbiol Infect Dis* 2007;58:275–81. doi:10.1016/j.diagmicrobio.2007.01.010 PMID:17350203
30. Bhutta ZA, Mansurali N. Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. *Am J Trop Med Hyg* 1999;61:654–7. PMID:10548305
31. Jesudason M, Esther E, Mathai E. Typhidot test to detect IgG & IgM antibodies in typhoid fever. *Indian J Med Res* 2002;116:70–2. PMID:12592993
32. Jesudason MV, Sivakumar S. Prospective evaluation of a rapid diagnostic test Typhidot for typhoid fever. *Indian J Med Res* 2006;123:513–6. PMID:16783041
33. Tam FC, Ling TK, Wong KT, Leung DT, Chan RC, Lim PL. The TUBEX test detects not only typhoid-specific antibodies but also soluble antigens and whole bacteria. *J Med Microbiol* 2008;57:316–23. doi:10.1099/jmm.0.47365-0 PMID:18287294