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Rapid Diagnostic Tests for Typhoid and Paratyphoid (Enteric) Fever (Protocol)

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[Diagnostic Test Accuracy Protocol]

Rapid Diagnostic Tests for Typhoid and Paratyphoid (Enteric) Fever

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ABSTRACT

This is a protocol for a Cochrane Review (Diagnostic test accuracy). The objectives are as follows:

To assess the diagnostic accuracy of RDTs for detecting typhoid and paratyphoid fever in persons living in endemic areas presenting to a healthcare facility with fever.

BACKGROUND

Target condition being diagnosed

Typhoid and paratyphoid (enteric) fever are diseases caused by *Salmonella enterica* serovar Typhi and Paratyphi A respectively. The more common typhoid is an important infectious disease in developing countries with over 22 million new cases worldwide, leading to an estimated 200,000 deaths (WHO 2002). South and South-East Asia are the most affected areas of the world with an estimated annual prevalence of > 100/100,000 (Crump 2004). Typhoid and paratyphoid fevers are prevalent in low- or middle-income countries with inadequate sanitation and hygiene, particularly regarding food, water, and disposal of human excrement. Despite advances in technology and public health strategies, enteric fever remains a major cause of morbidity in the developing world (Bhutta, 2006). Urbanisation, global warming, and traditional methods of water-side living have created even greater demands for clean water in developing countries (UNICEF 2006). Both typhoid and paratyphoid are most common where standards of personal and environmental hygiene are low, and only to this extent are these diseases tropical (Gill 2009).

The Gram-negative bacilli are transmitted by the faecal-oral route when food or water contaminated with infected faeces is ingested. The most important reservoirs of infection are short-term convalescent or chronic human carriers. Food handlers who are carriers are a particularly important source of transmission (Gill 2009).

The clinical presentation of typhoid and paratyphoid fever varies from a mild illness with a low-grade fever, malaise and slight dry cough to a severe clinical picture with multiple complications including intestinal perforation (Ismail 2006). Toxic apathy, blanching 'rose spots' on the trunk, abdominal organomegaly, and diarrhoea are also associated with enteric fever, but the clinical picture is highly variable between geographical location and age-groups. Typhoid and paratyphoid can present in many different and non-specific ways, thus posing a diagnostic challenge for the health professional. Most enteric fever is diagnosed on clinical grounds and treated presumptively. As a result the diagnosis may be delayed or missed, while other febrile illnesses are being considered (Parry 2002).

There are a number of reasons why there is significant resistance of *Salmonella enterica* serovar Typhi / Paratyphi A to antimicrobials worldwide. Health professionals in the tropics overprescribe antimicrobials for many reasons, including cultural factors and patient expectation (Okeke 2005). The purchase of drugs such as antimicrobials from untrained vendors and unlicensed pharmacists are commonplace in the developing world (Larsson 2008). A major challenge is the inability to confirm diagnoses in resource-limited settings where traditional laboratory methods of diagnosing typhoid and paratyphoid are not available. Health care workers are therefore reliant on their clinical skills to make an educated guess of the cause of illness, and/or prescribe an antimicrobial that targets several bacteria (Shetty 2008). This over-treatment has contributed to increasing resistance to fluoroquinolones (eg ciprofloxacin) and multi-drug resistance of *Salmonella enterica* serovar Typhi / Paratyphi A within endemic Asian countries (Chuang 2009).

Index test(s)

Simple, reliable, point-of-care rapid diagnostic tests (RDTs) for typhoid and paratyphoid (enteric) fever have been a long-felt need of clinicians working in endemic areas (Jesudason 2006). The tests need to be suitable for use in remote areas with limited diagnostic facilities and relatively untrained staff. They should be designed to yield a simple 'positive/negative' result at thresholds pre-set by the manufacturers, similar to a pregnancy test. These results should normally be made available within 15 minutes, so that they can be used while the healthcare provider is dealing with suspected patients (http://www.rapid-diagnostics.org/). Finally, such tests must be made available at low cost for use in resource-limited settings.

The lack of RDTs in areas without microbiology facilities means that the burden of enteric fever is underestimated worldwide (Parry 2002). RDTs could help rationalise antimicrobial treatment and thus contribute to tackling the problem of resistance in endemic areas (Bhutta, 2006). RDTs could be incorporated into clinical algorithms for patients with fever from endemic areas to help guide management.

Typhoid and paratyphoid RDTs comprise a heterogenous group of different methods and formats. RDTs have been applied to either blood or urine samples. Blood RDTs (using either venous and/or capillary samples) are more common than urine tests. These RDT products include test formats based on lateral flow, flow-through, agglutination or solid phase methods (Pastoor 2008).

RDTs may detect antigens (components of the causative *Salmonella* organism) or antibodies (markers of the human's immune response to the antigen). The type of antibody class or immunoglobulin detected could be either Immunoglobulin-M (IgM), which may be indicative of recent exposure, or Immunoglobulin-G (IgG), which can indicate recent or previous exposure. Examples of commercial RDTs for typhoid and paratyphoid which have been undergoing evaluation in recent years include *Typhidot®* , *Typhidot-M®* , and *TUBEX™ (* Baker 2010).

New RDT developments are likely to take a serological approach, although the identification of novel antigens free of cross-reacting materials and antigen pools is a major challenge (Baker 2010).

Alternative test(s)

The gold standard for diagnosing enteric fever has been culture of the *Salmonella enterica* serovar Typhi or Paratyphi A organism from either bone marrow or peripheral blood. The mainstay of diagnosis in clinical practice is a positive blood culture, although the test is only positive in 40 to 60% of cases, usually early in the course of the disease (WHO 2002). This lack of sensitivity may be due to the low number of bacteria circulating in the blood, or may be affected by prior antimicrobial therapy (Wain 1998). Bone marrow culture gives a higher culture-positive rate, probably because the concentration of organisms is higher than in the blood, and may even yield a positive culture after antibiotic therapy has been started (Wain 2001). Bone marrow culture is positive in 80 to 95% of patients with typhoid and paratyphoid, even in patients who have been taking antibiotics for several days regardless of the duration of the illness (Parry 2002). Although bone marrow cultures are more sensitive, they are difficult to obtain, relatively invasive, and of little use in public health settings (Wain 2001). Even with sophisticated

laboratories, confirming the diagnosis of enteric fever can still be difficult as samples of blood or bone marrow may still not show evidence of the disease despite a patient actually having typhoid or paratyphoid.

False negative blood cultures depend on numerous factors including: volume of blood sample taken; the type of culture medium used; and the length of the incubation period (Massi 2005). The sensitivity of blood culture is higher in the first week of illness (Parry 2002). Widespread antimicrobial availability and prescribing contributes to the low sensitivity of blood culture (WHO 2002). The ratio of blood to broth in preparing the blood culture could affect culture positivity rates (Parry 2002) and highlights the issues of the quality assurance of laboratories in endemic countries.

The Widal test (WT) is an example of a serological test. It detects agglutinating antibodies to lipopolysaccharide (LPS) (O antigen) and flagella (H antigen). It is still widely used for the serological diagnosis of typhoid (House 2001). In its original format the WT required both acute and convalescent-phase serum samples taken approximately 10 days apart. More recently, the test has been evaluated for use as a single, acute-phase serum sample (Saha 1996). In enteric fever, titres often rise before the clinical onset, making it very difficult to demonstrate the diagnostic four-fold rise between initial and subsequent samples (Gill 2009).

The role of the WT is controversial because the sensitivity, specificity, and predictive values vary considerably among geographic areas (Parry 2002). Test results need to be interpreted carefully in the light of previous history of typhoid / paratyphoid and vaccination. Interpretation of the result is also greatly helped by knowledge of the background levels of antibodies in the local healthy population (House 2001). The widespread use of typhoid vaccines, and the large number of cases of repeated exposure to *Salmonella* species, are found to lower the specificity of the WT (House 2001). Several other diseases caused by non-*Salmonella* organisms (eg malaria, dengue, brucellosis) have been shown to exhibit cross-reactivity in typhoid-endemic regions (Olopoenia, 2000). There is considerable variation in agglutinin levels among non-infected populations. These levels are susceptible to change over time, and depend on the degree of endemicity (Parry 2002). Despite these shortcomings of both sensitivity and specificity the WT, both simple and inexpensive, is still widely used as a diagnostic test (Fadeel 2004).

Nucleic acid amplification tests (NAATs) for typhoid and paratyphoid diagnosis, such as polymerase chain reaction (PCR), are being explored. Theoretically, NAATs could amplify DNA from dead or unculturable bacteria, thus addressing the concern of poor culture positivity because of pre-treatment with antimicrobials (Wain 2001). However, a novel three-colour real-time PCR technique has been found to have the same limitations as culture in terms of sensitivity, and deemed an unsuitable methodology for the routine diagnosis of typhoid and paratyphoid (Nga 2010). Methods combining culture and PCR methods have been also been explored (Zhou 2010). However, the use of NAATs in developing countries will most likely be limited in the medium-term for reasons of cost (Olsen 2004).

Rationale

RDTs have the potential to be useful for clinicians working in resource-limited settings in the tropics. Differentiating the common causes of the febrile patient by clinical criteria can be very challenging without the laboratory support for blood films, serology, or blood cultures (Bhutta, 2006). A diagnostic test in such settings must be cheap, simple to perform, able to deliver a quick result, and be both sensitive and specific (www.rapid-diagnostics.org). Such a test should correctly identify true cases of typhoid and paratyphoid among febrile patients, ensuring prompt and typhoid / paratyphoid-specific treatment, allowing the avoidance of broad-spectrum medication that covers all common causes of fever. In many endemic areas, treatment for typhoid may be given to all patients with fever (Larsson 2008). Diagnosis of enteric fever by an RDT could reduce unnecessary prescription of antimicrobials, reduce drug expenditure, and limit the development of antimicrobial resistance.

The evaluation of RDTs in enteric fever is complicated by the lack of a suitable gold standard. The isolation and culture of *Salmonella enterica* serovar Typhi or Paratyphi A from blood or bone marrow is the available reference standard, but does not have 100% sensitivity (Baker 2010). The sensitivity and specificity of the RDT may be difficult to interpret in that it is possible that RDTs are more sensitive than blood and/or bone marrow culture.

OBJECTIVES

To assess the diagnostic accuracy of RDTs for detecting typhoid and paratyphoid fever in persons living in endemic areas presenting to a healthcare facility with fever.

Investigation of sources of heterogeneity

In the primary analysis, the studies will be grouped by:

- *Salmonella enterica* serovars (Typhi or Paratyphi A);
- study design (see 'Types of studies');
- test population (clinically-suspected typhoid / paratyphoid or unselected febrile patients)
- index test type (individual commercial test / test format - see 'Index tests'); and
- reference test (Grade 1 or Grade 2 - see 'Reference standards').

We plan to investigate the following sources of heterogeneity (see 'Investigations of heterogeneity'):

- degree of typhoid endemicity (low / medium /high as per Crump 2004);
- patient age (adults / children / mixed);
- geographical area (sub-Saharan Africa / rest of the world). Non-typhoidal *Salmonella* (NTS) are emerging as a prominent cause of bacteraemia in sub-Saharan Africa. Endemicity may affect RDT performance in this compared to other locations.

METHODS

Criteria for considering studies for this review

Types of studies

We will include:

- Randomized controlled trials in which patients are randomized to one of several index tests and all receive the reference standard.

- Paired comparative trials in which a series of patients receive two or more index tests and a reference standard.
- Prospective cohort studies in which a series of patients from a given population are recruited and receive one or more index test and the reference standard.
- Retrospective case-control studies comparing a group of patients with laboratory-confirmed typhoid / paratyphoid cases (positive reference standard) and a group of patients without typhoid / paratyphoid (negative reference standard). Each group receives the index test(s) and the index test(s) are analysed and compared between the two groups.

Participants

Patients living in typhoid- or paratyphoid-endemic areas attending a healthcare facility with fever are eligible. This may or may not include patients with a clinical suspicion of typhoid or paratyphoid.

When only a subgroup of participants in a study are eligible for inclusion in the review, the study will be included provided that it is possible to extract relevant data specific to that subgroup.

Index tests

All RDTs specifically designed to detect typhoid or paratyphoid cases. The tests will be categorised as follows:

- RDTs that are applied to blood samples (venous or capillary) to detect antigens;
- RDTs that are applied to blood samples (venous or capillary) to detect antibodies (IgG or IgM);
- RDTs that are applied to urine samples to detect antigens; and
- RDTs that are applied to urine samples to detect antibodies (IgG or IgM).

The RDTs will be further classified by format, eg lateral flow, flow-through, agglutination or solid phase kits.

Comparator tests

Studies may compare one or more RDT against one or more reference standard.

Target conditions

Typhoid fever caused by *Salmonella enterica* serovar Typhi.

Paratyphoid fever caused by *Salmonella enterica* serovar Paratyphi A.

Reference standards

Studies are required to diagnose typhoid or paratyphoid using one of the following reference standards:

- Bone marrow culture;

and/or

- Peripheral blood culture.

A **Grade 1 study** will be defined as one using both bone marrow **and** peripheral blood culture as the reference standard.

In Grade 1 studies, either bone marrow or peripheral blood culture positivity will be considered a positive reference standard.

A **Grade 2 study** will be defined as using peripheral blood culture **only** as the reference standard.

Because overall estimates of accuracy ignoring the use of different reference standards are difficult to interpret, the results will be reported separately for each grade of reference standard (Reitsma 2009).

Search methods for identification of studies

We will attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, ongoing).

We will limit our searches to studies conducted in humans.

Electronic searches

To identify all relevant studies, we will search the following databases using the search terms and strategy described in Appendix 1:

Cochrane Infectious Diseases Group Specialized Register; MEDLINE; EMBASE; MEDION; Science Citation Index; LILACS; IndMED; African Index Medicus.

MeSH and other search terms will include: Typhoid; Enteric Fever; Paratyphoid; *Salmonella* Typhi; *Salmonella* Paratyphi; rapid diagnostic tests; RDT; diagnostics; antigen detection; antibody detection; blood culture; bone marrow culture; Widal Test; Typhidot; Typhidot-M; TUBEX; lateral flow; agglutination; solid phase.

Searching other resources

We will also check the reference lists of all studies identified by the above methods, and we will manually search World Health Organization (WHO) reports.

We will also manually search papers from the 3rd (1997) to the 7th (2009) International Conferences on Typhoid Fever and other Salmonellosis.

We will contact test manufacturers to identify ongoing or unpublished studies.

Data collection and analysis

Selection of studies

The first author will initially assess the titles and abstracts identified by the search strategy and exclude those which are not related to RDTs and typhoid. Letters and review articles related to RDTs and typhoid will be included in the table of excluded studies. All potentially relevant articles will be retrieved and independently examined by Lalith Wijedoru (LW) and Chris Parry (CMP), using a pro forma as a guide. Discrepancies between decisions on inclusion of studies will be discussed with Sarah Donegan (SD).

Data extraction and management

LW and CMP will independently extract a standard set of data from each study article (see Appendix 2), using a pre-piloted specifically designed data extraction form. The data extraction will be cross checked and any discrepancies will be resolved by discussion and consultation with SD. If information is missing or not clear we will write to the study investigators.

The number of true positives, true negatives, false positives and false negatives based only on the *Salmonella enterica* serovars the test is designed to detect (Typhi or Paratyphi A) will be extracted as a two by two table for each study along with the corresponding threshold value. If data for multiple two by two tables are presented based on more than one threshold for a single study, we will extract each table and the threshold values. If this data (two by two table) is also available for a subgroup of patients in the study we will extract this data if the subgroup of patients is of interest (ie grouped by patient age). In cases of studies where only a subgroup of participants is included in the review, this data will only be extracted and presented for that particular subgroup.

Where multiple index tests or reference standards were applied in a study, data will be extracted for each test. Since blood culture and bone marrow are poor reference standards, where possible we will extract the results of a composite reference standard (blood culture and bone marrow culture), such that a negative result is documented if both cultures are negative (Reitsma 2009). We will extract the number of uninterpretable or invalid test results.

Assessment of methodological quality

LW and CMP will independently assess the quality of each individual study using a modified QUADAS tool (Whiting 2003; see Appendix 3). Each quality indicator on the checklist will be answered with a 'yes', 'no' or 'unclear' response for each study, and the reason for the judgement made given.

Statistical analysis and data synthesis

In the description of studies we will describe the number of uninterpretable or invalid test results. The analyses will be stratified according to the following hierarchy:

1. *Salmonella enterica* serovars the test is detecting (Typhi, Paratyphi A, or both);

2. Reference standard test applied (bone marrow *and* blood culture [Grade 1], blood culture [Grade 2]);

3. Study design (case control, prospective cohort, randomized controlled trial, paired comparative trial);

4. Test population (clinically-suspected typhoid / paratyphoid, unselected febrile patients);

5. Index test type (split by blood or urine);

6. Test based on antigens or antibodies (IgM / IgG); and

7. Commerical name (eg *Typhidot*, *Typhidot-M*, *TUBEX*).

Data from the same study may contribute to different comparisons (eg RDT vs blood culture; RDT vs bone marrow and blood culture) but data from the same study will not be combined in the same meta-analysis as if it is from different studies.

To demonstrate the variation in accuracy between studies, for each test, estimates of the observed sensitivities and specificities will be plotted in forest plots.

Where adequate data are available, meta-analyses will be undertaken to estimate and compare the performance of the tests. The analyses will estimate and compare ROC curves though regression modelling using hierarchical summary ROC random-effects models (Rutter 2001). The data will be exported from RevMan (Review Manager 5) into SAS, models will be fitted using SAS, and then we will enter the appropriate parameter estimates (HSROC model parameter estimates and confidence and prediction region parameters) into RevMan. We will include covariates in the models regarding index test type (eg urine/blood, antigen/antibody, commercial name) providing we have several studies contributing to each test type. A secondary analysis based only on studies that directly compared index tests will be carried out.

Summary estimates of sensitivity and specificity (with 95% confidence intervals) will be reported in additional tables. We will also present summary sensitivities and specificities and 95% confidence regions in ROC space. When covariates regarding index test type are included in the models, we will present P values for the hypothesis test to help indicate differences between test types.

As a secondary analysis, we will compare the composite reference standard (blood and bone marrow cultures) with the reference standards individually in order to explore the accuracy of the blood culture and bone marrow culture individually.

Investigations of heterogeneity

We will assess heterogeneity between studies by visually inspecting the forest plots and summary ROC plots and by estimating the between-study heterogeneity from the hierarchical meta-analysis model. We will also assess heterogeneity though regression modelling using hierarchical summary ROC random-effects models by presenting summary specificities and sensitivities for groups of studies (categories of covariate) and carrying out significance tests to detect a difference between categories. We intend to conduct meta-regression by including the following covariates in the regressions models:

- format (ie lateral flow vs flow-through vs agglutination vs solid phase kits);
- antibodies (IgM vs IgG);
- endemicity (low vs medium vs high vs unclear) (Crump 2004);
- patient age (adults vs children vs mixed vs unclear);
- geographical location (by sub-Saharan Africa vs rest of the world)

Sensitivity analyses

We will carry out sensitivity analyses to assess the robustness of the meta-analyses based on quality components.

Assessment of reporting bias

We will not attempt to assess reporting bias.

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APPENDICES

Appendix 1. Detailed Search Strategy

Search set	MEDLINE	EMBASE
1	Exp Typhoid fever [MeSH]	Exp Typhoid fever [Emtree]
2	Exp Salmonella Typhi [MeSH]	Exp Salmonella Typhi [Emtree]
3	Typhoid fever ti, ab	Typhoid fever ti, ab
4	Enteric fever ti, ab	Enteric fever ti, ab
5	Exp Paratyphoid fever [MeSH]	Exp Paratyphoid fever [Emtree]
6	Exp Salmonella Paratyphi A [MeSH]	Exp Salmonella Paratyphi [Emtree]
7	Exp Salmonella Paratyphi B [MeSH]	Paratyphoid fever ti, ab
8	Exp Salmonella Paratyphi B [MeSH]	1-7/OR
9	Paratyphoid fever ti, ab	Rapid diagnostic test* ti, ab
10	1-9/OR	RDT ti, ab
11	Rapid diagnostic test* ti, ab	Antigen detection [Emtree]
12	RDT ti, ab	Antibody detection [Emtree]
13	Antigen* detect* ti, ab	Blood culture [Emtree]
14	Antibod* detect* ti, ab	Bone marrow culture [Emtree]
15	Blood culture* ti, ab	Serodiagnostic test* ti, ab

(Continued)

16	Bone marrow culture ti, ab	Widal ti, ab
17	Serodiagnostic test* ti, ab	DOT enzyme immunoassay ti, ab
18	Widal ti, ab	Typhidot ti, ab
19	DOT enzyme immunoassay ti, ab	TUBEX ti, ab
20	Typhidot ti, ab	immunochromatographic lateral flow assay ti, ab
21	TUBEX ti, ab	solid-phase ti, ab
22	immunochromatographic lateral flow assay ti, ab	Dot blot ti, ab
23	solid-phase ti, ab	PCR ti, ab
24	Dot blot ti, ab	Serodiagnosis [Emtree]
25	PCR ti, ab	Immunoblotting [Emtree]
26	Reagent kits, diagnostic [MeSH]	9-25/OR
27	Immunoblotting [MeSH]	8 AND 26
28	Serological tests [MeSH]	
29	11-28/OR	
30	10 AND 29	

Appendix 2. Data Extraction

Study ID	First author, year of publication
Clinical features and setting	Clinical Features: presenting signs and symptoms; index of suspicion for enteric fever (ie suspected vs unselected febrile); and recent prior antimicrobial treatment. Setting: health care facility; country; endemicity; and endemic subspecies.
Participants	Sample size; age;

(Continued)

	gender; comorbidities; point of recruitment (in-patients/ out-patients); and pregnancy.
Study design	Whether patients enrolled prospectively or retrospectively; Whether sampling methods were consecutive or random; If the study enrolled more than one RDT, how were tests allocated to individuals or did individuals receive all the tests? Were RDTs used on suspected typhoid / paratyphoid cases or unselected febrile patients?
Target condition	Typhoid Fever and/or Paratyphoid fever
Reference standard	Which reference standard was used (bone marrow/ blood culture/both)? Who performed the reference standard test(s)? Where was the test performed? How many repeats were used? Number of observers/operators Methods of inter-observer discrepancy resolution Has the laboratory received quality accreditation by an external agency?
Index tests	*Salmonella enterica* serovars designed to detect ie Typhi (typhoid), Paratyphi A (paratyphoid), or both; Commercial name; Blood or urine; If blood RDT, capillary or venous blood; Antigen or antibody detection; If antibody detection, subclass detected (ie IgG / IgM) Format; Transport and storage conditions; Details of test operators, including any special training provided; Where was the test performed? Number of observers/operators and methods of inter-observer discrepancy resolution; Threshold ie what constituted a positive result?
Data	Numbers of true positives, false positives, true negative and false negatives.
Notes	Source (s) of funding

Appendix 3. Assessment of methodological quality

Quality Indicator	Notes
Was the spectrum of patients representative of the spectrum of patients who will receive the test in practice?	**Yes** - patients with fever **and** recruited from an area of high or medium endemicity for typhoid and/or paratyphoid fever as defined by Crump 2004 **No -** patients without fever **or** recruited from an area of low endemicity (Crump 2004) for typhoid and/or paratyphoid fever **Unclear** - if the location or clinical characteristics of participants is not adequately described
Were selection criteria clearly described?	**Yes** - Inclusion and exclusion criteria clearly described eg patients with fever and/or patients suspected to have typhoid/paratyphoid **No** - Inclusion and exclusion criteria not included **Unclear** - If selection criteria are partially reported
Is the reference standard likely to correctly identify the target condition?	**Yes -** if bone marrow **and** blood culture (Grade 1 Reference standard) are performed at an externally accredited laboratory **and** adequate blood/marrow volumes are taken(Wain 1998, Wain 2001) **No -** If inadequate blood/marrow volumes are taken (Wain 1998, Wain 2001) **Unclear** - if blood culture alone (Grade 2 Reference standard) is performed, **or** if external quality assurance accreditation of the relevant laboratory **or** blood/marrow volumes are not described
Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	**Yes** - if the index test and reference standard(s) are collected on the same patients at the same time **or** within 24 hours of each other **No** - if the time period between index test and reference standard(s) collection is > 24 hours **Unclear** - if the time period between index test and reference standard collection is not described
Is partial verification avoided?	**Yes** - if all participants received both index and reference test(s) **No** - if not all participants received both index and reference tests **Unclear** - if insufficient information is provided.
Is differential verification avoided?	**Yes** - if the same reference test(s) was / were used in all participants **No** - if different reference test(s) is / are used depending on index test results **Unclear** if insufficient information is provided
Is incorporation avoided? ie the index test does not perform part of the reference standard	**Yes** - the reference standards are culture: bone marrow **and** peripheral blood (Grade 1); and peripheral blood culture **only** (Grade 2). RDTs do not form part of either of these individual or composite reference standards.
Are the reference standard tests results blinded?	**Yes** - person undertaking the reference test did not know the results of the index tests, or if the tests were carried out in different places **No** - if the same person performed both tests, or the results of the index tests were known to the person undertaking the reference tests **Unclear** - if insufficient information provided

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Are the index test results blinded?	**Yes** - person undertaking the index test did not know the results of the reference tests, or if the tests carried out in different places **No** - if the same person performed both tests, or the results of the reference tests were known to the person undertaking the index tests **Unclear** - if insufficient information provided
Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	**Yes** - if clinical data is available when interpreting the index test eg index test performed at the point-of-care of patients **or** if tests are interpreted by the same individual assessing the patients **or** if interpreted by remote staff provided with relevant clinical details eg a request form **No** - if index tests are interpreted by staff not involved with the clinical assessment of the patient **or** who are not given any clinical information accompanying the test Unclear - if the above details about the study design are not provided
Were uninterpretable/ intermediate results reported?	**Yes** - if the number of participants in the two-by-two table matches the number of participants recruited into the study or if sufficient explanation is provided for any discrepancy. **No** - number of participants in the two-by-two table does not match the number of participants recruited into the study and insufficient explanation is provided for any discrepancy **Unclear** - if insufficient information is given to permit judgement
Were any withdrawals explained?	**Yes** if there are no participants excluded from the analysis, or if exclusions are adequately described. **No** if there are unexplained exclusion of participants **Unclear** if insufficient information is given to assess whether any participants were excluded from the analysis

CONTRIBUTIONS OF AUTHORS

LW and CP came up with the concept of the review. LW wrote the protocol. SD and CP edited the protocol.

DECLARATIONS OF INTEREST

There are no declarations of interest to be made.

INDEX TERMS

Medical Subject Headings (MeSH)

False Negative Reactions; False Positive Reactions; Immunoassay [*methods]; Paratyphoid Fever [blood] [*diagnosis]; Polymerase Chain Reaction [standards]; Reagent Kits, Diagnostic [*standards]; Reference Standards; Sensitivity and Specificity; Typhoid Fever [blood] [*diagnosis]

MeSH check words

Adult; Child; Humans