Rapid diagnostic tests for non-malarial febrile illness in the tropics

F. Chappuis[1,2], E. Alirol[1,2], V. d'Acremont[3,4], E. Bottieau[5] and C. P. Yansouni[6]

1) *Division of International and Humanitarian Medicine, Geneva University Hospitals and University of Geneva,* 2) *Médecins Sans Frontières, Operational Centre Geneva, Geneva, Switzerland,* 3) *Department of Ambulatory Care and Community Medicine, University of Lausanne, Lausanne,* 4) *Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* 5) *Department of Clinical Sciences, Institute of Tropical Medicine (ITM), Antwerp, Belgium and* 6) *Divisions of Infectious Diseases and Medical Microbiology, J.D. MacLean Centre for Tropical Diseases, McGill University Health Centre, Montreal, Canada*

Abstract

The recent roll-out of rapid diagnostic tests (RDTs) for malaria has highlighted the decreasing proportion of malaria-attributable illness in endemic areas. Unfortunately, once malaria is excluded, there are few accessible diagnostic tools to guide the management of severe febrile illnesses in low resource settings. This review summarizes the current state of RDT development for several key infections, including dengue fever, enteric fever, leptospirosis, brucellosis, visceral leishmaniasis and human African trypanosomiasis, and highlights many remaining gaps. Most RDTs for non-malarial tropical infections currently rely on the detection of host antibodies against a single infectious agent. The sensitivity and specificity of host-antibody detection tests are both inherently limited. Moreover, prolonged antibody responses to many infections preclude the use of most serological RDTs for monitoring response to treatment and/or for diagnosing relapse. Considering these limitations, there is a pressing need for sensitive pathogen-detection-based RDTs, as have been successfully developed for malaria and dengue. Ultimately, integration of RDTs into a validated syndromic approach to tropical fevers is urgently needed. Related research priorities are to define the evolving epidemiology of fever in the tropics, and to determine how combinations of RDTs could be best used to improve the management of severe and treatable infections requiring specific therapy.

Keywords: African trypanosomiasis, brucellosis, dengue, fever, leptospirosis, rapid diagnostic tests, tropics, typhoid, visceral leishmaniasis

Clin Microbiol Infect

Corresponding author: F. Chappuis, Division of International and Humanitarian Medicine, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 6, 1211 Geneva 14, Switzerland
E-mail: francois.chappuis@hcuge.ch

Introduction

The recent roll-out of rapid diagnostic tests (RDTs) for malaria has highlighted the decreasing proportion of malaria-attributable illness in endemic areas [1–4], and that the proportion of patients evaluated for fever suffering from a condition other than malaria is likely to continue increasing with time [5–8]. Unfortunately, once malaria is excluded, there are few accessible diagnostic tools to guide the management of severe febrile illnesses in low resource settings. Making matters worse, very little epidemiological data underpins clinicians' assessment of prior probability in vast areas of Africa and Asia. However, wherever systematically studied, various non-malarial

infections have been found to be major causes of febrile syndromes in tropical settings, such as relapsing fever [9], leptospirosis [10,11], rickettsial infection [12], dengue [13] or typhoid fever [14]. Such infections can be severe and most are treatable with specific therapy, but often clinically indistinguishable without confirmatory tests.

Based on the successful contribution of RDTs to malaria and HIV diagnosis, several point-of-care assays and RDTs designed for peripheral health facilities have been or are being developed for other tropical infections to improve patient care and epidemiological surveillance. The purpose of this article is to review currently available RDTs for the individual case-management of non-malarial tropical infections presenting with acute

or persistent fever. Priority has been given to RDTs detecting potentially severe illnesses of proven or suspected epidemiological importance, requiring specific management, for which 'immediate' diagnosis would be most useful. In this review, we will not focus on RDTs developed for febrile illnesses with predominantly focal symptoms, such as respiratory or diarrhoeal infections.

For this review, RDTs are defined as any test yielding results within minutes and that can be performed in health centres with little infrastructure or trained personnel, preferably without electricity. These are nearly all immunoassays, in various formats. New generations of such tests are increasingly single-step lateral flow assays (immunochromatographic tests in the form of a dipstick or cassette), which offer technical and operational advantages over older formats such as latex agglutination kits, flocculation assays and vertical flow-through assays [15–17].

Diseases

The epidemiological, clinical and reference diagnostic features of the diseases presented below are summarized in Table 1.

Dengue and chikungunya

The retrospective nature of classical serological tests to confirm dengue does not help clinicians to manage acutely ill patients (Table 1). The new combined RDTs that detect both IgM/IgG and the NS1 antigen provide immediate results and are able to diagnose dengue at different points in time after initiation of symptoms (Table 2).

The NS1 antigen is highly specific and detectable in serum from days 1 to 9 after fever onset [18,19]; its sensitivity depends on the type of test used and the time since onset of symptoms (it declines in parallel with viraemia), and is higher in primary than secondary dengue [20–22]. Tests detecting specific IgM antibodies are generally sensitive for diagnosing dengue, but not in the first days of fever. In a study among travellers in Israel, IgM became detectable between days 4 and 8 of fever [23]. Tests that combine detection of NS1 and IgM can therefore diagnose dengue throughout the febrile illness. The sensitivity of combined NS1/antibody tests was 76–93% in six recent studies conducted in endemic areas (Table 2). Sensitivity was even higher (96%) among travellers, who are more likely to present with primary dengue [24]. Whereas specificity of the NS1 band is very high (>95% in most studies), specificity of the IgM band is sometimes lower in endemic areas due to cross-reactivity with other pathogens, mainly Chikungunya virus [25]. Only two combined NS1/antibody RDTs that have been evaluated in endemic areas are commercially available: RDT from Panbio (Inverness Medical Innovations, Brisbane, Australia), presently

available in two separate cassettes (Panbio Dengue Early Rapid™, detecting NS1, and Panbio Dengue Duo Cassette™, detecting IgM/IgG), and the SD Bioline Dengue Duo™ (Standard Diagnostics, Kionggi, Korea), detecting both NS1 and IgM/IgG in two cassettes bonded together.

Chikungunya is an emerging alphavirus with wide geographical distribution [26]. RDTs based on IgM detection have been recently developed with limited field validation. Sensitivity was poor in the first week of illness but increased afterwards [27,28].

Enteric fever

The diagnosis of enteric fever is notoriously difficult, owing to its non-specific clinical presentation, a very low number of circulating bacteria in blood [29], and antigenic similarity to other members of the *Enterobacteraceae* [30]. The Widal test is a simple serological assay detecting antibodies against lipopolysaccharide (LPS; O) and flagellar (H) antigens of *S.* Typhi. Introduced over 115 years ago, this test continues to be widely used despite its unacceptably low accuracy [31–35].

Numerous RDTs based on host antibody detection have attempted to improve diagnostic performance, with limited success (Table 3). The IDL TUBEX TF™ (IDL Biotech AB, Bromma, Sweden) is a semi-quantitative colorimetric test detecting anti-0:9 IgM antibodies. Results are available within 3 min at room temperature and require minimal laboratory supplies. The Typhidot™ platform (Reszon Diagnostics International Sdn. Bhd., Selangor, Malaysia) qualitatively detects antibodies to the outer membrane protein (Vi; OMP) and is commercialized in three forms: the Typhidot™ detects either IgM or IgG antibody via an enzyme immunoblot assay and yields qualitative results in 60 min; the Typhidot Rapid IgM™ and Typhidot Rapid IgM IgG Combo™ are immunochromatographic (ICT) cassettes featuring separate lines for IgG and IgM and yield results in 15 min. Other commercialized RDTs include the RTI LPS IgM ICT™ (Royal Tropical Institute, Amsterdam, the Netherlands; now produced by LifeAssay Diagnostics Ltd) detecting anti-LPS (O antigen) IgM, and the Typhoid Rapid Test™ (SD Bioline, Kionggi, Korea) detecting total IgM/IgG against an unspecified *S.* Typhi antigen. All of these RDTs detect antibodies against antigens selected from *S.* Typhi isolates. TUBEX and Typhidot are non-reactive with sera from *S.* Paratyphi infections [36]. Conversely, the RTI LPS IgM ICT is equally sensitive for infections from *S.* Typhi and *S.* Paratyphi [37].

In general, the sensitivity of serological tests is unsatisfactory initially and increases with longer duration of illness. In Indonesia, the sensitivity of Widal and the RTI LPS IgM ICT increased from 43–48% at days 4–6 of fever to 90–100% after more than 9 days, compared with positive blood cultures [37]. The reported specificity of RDTs is affected by imperfect sensitivity of reference standard blood cultures, and rises from

TABLE 1. Epidemiology, clinical features and reference diagnostic tests of dengue fever, enteric fever, leptospirosis, brucellosis, human African trypanosomiasis and visceral leishmaniasis

Disease	Burden and epidemiology	Clinical features	Reference diagnostic testing
Dengue fever	Annual global incidence estimated at 50–100 million cases. 500 000 people with severe dengue require hospitalization each year, esentially children, of whom about 2.5% die. Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. About half of the world's population is now at risk. Transmitted by Aedes mosquitoes (e.g. *A. aegypti*). No animal reservoir	Suspect case defined by acute fever with at least two of the following: headache, retro-orbital pain, myalgia, arthralgia, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days. Severe dengue is characterized by shock, respiratory distress, severe bleeding or organ impairment. Warning signs occur 3–7 days after the first symptoms.	Isolation of the dengue virus from serum, plasma, leukocytes or tissues. Demonstration of a fourfold or greater rise in reciprocal IgG or IgM antibody titres to one or more dengue virus antigens in paired serum samples, Detection of viral genomic sequences in tissue, serum or CSF samples by PCR.
Enteric fever (*Salmonella* Typhi/Paratyphi)	Annual global incidence estimated at 27 million cases (22 million S. Typhi, 5 million S. Paratyphi) with 216 150 deaths [80]. Occurs worldwide in low/middle income countries, particularly south/southeast Asia. African burden less clear. Under-reporting and diagnostic difficulty make precise estimates difficult. Faecal-oral transmission. No animal reservoir.	Clinical spectrum ranging from fever and malaise to severe complications such as intestinal perforation and encephalopathy. Fever generally increases abruptly after first week of illness in untreated patients. Splenomegaly and fleeting rash may occur. Diarrhoea in less than 50% of cases.	Recovery of Salmonella Typhi/Paratyphi from blood or bone marrow (BM). Recovery from intestinal tract may represent chronic carriage. Sensitivity <100% for all specimens, depending on many factors. Combination of several samples (blood, BM, urine, gastric aspirates, rose spots) increases yield [81]. Drawing sufficient volume of blood (i.e $\geq$ 20–30 mL) is the most important factor affecting the yield of blood cultures [29,82].
Leptospirosis	Zoonotic disease caused by bacteria of the genus *Leptospira*. Worldwide distribution but true disease burden unknown. Most common in urban slums and rural tropics. Epidemics often seen during flooding. Main transmission to humans by exposure to water and soil contaminated by the urine of infected animals (e.g. rodents) [83].	Wide spectrum from asymptomatic to fulminant disease. Fever, malaise, headaches, severe myalgias, conjunctival suffusion, anorexia, nausea, vomiting followed by aseptic meningitis in up to 25% cases. Severe forms: jaundice, renal failure and haemorrhage (Weil's disease) and/or respiratory distress	Culture from blood or other body fluids has low sensitivity and very slow growth rate. The microscopic agglutination test (MAT) detects serogroup-specific IgM and IgG antibodies. Paired sera required for definitive diagnosis (seroconversion or fourfold increase in titres) Conventional and real-time PCR in blood or serum useful for early diagnosis, despite limited sensitivity [51,84].
Brucellosis (*B. melitensis*, *B. abortus*, *B. suis*)	Leading zoonosis in the world, with annual global incidence of ~500 000 cases, and prevalence $\geq$ 10/100 000 in some countries [85]. Transmission by contact with fluids from infected animals or derived food products. Reservoir is domestic livestock, differs by *Brucella* species. Worldwide occurrence, highest in Mediterranean basin, Indian subcontinent, Mexico and Central and South America.	Extremely wide clinical spectrum. Acute disease presents as fever $\pm$ focal symptoms (e.g. arthritis). Chronic disease (e.g. abscess) may be more difficult to diagnose. Treatment requires at least 6 weeks of dual drug therapy, and relapses are frequent.	Recovery of *Brucella* species in culture of any body fluid or tissue. Blood or bone marrow have highest yield, and may require prolonged incubation (up to 3 weeks) if manual culture systems are used.
Human African trypanosomiasis (HAT)	Exclusively in Africa; due to *Trypanosoma brucei gambiense* or *T. b. rhodesiense* *T. b. gambiense*: Foci in rural areas of west and central Africa; most prevalent in Democratic Republic of Congo, Central African Republic and southern Sudan;since 2009, less than 10 000 cases reported annually but proportion of under-reported cases unknown [86]. *T. b. rhodesiense*: Foci in rural areas of east and southern Africa; less than 200 cases reported in 2009 [86].	First (early) haematolymphatic stage: intermittent fever, headache, pruritus, lymphadenopathy. Second (late) meningo-encephalitic stage: sleep disturbances and neuropsychiatric disorders. More severe symptoms and faster evolution to the meningoencephalitic stage for *T. b. rhodesiense*; fatal if left untreated.	Microscopic examination of trypanosomes in body fluid (lymph, blood or cerebrospinal fluid [CSF]). Need for concentration methods for *T. b. gambiense* HAT (e.g. microhaematocrit centrifugation technique, mini-anion-exchange centrifugation technique) because of scanty parasites. Diagnosis of second stage HAT based on presence of trypanosomes and/or more than 5 white blood cells per μL in CSF [58].
Visceral leishmaniasis	Systemic protozoan infection due to *Leishmania donovani* in South Asia and East Africa or *L. infantum* in Latin America and the Mediterranean region. 200 000–400 000 cases occur annually in 70 countries. The five most affected countries are India, Bangladesh, Sudan, South Sudan and Ethiopia [87]. Fatal if left untreated.	Prolonged fever, malaise, weight loss, epistaxis, cough, enlarged liver and spleen, lymphadenopathies, progressive anaemia, concomitant infections (e.g. pneumonia).	Microscopic examination of *Leishmania* amatigote forms in aspirates from lymph node, bone marrow or spleen. Sensitivity >90% only with spleen aspirate examination but risk of major bleeding ~0.1%. Sensitivity improved by culture or PCR. Various serological methods with high sensitivity and specificity: ELISA, IF, Western blot, DAT [61,88].

46–80% when clinical suspects with negative blood cultures are used as controls, to 83–100% when controls have an established alternative diagnosis (Table 3). In addition, numerous other host factors may affect the specificity of antibody-detection tests. Thus, the modest accuracy of available RDTs for early enteric fever precludes their routine use, except perhaps in the setting of a high pre-test probability, such as during outbreaks [14].

TABLE 2. Selected studies evaluating RDTs for dengue fever[a] (adapted from Blacksell et al. [89])

Author	Year	Assay	Country	Sample timing (days of illness)	Reference comparator	Antigen or antibody detected	Sensitivity% (95% CI)	Specificity% (95% CI)
Shu et al. [90]	2009	BioRad STRIP	Taiwan	1–7 (median: 2)	PCR or paired ELISA	NS1	77.3 (0.54–0.92)	100
Hang et al. [91]	2009		Vietnam	1–6	PCR or paired ELISA	NS1	72.8 (64.1–80.3)	100 (91.6–100)
Chaiyaratana et al. [92]	2009		Thailand	1–8	NS1 Ag ELISA	NS1	98.9 (96.8–100)	90.6 (85.6–95.7)
Ramirez et al. [93]	2009		Venezuela	2–6	PCR or paired ELISA	NS1	67.8 (57.4–76.7)	94.4 (80.9–99.4)
Lima et al. [94]	2010		Brazil	1–6	Combinations of viral culture, PCR, NS1 Ag ELISA	NS1	89.6 (84.7–93.2)	99.1 (96.9–99.9)
Tricou et al. [95]	2010		Vietnam	1–6	PCR or paired ELISA	NS1	61.6 (55.2–67.8)	100 (93.8–100)
Osorio et al. [96]	2010		Colombia	2–7 (median: 4)	Viral culture, PCR or paired ELISA	NS1	57.7 (47.6–67.3)	95.3 (84.2–99.4)
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	AFRIMS ELISA paired samples	NS1	58.6 (48.2–68.4)	98.8 (95.6–99.9)
Tricou et al. [95]	2010	SD Bioline Dengue Duo	Vietnam	1–6	PCR or paired ELISA	NS1	62.4 (56.1–68.5)	100 (93.8–100)
Wang and Sekaran [97]	2010		Malaysia	1–15	Virus isolation, PCR, paired ELISA	NS1	65.4 (58.5–72.3)	98.8 (96.2–100)
Osorio et al. [96]	2010		Colombia	2–7 (median: 4)	Viral culture, PCR or paired ELISA	NS1	51 (44.1–57.7)	96.7 (90.8–99.3)
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	Paired ELISA	NS1	48.5 (38.5–58.7)	99.4 (96.6–100)
Fry et al. [98]	2011	Panbio Early Rapid NS1	Vietnam	1–5 (84.5%<3)	PCR or paired ELISA	NS1	69.2 (62.8–75.6)	96% (92.2–99.8)
Fry et al. [98]	2011		Malaysia	1–15 (70% ≤5)	PCR or paired ELISA	NS1	68.9 (61.8–76.1)	96.7 (82.8–99.9)
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	Paired ELISA	NS1	58.6 (48.2–68.4)	92.5 (87.3–96.1)
Wang and Sekaran [97]	2010	SD Bioline Dengue Duo	Malaysia	1–15	Virus isolation, PCR, rising titre in a paired sample using MAC ELISA	IgM	53.5	100
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	Paired ELISA	IgM	79.2 (70.5–87.2)	89.4 (83.5–93.7)
Blacksell et al. [25]	2011	Panbio Dengue Duo Cassette	Sri Lanka	Median 5; IQR 2–7	Paired ELISA	IgM	70.7 (60.7–79.4)	80.0 (73.0–85.9)
Blacksell et al. [25]	2011	Merlin IgM	Sri Lanka	Median 5; IQR 2–7	Paired ELISA	IgM	72.7 (62.9–81.2)	73.8 (66.2–80.4)
Blacksell et al. [25]	2011	Biosynex IgM	Sri Lanka	Median 5; IQR 2–7	Paired ELISA	IgM	79.8 (70.5–87.2)	46.3 (38.3–54.3)
Tricou et al. [95]	2010	SD Dengue Duo Bioline	Vietnam	1–6	PCR or paired ELISA	NS1/IgM NS1/IgM/IgG	75.5 (69.6–80.8) 83.7 (78.4–88.1)	100 (93.8–100) 97.9 (88.7–99.9)
Wang and Sekaran [97]	2010		Malaysia	1–15	Virus isolation, PCR, paired ELISA	NS1/IgM	88.7 (84.0–93.3)	98.8 (96.3–100)
Osorio et al. [96]	2010		Colombia	2–7 (median: 4)	Viral culture, PCR or paired ELISA	NS1/IgM NS1/IgM/IgG	78.4 (72.4–83.7) 80.7 (75–85.7)	91.3 (83.6–96.2) 89.1 (81–94.7)
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	Paired ELISA	NS1/IgM	92.9 (83.9–97.1)	88.8 (82.8–93.2
Fry et al. [98]	2011	Panbio Early Rapid NS1 and Duo assay	Malaysia	1–15 (70% ≤5)	PCR or paired ELISA	NS1/IgM NS1/IgM/IgG	89.0 (85.2–92.8) 93	Not reported Not reported
Blacksell et al. [25]	2011		Sri Lanka	Median 5; IQR 2–7	Paired ELISA	NS1/IgM	89.9 (82.2–95.0)	75.0 (67.6–81.5)

[a]Studies were included if: (i) study undertaken in a dengue endemic country; (ii) test detecting either NS1 or IgM or both; (iii) reference method including at least PCR or ELISA on paired samples when evaluating NS1 and at least ELISA on paired samples when evaluating IgM; (iv) sample timing described; (v) study published from 2009 onwards.

The detection of *S.* Typhi antigens in clinical samples has been shown to have comparable accuracy to antibody-detection tests [38–40]. A valuable alternative use for putative RDTs based on such assays could be the rapid identification of *S.* Typhi/Paratyphi in culture media, greatly simplifying blood cultures in basic laboratories.

Leptospirosis

The Lepto lateral flow™ has been developed by the Royal Tropical Institute (KIT, Amsterdam, the Netherlands) [41]. It is based on the binding of specific IgM antibodies to a whole-cell antigen prepared from the non-pathogenic Patoc 1 strain. In the Andaman Islands, India, its sensitivity increased with the duration of illness, from 34% on days 2–3 to 63% on days 4–5 and 85% at the end of the first week, with high specificity (94%) [42]. It is now commercialized as Test-it™ Leptospira (LifeAssay Diagnostics). Most publications of the late 1990s and early 2000s evaluated earlier formats of RDTs developed by the KIT or commercial RDTs that are, to our knowledge, no longer available [43–48].

The persistence of anti-leptospiral IgM after infection and frequent exposure to non-pathogenic leptospires (e.g. during farming) are likely to explain the limited specificity of ELISA and RDTs using whole-cell antigens, as reported by some [48–50]. Diagnostic accuracy could theoretically be improved with assays using more specific antigens [51]. A novel Dual Path Platform™(DPP) assay (Chembio Diagnostics Systems, Medford, USA) incorporates recombinant leptospiral immunoglobulin-like proteins as antigens. In a phase 2 study conducted in Latin America, the assay achieved sensitivity of 85% and 64% in severe and mild leptospirosis, respectively. Like the other serological tests, sensitivity increased with duration of symptoms. Specificity was high (>93%) in various control groups but moderate (86%) in healthy slum residents, suggesting some background immunity also against this antigen in highly exposed populations [52].

The detection of leptospiral antigen in the blood or urine is a promising approach, as it may allow for earlier diagnosis (i.e. before the appearance of specific IgM) and therefore prompter treatment to prevent late clinical complications [53].

Brucellosis

Serological tests detecting antibodies to *Brucella* spp. antigens are the most frequently used modality for acute and chronic

TABLE 3. Selected studies evaluating RDTs for enteric fever[a]

Author	Year	Assay	Country	Sample timing (days of illness)	True positive definition	True negative definition	Sensitivity% (95% CI)	Specificity% (95% CI)
Keddy et al. [99]	2011	IDL TUBEX® TF (IgM)	South Africa Tanzania	Not reported[a]	Automated blood culture (Bac-T Alert)	Febrile patients with negative blood cultures	73 (60.3–83.4)	69 (49.2–84.7)
Ley et al. [100]	2011		Tanzania	Not reported[a]	Automated blood culture (BACTEC)	All non-typhi Bacteraemia	79 (52–81)	89 (81–94)
Naheed et al. [101]	2008		Bangladesh	Median 3 (range 1–30)	Manual blood culture	Blood culture neg and other bacteraemia	60	64
Kawano et al. [102]	2007		Philippines	Not reported[a]	Manual blood culture and BACTEC	Febrile patients with negative blood cultures	94.7 (86–98)	80.4 (71–87)
Dutta et al. [36]	2006		India	Median 4 (range 3–60)	Automated blood culture (BACTEC)	Paratyphoid and malaria cases	56 (47–66)	88 (82–94)
Olsen et al. [103]	2004		Vietnam	Median 11 (range 4–55)	Manual blood culture and BACTEC	Other laboratory-confirmed diagnoses	78 (65–88)	94 (71–100)
House et al. [104]	2001		Vietnam	Median 12 (range 7–17)	Blood culture (not specified)	Febrile patients with negative blood cultures	87 (66–87)	76 (63–89)
Keddy et al. [99]	2011	Typhidot (IgM)	South Africa Tanzania	Not reported[a]	Automated blood culture (Bac-T Alert)	Febrile patients with negative blood cultures	75.0 (61.1–86.0)	60.7 (40.6–78.5)
Kawano et al. [102]	2007		Philippines	Not reported[a]	Manual blood culture and BACTEC	Febrile patients with negative blood cultures	55 (43–66)	65 (55–74)
Dutta et al. [36]	2006		India	Median 4 (range 3–60)	Automated blood culture (BACTEC)	Paratyphoid and malaria cases	47 (37–58)	83 (71–94)
Olsen et al. [103]	2004		Vietnam	Median 11 (range 4–55)	Manual blood culture and BACTEC	Other laboratory-confirmed diagnoses	79 (66–88)	89 (66–98)
Keddy et al. [99]	2011	Typhidot (IgG)	South Africa Tanzania	Not reported[a]	Automated blood culture (Bac-T Alert)	Febrile patients with negative blood cultures	69.2 (54.9–81.3)	70.4 (49.8–86.2)
Kawano et al. [102]	2007		Philippines	Not reported[a]	Manual blood culture and BACTEC	Febrile patients with negative blood cultures	73 (62–83)	46 (36–56)
Naheed et al. [101]	2008	Typhidot Rapid IgM IgG Combo (IgM/IgG)	Bangladesh	Median 3 (range 1–30)	Manual blood culture	Blood culture neg and other bacteraemia	67 (51–81)	54 (33–74)
Hatta et al. [37]	2002	RTI LPS IgM ICT (IgM)	Indonesia	Median (25–75IQR) 8 (5–11) 15 (12–18) 29 (25–31)	Manual blood culture	Febrile hospitalized patients with a final diagnosis other than typhoid fever.	77.0 (61–89) 82.1 (66–92) 97.4 (87–100)	100 (98.5–100)
Kawano et al. [102]	2007	SD Bioline Typhoid Rapid Test (IgM/IgG)	Philippines	Not reported[a]	Manual blood culture and BACTEC	Febrile patients with negative blood cultures	IgM: 69 (55–80) IgG: 71 (59–81)	IgM: 79 (70–87) IgG: 76 (70–83)

[a]Studies were included if (i) blood or bone marrow cultures were used as the reference standard, (ii) enteric fever clinical suspects were used for specificity calculations, (iii) 95% confidence intervals around performance estimates were given or calculable from presented data, and (iv) time elapsed since the onset of fever was indicated, because host antibody responses and test sensitivity are time-dependent [37]. The results from Keddy et al. and Ley et al. [99,100] are reported despite the lack of timing data because they are the only published high-quality studies in African populations. Results from Kawano et al. [102] are reported despite the lack of timing data because it is the only study evaluating the RDT from SD Bioline.

infection. The Rose Bengal test (RBT) is a rapid slide-agglutination assay that uses a stained *B. abortus* suspension to detect anti-*Brucella* antibodies. It has long been used as a screening test in low-resource settings, but confusion about its diagnostic accuracy led the WHO to recommend that positive RBT results be confirmed by another method. Concerns have focused on perceived low sensitivity for chronic infection, cross-reactivity with other pathogens and the prozone effect. However, the RBT was recently assessed in a large study using stored samples from culture-confirmed cases (*n* = 208) and controls with other illnesses (*n* = 1159) [54]. RBT results were highly concordant with other serological methods, except when positive titres were lower than 1:8, for which confirmation using another test appears necessary.

An RDT has recently been developed that could allow testing at the point of care. The Lateral Flow immunochromatography assay™ (LFiC; KIT) detects IgM and IgG to the polysaccharide section of the *Brucella* S. lipopolysaccharide (S-LPS; O) antigen. The test is simple, uses fingerprick blood, does not require refrigeration, and has shown sensitivity of 96–100% and specificity reaching 99% when used for disease confirmation,

compared with positive blood cultures [55,56], and high concordance with standard serological methods [54,57]. Further studies are needed to define LFiC performance in different human epidemiological and clinical settings (e.g. relapse). The LFiC is now produced by LifeAssay Diagnostics Ltd.

Human African trypanosomiasis

For decades, the card agglutination test for trypanosomiasis (CATT) has been used for screening and diagnosis of *T. b. gambiense* HAT. Although not an RDT *sensu stricto* (it requires electricity and other equipment), it can be performed in remote settings. Its diagnostic accuracy (sensitivity, 87–98% on undiluted whole blood; specificity, 95%) has been evaluated in the context of mass screening of predominantly asymptomatic individuals [58], but never in clinically suspect patients (e.g. with persistent fever or neurological disorders). A new and more practical format (CATT-D10), with similar performance, has been developed to screen a smaller number of patients in peripheral health facilities [59]. Two lateral flow immunochromatographic RDTs for the serodiagnosis of *T. b. gambiense* HAT have reached advanced stages of development: (i) the 'Immunochromato-

graphic HAT-RDT', manufactured by Standard Diagnostics, in collaboration with the Foundation for Innovative New Diagnostics (FIND) and (ii) the 'Gambiense-Sero-K-set' developed by Coris BioConcept (Gembloux, Belgium) in collaboration with the Institute of Tropical Medicine, Antwerp. Results of phase 2 evaluations should be published soon for both RDTs, while phase 3 studies among clinically suspect patients are ongoing.

For *T. b. rhodesiense* HAT, there is no RDT in development. Diagnosis is, however, straightforward with classic microscopy, because parasite load is usually high in the blood during clinical illness.

Visceral leishmaniasis
The first RDT detecting antibodies against rK39, a recombinant antigen from *Leishmania infantum*, was evaluated in India in the late 1990s [60]. A meta-analysis of 13 rK39 RDT evaluation studies revealed an overall sensitivity of 93.7% and a specificity of 95.3%, with a trend towards decreased sensitivity in East Africa [61]. This trend was confirmed in recent studies, particularly among HIV co-infected patients [62–64]. Only two rK39 RDTs have been sufficiently validated for use in clinical practice, the Kalazar Detect™ from Inbios, Seattle, WA, USA, and the IT-LEISH™ from BioRad, Marnes-la-Coquette, France (formerly from DiaMed AG, Switzerland). Both can confirm VL, provided that they are applied on strictly-defined clinically suspect patients (e.g. $\geq$ 2 weeks fever and splenomegaly), but a negative rK39 RDT test result confidently rules out VL in South Asia only, highlighting the need for the development of a more sensitive RDT for East Africa. Newly developed RDTs based on detection of antibodies against rK28, a synthetic polyprotein, showed promising sensitivity estimates (95.9–98.1%) in a limited number of VL patients in Sudan and Bangladesh [65].

As antibodies remain detectable for years after treatment [66], serological-based assays are useless to diagnose VL relapses. For this purpose, efforts are currently being made to transform an existing antigen detection test in urine (KAtex™, Kalon Biological Ltd, Guildford, UK) into a more practical lateral flow test.

Other febrile diseases
Rickettsial diseases are a large group of infections with worldwide (e.g. murine typhus) or limited (e.g. scrub typhus) distribution [67]. Diagnostic confirmation can sometimes be made clinically in the presence of a typical eschar (e.g. scrub typhus), but most often relies on the detection of specific antibodies, generally on paired samples. Serological RDTs have been developed and validated for scrub typhus only, with very variable sensitivity estimates (39–97%) [68–72]. Rapid diagnostic tests are in development for melioidosis, an infection due to *Burkholderia pseudomallei* that is a frequent cause of febrile illness and sepsis in southeastern Asia and northern Australia. Numerous species of *Borrelia spp* may cause relapsing fever, an underestimated cause of fever in some tropical areas [9]. Despite the low sensitivity of classic microscopy compared with molecular techniques [73], no RDT has been developed to improve diagnosis in remote settings.

Discussion

In many peripheral health facilities in the tropics, RDT-based diagnosis of malaria is nowadays straightforward. Unfortunately, once malaria is excluded, there are few accessible diagnostic tools to guide the management of myriad severe febrile illnesses [6]. This review summarizes the current state of RDT development for several key infections and highlights many remaining gaps.

Most RDTs for non-malarial tropical infections currently rely on the detection of specific antibodies against a single infectious agent. Antibodies usually take several days after the appearance of fever to be detectable in peripheral blood, which limits the sensitivity of serological RDTs in acute fever [42,52,68]. Their specificity can also be altered by cross-reactivity with other infectious agents, background seroprevalence in the healthy exposed population and long-term persistence of antibodies after infection [52,74]. The latter may also prevent the use of serological RDTs to monitor treatment response and/or to diagnose relapse [75]. Considering the above limitations, there is an urgent need to foster the development of sufficiently sensitive antigen-based RDTs, as successfully accomplished for malaria and dengue.

In many studies, evaluation of RDT accuracy is impaired by an imperfect reference standard (e.g. blood culture for typhoid fever, microscopic agglutination tests (MAT) for leptospirosis or bone marrow aspiration for VL), leading to over-estimation of RDT sensitivity and under-estimation of specificity. Optimizing the choice of reference standard (e.g. using a composite reference standard) or adjusting for the absence of reference standard (e.g. applying latent class analysis) should be strongly encouraged in all diagnostic studies evaluating the 'true' RDT performance [76,77].

In addition to being highly accurate, RDTs need to be affordable, user friendly, rapid and robust, equipment-free and delivered to end-users, as summarized in the ASSURED criteria [78]. Even if the ASSURED criteria are met, many potential obstacles to widespread correct use of RDTs and other point-of-curve tests remain, as recently reviewed [78,79]. A striking feature is the lack of current production

of many validated RDTs, possibly reflecting the fragile commitment of manufacturers to maintaining the availability of such tests, and the lack of regulatory and financial conditions that could facilitate or incentivise such commitments. The advantages and pitfalls of RDTs in low resource settings are summarized in the Box.

Box: Advantages and pitfalls of rdts in low-resource settings

Advantages of RDTs over conventional laboratory tests:

- Access to appropriate diagnosis-based management, i.e.

  - Comparatively low skill required for use
  - Minimal infrastructure requirements
  - Low cost

- Rapid results in a clinically relevant time-frame
- Potentially increased standardization of care

Pitfalls and limitations of immunoassay-based RDTs:

Technical

- RDTs based on detection of host antibodies generally have low sensitivity in the first several days of disease – when treatment might be most desirable. Moreover, the accuracy of such tests is affected by host factors and prior infections.
- Despite having a broad range of operating and storage temperatures, antibodies used for RDT assembly may degrade in extreme environments.
- Inherent limits in sensitivity when conventional colorimetric detection is used. Inter-reader variability can be significant, especially for faint test lines.
- May be susceptible to the prozone phenomenon (i.e. falsely negative or borderline results due to an excess of either the antigen or the antibody of interest).
- Specificity may be severely decreased (i.e. false positives) in the presence of concomitant conditions that cause polyclonal hyper-gammaglobulinaemia.

Operational

- *Training and quality assurance are essential*: even the simplest RDTs can be improperly used or misinterpreted, and inaccurate results can harm patients and undermine their confidence in local medical services.
- *Feasibility at the point-of-care*: unlike conventional laboratory services, health workers using RDTs might assume responsibility for specimen collection *and* testing, as well as for quality control and documentation. This will become an organizational challenge as RDTs for an ever increasing list of diseases become available.
- *User-interpretation of the signal, documentation and archiving of results:* these may be addressed by battery-operated automated RDT readers, which digitally photograph RDT test strips, uniformly interpret the results, and archive standardized photos for subsequent quality assurance.
- *One test = one disease*: while microscopy and bacterial culture have the ability to detect multiple pathogens at once, including unsuspected ones, most existing RDTs only detect a single pathogen. This limits the usefulness of current RDTs in the management of some important clinical syndromes such as sepsis.

Current approaches to fever in low-resource settings are most often fragmented or rely on non-specific clinical data and empirical therapy. There is abundant evidence that this approach is harmful and that integrated diagnostic pathways for febrile illness are urgently needed [8]. In addition to the development of locally validated RDTs, research priorities are (i) filling the void of epidemiological knowledge in much of the tropics (to assess pre-test probability) and (ii) developing validated pathways combining key epidemiological and clinical features with the use of RDTs, either alone or in tailored panels, to better manage severe and treatable infections requiring specific therapy.

Acknowledgements

Authors' Contributions

FC structured and coordinated the writing of the manuscript and drafted several chapters. EA did an extensive search of the literature and reviewed the manuscript. VDA and EB drafted several chapters and reviewed the manuscript. CY coordinated with FC the writing of the manuscript and wrote several chapters.

Transparency Declaration

References

1. World Health Organization. *Guidelines for the treatment of malaria -* 2nd edn; 2010.
2. Reyburn H, Mbatia R, Drakeley C et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* 2004; 329: 1212.
3. Doudou MH, Mahamadou A, Ouba I et al. A refined estimate of the malaria burden in Niger. *Malar J* 2012; 11: 89.
4. Feachem RG, Phillips AA, Hwang J et al. Shrinking the malaria map: progress and prospects. *Lancet* 2010; 376: 1566–1578.
5. Crump JA. Typhoid Fever and the challenge of nonmalaria febrile illness in sub-saharan Africa. *Clin Infect Dis* 2012; 54: 1107–1109.
6. Crump JA, Gove S, Parry CM. Management of adolescents and adults with febrile illness in resource limited areas. *BMJ* 2011; 343: d4847.
7. Naing C, Kassim AI. Scaling-up attention to nonmalaria acute undifferentiated fever. *Trans R Soc Trop Med Hyg* 2012; 106: 331–332.
8. Yansouni CP, Bottieau E, Chappuis F et al. Rapid diagnostic tests for a coordinated approach to fever syndromes in low-resource settings. *Clin Infect Dis* 2012; 55: 610–611.

9. Vial L, Diatta G, Tall A *et al.* Incidence of tick-borne relapsing fever in west Africa: longitudinal study. *Lancet* 2006; 368: 37–43.

10. Biggs HM, Bui DM, Galloway RL *et al.* Leptospirosis among hospitalized febrile patients in northern Tanzania. *Am J Trop Med Hyg* 2011; 85: 275–281.

11. Reller ME, Bodinayake C, Nagahawatte A *et al.* Leptospirosis as frequent cause of acute febrile illness in southern Sri Lanka. *Emerg Infect Dis* 2011; 17 : 1678–1684.

12. Prabhu M, Nicholson WL, Roche AJ *et al.* Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. *Clin Infect Dis* 2011; 53: e8–e15.

13. Kasper MR, Blair PJ, Touch S *et al.* Infectious etiologies of acute febrile illness among patients seeking health care in south-central Cambodia. *Am J Trop Med Hyg* 2012; 86: 246–253.

14. Lutterloh E, Likaka A, Sejvar J *et al.* Multidrug-resistant typhoid fever with neurologic findings on the Malawi-Mozambique border. *Clin Infect Dis* 2012; 54: 1100–1106.

15. Josko D. Updates in immunoassays: introduction. *Clin Lab Sci* 2012; 25: 170–172.

16. Josko D. Updates in immunoassays: bacteriology. *Clin Lab Sci* 2012; 25: 173–178.

17. Josko D. Updates in immunoassays: virology. *Clin Lab Sci* 2012; 25: 179–184.

18. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol* 2002; 40: 376–381.

19. Huang JH, Wey JJ, Sun YC, Chin C, Chien LJ, Wu YC. Antibody responses to an immunodominant nonstructural 1 synthetic peptide in patients with dengue fever and dengue hemorrhagic fever. *J Med Virol* 1999; 57: 1–8.

20. Bessoff K, Delorey M, Sun W, Hunsperger E. Comparison of two commercially available dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single clinical sample for diagnosis of acute DENV infection. *Clin Vaccine Immunol* 2008; 15: 1513–1518.

21. Kumarasamy V, Wahab AH, Chua SK *et al.* Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J Virol Methods* 2007; 140: 75–79.

22. McBride WJ. Evaluation of dengue NS1 test kits for the diagnosis of dengue fever. *Diagn Microbiol Infect Dis* 2009; 64: 31–36.

23. Schwartz E, Mileguir F, Grossman Z, Mendelson E. Evaluation of ELISA-based sero-diagnosis of dengue fever in travelers. *J Clin Virol* 2000; 19: 169–173.

24. Huhtamo E, Hasu E, Uzcategui NY *et al.* Early diagnosis of dengue in travelers: comparison of a novel real-time RT-PCR, NS1 antigen detection and serology. *J Clin Virol* 2010; 47: 49–53.

25. Blacksell SD, Jarman RG, Bailey MS *et al.* Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. *Clin Vaccine Immunol* 2011; 18: 2095–2101.

26. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. *Lancet* 2012; 379: 662–671.

27. Rianthavorn P, Wuttirattanakowit N, Prianantathavorn K, Limpaphayom N, Theamboonlers A, Poovorawan Y. Evaluation of a rapid assay for detection of IgM antibodies to chikungunya. *Southeast Asian J Trop Med Public Health* 2010; 41: 92–96.

28. Kosasih H, Widjaja S, Surya E *et al.* Evaluation of two IgM rapid immunochromatographic tests during circulation of Asian lineage Chikungunya virus. *Southeast Asian J Trop Med Public Health* 2012; 43: 55–61.

29. Wain J, Diep TS, Ho VA *et al.* Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical

30. features, transmissibility, and antibiotic resistance. *J Clin Microbiol* 1998; 36: 1683–1687.

31. Espersen F, Hoiby N, Hertz JB. Cross-reactions between Salmonella typhi and 24 other bacterial species. *Acta Pathol Microbiol Scand B* 1980; 88: 243–248.

32. Widal F. Sérodiagnostique de la fièvre typhoïde. *La Semaine Médicale* 1896; 16: 259.

33. Olopoenia LA, King AL. Widal agglutination test - 100 years later: still plagued by controversy. *Postgrad Med J* 2000; 76: 80–84.

34. Parry CM, Hoa NT, Diep TS *et al.* Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. *J Clin Microbiol* 1999; 37: 2882–2886.

35. Parry CM, Wijedoru L, Arjyal A, Baker S. The utility of diagnostic tests for enteric fever in endemic locations. *Expert Rev Anti Infect Ther* 2011; 9: 711–725.

36. Lunguya O, Phoba MF, Mundeke SA *et al.* The diagnosis of typhoid fever in the Democratic Republic of the Congo. *Trans R Soc Trop Med Hyg* 2012; 106: 348–355.

37. Dutta S, Sur D, Manna B *et al.* Evaluation of new-generation serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. *Diagn Microbiol Infect Dis* 2006; 56: 359–365.

38. Hatta M, Goris MG, Heerkens E, Gooskens J, Smits HL. Simple dipstick assay for the detection of Salmonella typhi-specific IgM antibodies and the evolution of the immune response in patients with typhoid fever. *Am J Trop Med Hyg* 2002; 66: 416–421.

39. West B, Richens JE, Howard PF. Evaluation in Papua New Guinea of a urine coagglutination test and a Widal slide agglutination test for rapid diagnosis of typhoid fever. *Trans R Soc Trop Med Hyg* 1989; 83: 715–717.

40. Rockhill RC, Rumans LW, Lesmana M, Dennis DT. Detection of Salmonella typhi D, Vi, and d antigens, by slide coagglutination, in urine from patients with typhoid fever. *J Clin Microbiol* 1980; 11: 213–216.

41. Fadeel MA, Crump JA, Mahoney FJ *et al.* Rapid diagnosis of typhoid fever by enzyme-linked immunosorbent assay detection of Salmonella serotype typhi antigens in urine. *Am J Trop Med Hyg* 2004; 70: 323–328.

42. Smits HL, Eapen CK, Sugathan S *et al.* Lateral-flow assay for rapid serodiagnosis of human leptospirosis. *Clin Diagn Lab Immunol* 2001; 8: 166–169.

43. Sehgal SC, Vijayachari P, Sugunan AP, Umapathi T. Field application of Lepto lateral flow for rapid diagnosis of leptospirosis. *J Med Microbiol* 2003; 52: 897–901.

44. Bajani MD, Ashford DA, Bragg SL *et al.* Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *J Clin Microbiol* 2003; 41: 803–809.

45. Vijayachari P, Sugunan AP, Sehgal SC. Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis. *Epidemiol Infect* 2002; 129: 617–621.

46. Levett PN, Branch SL, Whittington CU, Edwards CN, Paxton H. Two methods for rapid serological diagnosis of acute leptospirosis. *Clin Diagn Lab Immunol* 2001; 8: 349–351.

47. Sehgal SC, Vijayachari P, Sharma S, Sugunan AP. LEPTO Dipstick: a rapid and simple method for serodiagnosis of acute leptospirosis. *Trans R Soc Trop Med Hyg* 1999; 93: 161–164.

48. Effler PV, Bogard AK, Domen HY, Katz AR, Higa HY, Sasaki DM. Evaluation of eight rapid screening tests for acute leptospirosis in Hawaii. *J Clin Microbiol* 2002; 40: 1464–1469.

49. Wagenaar JF, Falke TH, Nam NV *et al.* Rapid serological assays for leptospirosis are of limited value in southern Vietnam. *Ann Trop Med Parasitol* 2004; 98: 843–850.

50. Tanganuchitcharnchai A, Smythe L, Dohnt M *et al.* Evaluation of the Standard Diagnostics Leptospira IgM ELISA for diagnosis of acute leptospirosis in Lao PDR. *Trans R Soc Trop Med Hyg* 2012; 106: 563–566.

50. Blacksell SD, Smythe L, Phetsouvanh R *et al.* Limited diagnostic capacities of two commercial assays for the detection of Leptospira immunoglobulin M antibodies in Laos. *Clin Vaccine Immunol* 2006; 13: 1166–1169.

51. Toyokawa T, Ohnishi M, Koizumi N. Diagnosis of acute leptospirosis. *Expert Rev Anti Infect Ther* 2011; 9: 111–121.

52. Nabity SA, Ribeiro GS. Lessa Aquino C et al. Accuracy of a Dual Path Platform (DPP) Assay for the rapid point-of-care diagnosis of human leptospirosis. *PLoS Negl Trop Dis* 2012; 6: e1878.

53. Saengjaruk P, Chaicumpa W, Watt G *et al.* Diagnosis of human leptospirosis by monoclonal antibody-based antigen detection in urine. *J Clin Microbiol* 2002; 40: 480–489.

54. Diaz R, Casanova A, Ariza J, Moriyon I. The Rose Bengal Test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Negl Trop Dis* 2011; 5: e950.

55. Smits HL, Abdoel TH, Solera J, Clavijo E, Diaz R. Immunochromatographic Brucella-specific immunoglobulin M and G lateral flow assays for rapid serodiagnosis of human brucellosis. *Clin Diagn Lab Immunol* 2003; 10: 1141–1146.

56. Irmak H, Buzgan T, Evirgen O *et al.* Use of the Brucella IgM and IgG flow assays in the serodiagnosis of human brucellosis in an area endemic for brucellosis. *Am J Trop Med Hyg* 2004; 70: 688–694.

57. Roushan MR, Amin MJ, Abdoel TH, Smits HL. Application of a user-friendly Brucella-specific IgM and IgG antibody assay for the rapid confirmation of Rose Bengal-positive patients in a hospital in Iran. *Trans R Soc Trop Med Hyg* 2005; 99: 744–750.

58. Chappuis F, Loutan L, Simarro P, Lejon V, Buscher P. Options for field diagnosis of human african trypanosomiasis. *Clin Microbiol Rev* 2005; 18: 133–146.

59. Hasker E, Mitashi P, Baelmans R *et al.* A new format of the CATT test for the detection of human African Trypanosomiasis, designed for use in peripheral health facilities. *Trop Med Int Health* 2010; 15: 263–267.

60. Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW. Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* 1998; 351: 563–565.

61. Chappuis F, Rijal S, Soto A, Menten J, Boelaert M. A meta-analysis of the diagnostic performance of the direct agglutination test and rK39 dipstick for visceral leishmaniasis. *BMJ* 2006; 333: 723.

62. Cunningham J, Hasker E, Das P *et al.* A Global Comparative Evaluation of Commercial Immunochromatographic Rapid Diagnostic Tests for Visceral Leishmaniasis. *Clin Infect Dis* 2012; 55: 1312–1319.

63. Boelaert M, El-Safi S, Hailu A *et al.* Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in East Africa and the Indian subcontinent. *Trans R Soc Trop Med Hyg* 2008; 102: 32–40.

64. ter Horst R, Tefera T, Assefa G, Ebrahim AZ, Davidson RN, Ritmeijer K. Field evaluation of rK39 test and direct agglutination test for diagnosis of visceral leishmaniasis in a population with high prevalence of human immunodeficiency virus in Ethiopia. *Am J Trop Med Hyg* 2009; 80: 929–934.

65. Pattabhi S, Whittle J, Mohamath R *et al.* Design, development and evaluation of rK28-based point-of-care tests for improving rapid diagnosis of visceral leishmaniasis. *PLoS Negl Trop Dis* 2010; 4: e822.

66. Gidwani K, Picado A, Ostyn B *et al.* Persistence of Leishmania donovani antibodies in past visceral leishmaniasis cases in India. *Clin Vaccine Immunol* 2011; 18: 346–348.

67. Parola P, Raoult D. Tropical rickettsioses. *Clin Dermatol* 2006; 24: 191–200.

68. Blacksell SD, Jenjaroen K, Phetsouvanh R *et al.* Accuracy of rapid IgM-based immunochromatographic and immunoblot assays for diagnosis of acute scrub typhus and murine typhus infections in Laos. *Am J Trop Med Hyg* 2010; 83: 365–369.

69. Coleman RE, Sangkasuwan V, Suwanabun N *et al.* Comparative evaluation of selected diagnostic assays for the detection of IgG and IgM antibody to Orientia tsutsugamushi in Thailand. *Am J Trop Med Hyg* 2002; 67: 497–503.

70. Blacksell SD, Jenjaroen K, Phetsouvanh R *et al.* Accuracy of AccessBio Immunoglobulin M and total antibody rapid immunochromatographic assays for the diagnosis of acute scrub typhus infection. *Clin Vaccine Immunol* 2010; 17: 263–266.

71. Ching WM, Rowland D, Zhang Z *et al.* Early diagnosis of scrub typhus with a rapid flow assay using recombinant major outer membrane protein antigen (r56) of Orientia tsutsugamushi. *Clin Diagn Lab Immunol* 2001; 8: 409–414.

72. Silpasakorn S, Srisamut N, Ekpo P *et al.* Development of new, broadly reactive, rapid IgG and IgM lateral flow assays for diagnosis of scrub typhus. *Am J Trop Med Hyg* 2012; 87: 148–152.

73. Parola P, Diatta G, Socolovschi C *et al.* Tick-borne relapsing fever borreliosis, rural Senegal. *Emerg Infect Dis* 2011; 17: 883–885.

74. Sundar S, Maurya R, Singh RK *et al.* Rapid, noninvasive diagnosis of visceral leishmaniasis in India: comparison of two immunochromatographic strip tests for detection of anti-K39 antibody. *J Clin Microbiol* 2006; 44: 251–253.

75. Chappuis F, Sundar S, Hailu A *et al.* Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat Rev Microbiol* 2007; 5: 873–882.

76. Limmathurotsakul D, Turner EL, Wuthiekanun V *et al.* Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clin Infect Dis* 2012; 55: 322–331.

77. Boelaert M, el Safi S, Goetghebeur E, Gomes-Pereira S, Le Ray D, Van der Stuyft P. Latent class analysis permits unbiased estimates of the validity of DAT for the diagnosis of visceral leishmaniasis. *Trop Med Int Health* 1999; 4: 395–401.

78. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. *Clin Microbiol Infect* 2010; 16: 1062–1069.

79. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Med* 2012; 9: e1001306.

80. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ* 2004; 82: 346–353.

81. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of Salmonella typhi in typhoid fever. *Lancet* 1975; 1: 1211–1213.

82. Baron E, Weinstein M, Yagupsky P, Welch D, Wilson D. *Cumitech 1C, Blood Cultures IV*. Washington, D.C.: ASM Press, 2005.

83. Bharti AR, Nally JE, Ricaldi JN *et al.* Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003; 3: 757–771.

84. Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: association of level of leptospiremia and clinical manifestations in Sri Lanka. *Clin Infect Dis* 2012; 54: 1249–1255.

85. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. *Lancet Infect Dis* 2006; 6: 91–99.

86. Simarro PP, Cecchi G, Paone M *et al.* The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int J Health Geogr* 2010; 9: 57.

87. Alvar J, Velez ID, Bern C *et al.* Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE* 2012; 7: e35671.

88. Srivastava P, Dayama A, Mehrotra S, Sundar S. Diagnosis of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 2011; 105: 1–6.

89. Blacksell SD. Commercial dengue rapid diagnostic tests for point-of-care application: recent evaluations and future needs? *J Biomed Biotechnol* 2012; 2012: 151967.

90. Shu PY, Yang CF, Kao JF *et al.* Application of the dengue virus NS1 antigen rapid test for on-site detection of imported dengue cases at airports. *Clin Vaccine Immunol* 2009; 16: 589–591.

91. Hang VT, Nguyet NM, Trung DT *et al.* Diagnostic accuracy of NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. *PLoS Negl Trop Dis* 2009; 3: e360.

92. Chaiyaratana W, Chuansumrit A, Pongthanapisith V, Tangnararatchakit K, Lertwongrath S, Yoksan S. Evaluation of dengue nonstructural protein 1 antigen strip for the rapid diagnosis of patients with dengue infection. *Diagn Microbiol Infect Dis* 2009; 64: 83–84.

93. Ramirez AH, Moros Z, Comach G *et al.* Evaluation of dengue NS1 antigen detection tests with acute sera from patients infected with dengue virus in Venezuela. *Diagn Microbiol Infect Dis* 2009; 65: 247–253.

94. Lima Mda R, Nogueira RM, Schatzmayr HG, dos Santos FB. Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of dengue in Brazil. *PLoS Negl Trop Dis* 2010; 4 : e738.

95. Tricou V, Vu HT, Quynh NV *et al.* Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. *BMC Infect Dis* 2010; 10: 142.

96. Osorio L, Ramirez M, Bonelo A, Villar LA, Parra B. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. *Virol J* 2010; 7: 361.

97. Wang SM, Sekaran SD. Early diagnosis of dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. *Am J Trop Med Hyg* 2010; 83: 690–695.

98. Fry SR, Meyer M, Semple MG *et al.* The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis* 2011; 5: e1199.

99. Keddy KH, Sooka A, Letsoalo ME *et al.* Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. *Bull World Health Organ* 2011; 89: 640–647.

100. Ley B, Thriemer K, Ame SM *et al.* Assessment and comparative analysis of a rapid diagnostic test (Tubex®) for the diagnosis of typhoid fever among hospitalized children in rural Tanzania. *BMC Infect Dis* 2011; 11: 147.

101. Naheed A, Ram PK, Brooks WA *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: 381–386.

102. Kawano RL, Leano SA, Agdamag DM. Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. *J Clin Microbiol* 2007; 45: 246–247.

103. Olsen SJ, Pruckler J, Bibb W *et al.* Evaluation of rapid diagnostic tests for typhoid fever. *J Clin Microbiol* 2004; 42: 1885–1889.

104. House D, Wain J, Ho VA *et al.* Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. *J Clin Microbiol* 2001; 39: 1002–1007.