Rapid diagnostic tests for neurological infections in central Africa

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Infections are a leading cause of life-threatening neuropathology worldwide. In central African countries affected by endemic diseases such as human African trypanosomiasis, tuberculosis, HIV/AIDS, and schistosomiasis, delayed diagnosis and treatment often lead to avoidable death or severe sequelae. Confirmatory microbiological and parasitological tests are essential because clinical features of most neurological infections are not specific, brain imaging is seldom feasible, and treatment regimens are often prolonged or toxic. Recognition of this diagnostic bottleneck has yielded major investment in application of advances in biotechnology to clinical microbiology in the past decade. We review the neurological pathogens for which rapid diagnostic tests are most urgently needed in central Africa, detail the state of development of putative rapid diagnostic tests for each, and describe key technical and operational challenges to their development and implementation. Promising field-suitable rapid diagnostic tests exist for the diagnosis of human African trypanosomiasis and cryptococcal meningoencephalitis. For other infections—eg, syphilis and schistosomiasis—highly accurate field-validated rapid diagnostic tests are available, but their role in diagnosis of disease with neurological involvement is still unclear. For others—eg, tuberculosis—advances in research have not yet yielded validated tests for diagnosis of neurological disease.

Introduction

Africa has the world's heaviest burden of several neurotropic infections,[1] as well as that of neurological disorders more generally,[2] while having the least services for neurological care.[3] By contrast with other causes of neurological disorders, infections of the CNS—eg, human African trypanosomiasis, cerebral malaria, neuroschistosomiasis, and tuberculous, cryptococcal, and bacterial meningitis—can be cured with inexpensive drug regimens. For many of these infections, access to accurate diagnostic tests is the principal factor limiting access to life-saving care.[4]

The Democratic Republic of the Congo has the greatest burden of neglected tropical diseases—including those causing neuropathology—in sub-Saharan Africa.[5] The success of initiatives to control human African trypanosomiasis (caused by *Trypanosoma brucei gambiense*) has substantially increased the average cost per new case detected. With diminishing returns, such stand-alone control programmes are increasingly difficult to justify in fragile health systems with competing priorities (figure 1).[6,7] Thus, DR Congo and neighbouring countries are planning to integrate human African trypanosomiasis control into primary care.[7] Are the primary care facilities ready for the challenge? Frontline medical personnel in central Africa need to distinguish between patients with human African trypanosomiasis and those with other neurological disorders, which constitute 9–24% of all admissions to African hospitals.[8–10] Confirmatory microbiological and parasitological tests are essential, since the clinical features of most neurological infections are non-specific[11–13] and treatment regimens are often prolonged or toxic, which complicates decisions about empirical treatment. Thus, with the change in the approach to control of human African trypanosomiasis comes an urgent need for rapid diagnostic tests that can be used at or near the point of care (figure 2).

We define rapid diagnostic tests as any test that yields results during the same clinic visit[14] and that can be used in health centres with little infrastructure or trained personnel, preferably without electricity. Existing rapid diagnostic tests are often done in laboratories by trained staff, but many have been assessed for use at the point of care—ie, by the health provider, at the patient's side. In the past decade, the use of rapid diagnostic tests has improved the management of malaria and HIV in low-resource settings,[15,16] and similar gains could be made elsewhere if integrated approaches using several rapid diagnostic tests were applied to patients presenting with signs attributable to several possible pathogens.[17,18]

We review the neurological infections for which rapid diagnostic tests are most urgently needed in rural central Africa, detail the state of development of rapid diagnostic tests for each infection, and discuss key technical and operational challenges to their development and implementation. We focus on rapid diagnostic tests that might realistically be available or scaled up within the next 5 years. We do not address the many non-communicable causes of neurological disorders in Africa, such as psychological distress caused by insecurity, nutritional toxicities or deficiencies, or emerging epileptic disorders such as nodding disease.

Pathogen selection and their epidemiology

In this Review we focus on neurological infections that should be prioritised for diagnostic research based on the following criteria. First, we prioritise neurotropic pathogens of proven or suspected epidemiological importance in central Africa. A major limitation is the paucity of up-to-date and accurate epidemiological data for nearly all countries in the region.[5] Second, we prioritised disorders that are severe and treatable over those that are either less serious or for which no treatment is available. Third, rapid diagnostic tests should ideally be useful for guiding treatment decisions without the need for additional data from diagnostic brain imaging, which is seldom available in rural regions in low-resource settings. Thus, some potentially severe and treatable conditions such as neurocysticercosis—for which the dose, safety, and efficacy of treatment is related to the number, location, and inflammatory response of lesions[19]—are a low priority at present in terms of diagnostics. In such cases, treatment would usually be limited to symptomatic interventions, such as antiepileptic drugs and corticosteroids, irrespective of microbiological testing. Unfortunately, management of several diseases of public health importance would not be changed by the availability of rapid diagnostic tests. In addition to neurocysticercosis, examples include rabies, human T-lymphotropic virus 1, and several arboviral infections, for which effective treatments have not yet been developed.[20,21] Finally, we do not include diseases for which clinical diagnosis is straightforward—eg, tetanus and leprosy.

Table 1 shows the prevalence of priority diseases and the frequency of neurological involvement for each, ranked by disease burden. Because no aggregate data are available for central Africa as a sub-region, we present epidemiological information for Africa as a whole along with data for DR Congo when available, because it is by far the largest and most populous country in the region. The complexity of most reference diagnostic techniques and the difficulty in identifying a specific cause of neurological infection largely explains the major gaps in our knowledge of epidemiology. This limitation impairs estimation of pretest disease probabilities and therefore clinical decision making. Epidemiological and hospital-based diagnostic studies are needed to fill this void and guide the development and implementation of new diagnostic tests.

Development of rapid diagnostic tests for key pathogens

Several promising technological advances could transform diagnostic microbiology in the coming decade, and bring access to diagnostic tests to patients who at present have none. Innovations in engineering and chemistry have led to radical changes in test design, including the use of microfluidics, mass spectrometry, optomechanical detection platforms, and nanoparticle-based devices. However, according to the infrastructure paradox,[53] technologically advanced diagnostics designed to bypass the need for well-developed health-care infrastructure are most likely to fail in low-resource settings, at least in the short term, because of a lack of technical support and extreme operating conditions. Thus, we focus on tests that use technologies that are simple, robust, affordable, and have the potential to be implemented in rural central Africa within the next 5 years. For the most part, these tests are based on a new generation of immunoassays. Table 2 summarises the characteristics of the rapid diagnostic tests that we discuss.

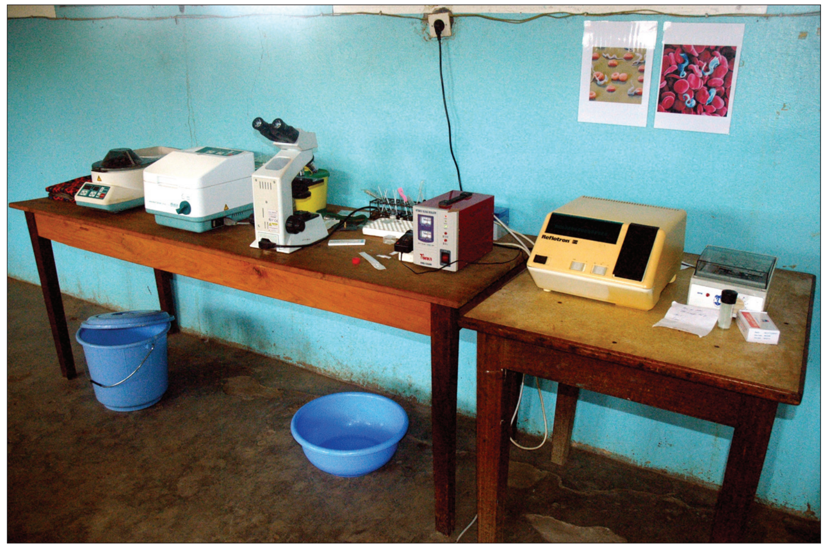

Figure 1: **Equipment used for the diagnosis of stages 1 and 2 of human African trypanosomiasis in the Democratic Republic of the Congo**
Photograph courtesy of C P Yansouni.

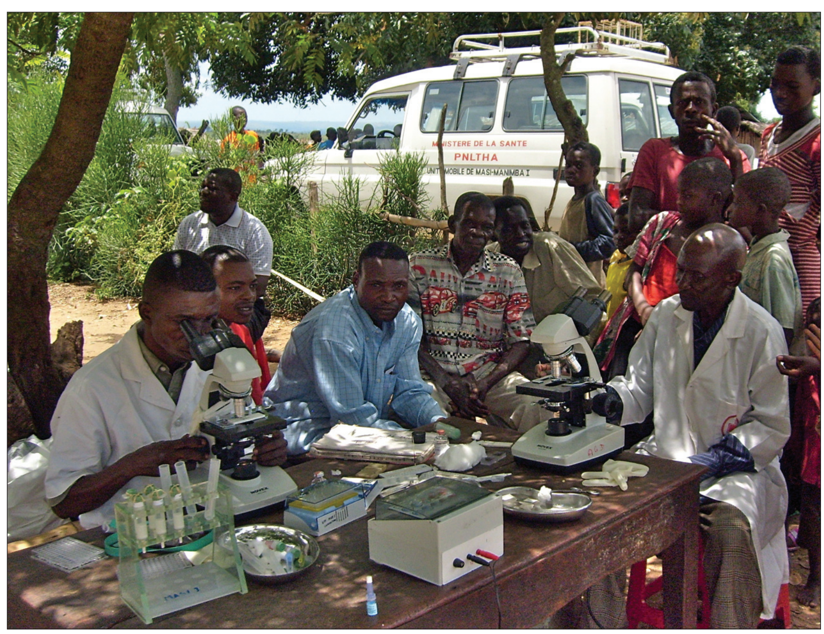

Figure 2: **Mass screening for human African trypanosomiasis by mobile teams of the national control programme in the Democratic Republic of the Congo**
Photograph courtesy of V Lejon.

Human African trypanosomiasis

Other than direct identification of parasites and white blood cell count in CSF, no existing or projected blood test for *T b gambiense* human African trypanosomiasis

	Epidemiology	Neurological effects and estimated burden
Malaria	Estimated prevalence of 174 million (110 million–242 million) cases in Africa in 2010;[22] about 7 million microscopically confirmed cases in 2011 in DR Congo;[22] mortality estimates for 2010 range from 596 000 (429 000–772 000) deaths[22] to 1 133 000 (848 000–1 591 000) deaths;[23] prevalence of parasitaemia in adults of 33·5% in DR Congo, 80% in some rural provinces, 90% from *Plasmodium falciparum*;[24] incidence decreasing in many parts of Africa, but not in central Africa[25,26]	Severe malaria in 2–10% of all cases, mainly with cerebral malaria in non-immune patients (ie, children aged <5 years in central Africa); mortality of untreated cerebral malaria roughly 100%, falling to 15–20% with prompt and effective treatment[27]
Tuberculosis	Prevalence in 2011 was 293 (243–347) cases per 100 000 people in Africa (512 [263–842] in DR Congo); incidence in 2011 was 262 (242–283) cases per 100 000 people in Africa (327 [282–375] in DR Congo); mortality in HIV-negative people in 2011 was 26 (21–31) deaths per 100 000 people in Africa (54 [24–96] in DR Congo); proportion of patients with tuberculosis who also had HIV was 39% in Africa and 15% in DR Congo[28]	Neurological involvement in about 1% of all patients with tuberculosis (higher in children and patients with HIV co-infection)[29]
HIV and related opportunistic infections	23 (22–25) million people had HIV in Africa in 2011, of whom 1·2 (1·1–1·3) million died;[30] HIV prevalence in people aged 15–49 years was 4·6% in Africa[30] in 2011 and 1·3% in DR Congo in 2009[31]	>20% of patients with AIDS have neurological complications of various causes, including cryptococcal meningitis, HIV-associated neurological disease, toxoplasmic encephalitis, stroke, and tuberculosis of the CNS[32]
Bacterial meningitis	30 000 cases reported in 2009;[31] non-epidemic cases in Africa are often under-reported; sporadic information is provided from case series;[33] microbiological surveillance is being introduced in DR Congo	Mainly caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and non-typhoid salmonellae;[33] case–fatality rate >30% and severe sequelae occur in roughly 25% of patients despite appropriate treatment[34]
Human African trypanosomiasis	Exclusively in Africa; 95% of cases caused by *Trypanosoma brucei gambiense*; 50 000 estimated new cases per year (70% of all cases occur in DR Congo); epidemic peaks in 1990s in several central African countries, falling incidence since 2000;[35] in 2011, roughly 7000 cases reported in Africa,[36] including about 5500 in DR Congo;[37] incidence is severely underestimated;[38,39] roughly 70 million people at risk in Africa, including 36 million in DR Congo[36]	Almost all cases of human African trypanosomiasis caused by *T b gambiense* develop to the meningoencephalitic stage, with almost 100% mortality if untreated; 50% of all cases of human African trypanosomiasis in DR Congo are diagnosed in the meningoencephalitic stage
Schistosomiasis	Roughly 90% of the estimated 207 million cases worldwide[40] occur in Africa (very little epidemiological information for DR Congo for >40 years); mortality of 280 000 deaths per year in Africa;[40] efforts are underway to create a global repository for spatial risk modeling of schistosomiasis in Africa[41]	Neurological complications (caused by ectopic migration of eggs or worms) in 2–4% of patients with schistosomiasis;[42] important contribution to non-traumatic myelopathy in endemic regions[43]
Syphilis	3 400 000 new infections in Africa in 2008;[44] about 700 000 cases of maternal syphilis yearly in Africa;[45] pooled estimates of syphilis seroprevalence in pregnant women between 1990, and 2011, were 4·5% for Africa,[46] and 4·2% for DR Congo;[47] 50–75% of children of seropositive mothers develop congenital syphilis, which accounted for roughly 2% of infant mortality worldwide in 2010[48]	Neurological complications in 10–15% of patients with symptomatic syphilis, higher in those with HIV co-infection[49]
Herpes simplex virus type 1 encephalitis	Unknown	Herpes simplex virus is a leading cause of viral encephalitis worldwide;[50,51] high mortality (70%) and major sequelae in survivors; mortality falls to 15% with early specific treatment[52]

Infections are ordered according to their burden. Data in parentheses are 95% CIs.

Table 1: **Burden of severe and treatable infections causing neurological disorders in Africa, with a focus on the Democratic Republic of the Congo**

can distinguish haemolymphatic (stage 1) from meningo-encephalitic invasion (stage 2), which is necessary to choose the appropriate treatment. Thus, most diagnostic development is focused on serological tests to identify which patients should have parasitological confirmation, followed by a lumbar puncture for staging.

The card agglutination test for trypanosomiasis—designed to run 50 tests at once—has been the corner-stone of mass screening of predominantly asymptomatic individuals for decades. Unfortunately, its diagnostic accuracy has only been assessed in the context of mass screening. Although not strictly a rapid diagnostic test because it requires electricity and other equipment, the card agglutination test for trypanosomiasis can be used in remote settings. A new format (CATT-D10) now also enables fewer patients to be tested in peripheral health facilities.[73] Phase 3 diagnostic studies—ie, in patients clinically suspected of having human African trypanosomiasis—are needed. Such studies will establish the diagnostic accuracy of card agglutination tests for trypanosomiasis, either as a qualitative result or by applying a dilution cutoff to increase specificity.[74]

Two lateral flow immunochromatographic rapid diagnostic tests for serodiagnosis of *T b gambiense* human African trypanosomiasis in remote regions are in advanced stages of development. The advantages of these single-format immunochromatographic tests over the card agglutination test for trypanosomiasis are that (1) they are intended for testing one person at a time (single-patient reagent format) and do not use reagent kits intended for batched tests, and (2) they are designed to be more sensitive than the card agglutination test and might therefore be useful in regions where *T b gambiense* strains do not elicit antibodies that react in card agglutination tests for trypanosomiasis. The Immunochromatographic HAT-RDT, manufactured by Standard Diagnostics

(Pajang-dong, South Korea) in collaboration with the Foundation for Innovative New Diagnostics (Foundation for Innovative New Diagnostics, Geneva, Switzerland) and the Institute of Tropical Medicine (Antwerp, Belgium), is undergoing phase 2 assessment in Angola, DR Congo, and Central African Republic. Two other immunochromatographic tests—Gambiense-Sero-K-SeT and Gambiense-Sero-Stip—developed by Coris BioConcept (Gembloux, Belgium) in collaboration with the Institute of Tropical Medicine, have completed phase 1 tests using

	Analyte detected	Specimen types	Indication for which test was assessed	Specific for CNS disease?	Stage of validation*, test format, comments	Proposed reference standard for validation
Human African trypanosomiasis (*Trypanosomiasis brucei gambiense*)						
CATT (Institute of Tropical Medicine, Antwerp, Belgium)	Antibody	Serum, whole blood	Mass screening	No	Phase 2	Antibody detection tests: the immune trypanolysis test (Institute of Tropical Medicine, Antwerp, Belgium); infection status: parasites in any body fluid (lymph, blood, CSF)
Immunochromatographic HAT (Standard Diagnostics, Pajang-Dong, South Korea/ Foundation for Innovative New Diagnostics, Geneva, Switzerland)	Antibody	Serum, whole blood	Stored samples	No	Phase 2, immunochromatographic strip	Antibody detection tests: the immune trypanolysis test; infection status: parasites in any body fluid (lymph, blood, CSF)
SeroStrip HAT (Coris BioConcept, Gembloux, Belgium)	Antibody	Serum, whole blood	Stored samples	No	Phase 2, immunochromatographic strip	Antibody detection tests: the immune trypanolysis test; infection status: parasites in any body fluid (lymph, blood, CSF)
LAMP-HAT (Eiken Chemical, Japan/Foundation for Innovative New Diagnostics)	DNA	Whole blood, CSF	NA	NA	Phase 2 in progress, nucleic acid amplification test	Antibody detection tests: the immune trypanolysis test; infection status: parasites in any body fluid (lymph, blood, CSF)
Cerebral malaria (*Plasmodium falciparum*)						
Several[54]	Antigen	Whole blood	Clinically suspected malaria	No	Phase 3 field studies, immunochromatographic cassette, specificity for malaria-attributable fever and neurological disease varies by setting	Malarial retinopathy† detected by skilled operators is an acceptable surrogate for histopathological finding of sequestration of asexual malaria parasites in CNS microvasculature[55–57]
HIV-associated toxoplasmic encephalitis						
None	NA	NA	NA	NA	Good candidate for loop-mediated isothermal amplification process or antigen detection	Compatible brain imaging and response to treatment within 14 days is an acceptable surrogate for histopathology
Neuroschistosomiasis (*Schistosoma mansoni*)						
CCA (Rapid Medical Diagnostics, Pretoria, South Africa)[58,59]	Circulating cathodic antigen	Urine	Mass screening	No	Phase 3, immunochromatographic strip or cassette with data for *S mansoni*; also detects moderate-to-high burden infections of other species—eg, *Schistosoma haematobium*, *Schistosoma japonicum*	Confirmed by histopathology showing parasite eggs in granulomas in neural tissues; preoperative diagnosis can be based on a composite scoring system[60]
HIV-associated cryptococcal meningitis						
CrAg LFA (Immuno-Mycologics, Norman, USA)[61,62]	Antigen	CSF, serum, plasma, urine	Confirmed meningitis, and acute respiratory illness	Yes, on CSF	Phase 2, immunochromatographic strip, no boiling of samples is needed	Positive culture or detection of cryptococcal antigen in CSF (using traditional cryptococcal antigen assays) or positive microscopy on CSF, or either positive result from blood (culture or cryptococcal antigen) in conjunction with a clinically compatible illness[63]
Tuberculous meningitis						
Xpert MTB/RIF (Cepheid, Sunnyvale, USA)	DNA	Sputum	Patients with suspected pulmonary tuberculosis	No	Phase 3 field studies are complete, nucleic acid amplification test, few data for use on CSF	Uniform case definition for tuberculous meningitis taking account of available resources and the ability to exclude competing diagnoses[64]
LAMP (Eiken Chemical, Japan/ Foundation for Innovative New Diagnostics)	DNA	Sputum	Patients with suspected pulmonary tuberculosis	No	Phase 2, nucleic acid amplification test	Uniform case definition for tuberculous meningitis taking account of available resources and the ability to exclude competing diagnoses[64]
Determine TB-LAM (Alere, Waltham, USA)[65]	Antigen	Urine	Screening for suspected pulmonary tuberculosis in patients with HIV	No	Phase 3	Uniform case definition for tuberculous meningitis taking account of available resources and the ability to exclude competing diagnoses[64]

(Continues on next page)

	Analyte detected	Specimen types	Indication for which test was assessed	Specific for CNS disease?	Stage of validation*, test format, comments	Proposed reference standard for validation
(Continued from previous page)						
Bacterial meningitis						
BinaxNOW *S pneumoniae* test (Alere, Waltham, USA)[66,67]	Antigen	Urine	Patients with suspected epidemic meningitis	No	Immunochromatographic strip	Confirmed by having clinically compatible case with isolation of a bacterial species from either CSF or blood (if isolation from blood only, CSF pleiocytosis must be present); can also be confirmed by a clinically compatible case with pleiocytosis in CSF (>10 white blood cells per μL) and positive CSF Gram stain; probable if a clinically compatible case with CSF white blood cells >30 cells per μL (>50% polymorphonuclear cells) and clinical response to antibiotics
RDT *N meningitidis* serogroups ACYW135 (Pasteur Institute, Paris, France and CERMES, Niamey, Niger)[68–70]	Antigen	CSF	Epidemic meningitis suspects	Yes	Immunochromatographic strip	Confirmed by having clinically compatible case with isolation of a bacterial species from either CSF or blood (if isolation from blood only, CSF pleiocytosis must be present); can also be confirmed by a clinically compatible case with pleiocytosis in CSF (>10 white blood cells per μL) and positive CSF Gram stain; probable if a clinically compatible case with CSF white blood cells >30 cells per μL (>50% polymorphonuclear cells) and clinical response to antibiotics
Neurosyphilis						
Treponemal (several)	Antibody	Serum, blood	Screening	No	Phase 3 field studies complete, immunochromatographic strip or cassette	Clinically compatible illness with a reactive non-treponemal and treponemal blood test for syphilis, plus for confirmed disease, a reactive CSF-VDRL test; for probable disease, a non-reactive CSF-VDRL test, but increased CSF protein concentration or >5 white blood cells per μL, with no alternative diagnosis and clinical response to penicillin
Non-treponemal (several)	Antibody	Serum	Screening	Yes, in CSF	Phase 2 complete, immunochromatographic strip or cassette, no assessments of non-treponemal rapid diagnostic tests on CSF, compared with CSF VDRL	Clinically compatible illness with a reactive non-treponemal and treponemal blood test for syphilis, plus for confirmed disease, a reactive CSF-VDRL test; for probable disease, a non-reactive CSF-VDRL test, but increased CSF protein concentration or >5 white blood cells per μL, with no alternative diagnosis and clinical response to penicillin
Herpes simplex virus type 1 encephalitis						
None	NA	NA	NA	NA	Good candidate for loop-mediated isothermal amplification process assay	Positive CSF PCR for herpes simplex virus type 1

RDT=rapid diagnostic test. NA=not applicable. VDRL=Venereal Disease Research Laboratory. CERMES=Centre de Recherche Médicale et Sanitaire. *See the panel for definitions of phases 1, 2, and 3. †Used for patients meeting standard criteria for cerebral malaria—eg, any sign of cerebral dysfunction with asexual *Plasmodium falciparum* parasitaemia and no other evident cause of coma.[16,72]

Table 2: **Key neurological pathogens and rapid diagnostic tests with phase 1 validation or higher, intended for individual case management**

stored serum samples modified to mimic whole blood and have a higher specificity than the preset lower limit of 95%, and sufficient sensitivity. Phase 2 and phase 3 trials are underway.[75] Finally, neopterin is a promising candidate CSF biomarker that is being assessed with the hope of replacing the tedious search for trypanosomes (involving manual searching with many steps) and imprecise leucocyte quantification used to stage human African trypanosomiasis at present.[76] If their accuracy is confirmed, these biomarkers might provide a basis for future rapid diagnostic tests for diagnosing stage 2 human African trypanosomiasis.

Cerebral malaria

Several studies[55,56] show that a characteristic malarial retinopathy is the best single discriminator between malarial and non-malarial coma for patients who meet standard definitions of cerebral malaria. However, the skill and equipment needed to assess this retinopathy restrict implementation. Rapid diagnostic tests for detection of *Plasmodium falciparum* in blood have been field-validated[77,78] and some are recommended by WHO.[16] Unfortunately, distinguishing people with cerebral malaria from those with coma from other causes is difficult, particularly in regions with high rates of asymptomatic parasitaemia. Standard definitions of cerebral malaria (any sign of cerebral dysfunction with asexual *P falciparum* parasitaemia and no other evident cause of coma)[16,72] are less specific than are postmortem findings, with one prospective series[55] documenting a false-positive diagnosis in 23% (seven of 31 clinically diagnosed deceased patients had no signs of cerebral malaria at autopsy). Increased plasma concentrations of pHRP2 have been used[79] to reliably identify Malawian children with histologically confirmed or retinopathy-positive cerebral malaria, suggesting that rapid diagnostic tests based on quantitation of pHRP2 might have promise for diagnosis of cerebral malaria.

HIV-associated toxoplasmic encephalitis

No commercially available rapid diagnostic test has been developed for the diagnosis of encephalitis caused by reactivation of *Toxoplasma gondii* infection. Diagnosis is difficult and is often presumptive, based on new-onset focal neurological signs, brain imaging, and response to empirical treatment. Serum IgG reactivity to *T gondii* defines those at risk for reactivation, but its use is limited by high seropositivity in African populations.[80–82] The use of detection of antigen in CSF has shown little promise.[83]

Neuroschistosomiasis

Neuroschistosomiasis—caused by *Schistosoma* spp eggs or worms that are transported to the spinal cord or the brain—is the most severe clinical outcome associated with infection with *Schistosoma mansoni* or *Schistosoma haematobium*.[84,85] Three target schistosoma antigens have been identified in CSF, urine, and serum, and a highly accurate rapid diagnostic test is commercially available for detection of *S mansoni* in urine. The diagnostic performance of several monoclonal antibodies against the soluble egg antigen of *S mansoni* has been tested in CSF, and IgG1 was the most discriminating isotype for diagnosis of neuroschistosomiasis.[86,87] These antibodies might serve as a basis for future rapid diagnostic tests for neuroschistosomiasis based on soluble egg antigen.

For urine, studies[58,59] in east and west Africa have shown that a commercially available circulating cathodic antigen cassette test is more sensitive than multiple Kato-Katz thick smears derived from stool samples for diagnosis of *S mansoni* infection. In a latent class analysis, Shane and colleagues[58] report that circulating cathodic antigen cassette has a sensitivity of 96·3% and a specificity of 74·7%. These findings were confirmed in a multicountry study funded by the Schistosomiasis Consortium for Operational Research and Evaluation.[88] The accuracy of the readily available urine circulating cathodic antigen cassette test for diagnosis of neuro-schistosomiasis should be assessed.

Finally, efforts to develop a diagnostic test that is accurate for all *Schistosoma* species have led to a promising assay with very high sensitivity based on the serum circulating anionic antigen.[89] Another study by the Schistosomiasis Consortium of Operational Research and Evaluation has provided encouraging results using both serum and urine samples and work on the development of a rapid test is ongoing (Van Dam GJ, Leiden University Medical Center, personal communication). Rapid diagnostic tests using extraneural specimens need to be validated for neuroschistosomiasis, and their optimum use should be defined by local epidemiology.

HIV-associated cryptococcal meningitis

Traditional cryptococcal antigen detection assays have high sensitivity and specificity. Positive results can be used for diagnosis of cryptococcal meningitis, whereas negative results can be used to make empirical treatment decisions for other diseases for which confirmed diagnosis is often impossible, such as tuberculous meningitis. Unfortunately, these assays usually require refrigeration or freezing of reconstituted reagents and lengthy sample processing, which includes boiling samples and inactivation of competing proteins for up to 1 h.

A lateral flow immunochromatographic cryptococcal antigen assay (CrAg LFA; Immuno-Mycologics, Norman, USA) has very high agreement with traditional cryptococcal antigen assays that use enzyme immunoassay or latex agglutination platforms. It has been tested[61] using serum and urine from patients with suspected pulmonary cryptococcosis with positive serum cryptococcal antigen enzyme immunoassay or blood cultures. It has also been tested[62] with serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis confirmed by CSF cryptococcal antigen enzyme immunoassay or CSF India ink staining. Advantages of the CrAg LFA over latex agglutination assays and enzyme immunoassays include lower cost, 5–15 min turnaround time, and that reagents do not need to be refrigerated and specimens do not need to be boiled, leading to its recommendation by the WHO.[63] Its specificity has not been tested; however, according to the manufacturer, CrAg LFA has

Panel: Definitions of phases 1, 2, and 3 of validation for diagnostic studies[71]

Phase 1

The aim of phase 1, early exploratory studies is to provide proof-of-principle that the marker is strongly associated with the disease of interest. Such studies need a small number (10–100) of samples, usually archived specimens.

Phase 2

The candidate test is assessed in a case–control study, of several series of patients (or archived samples), who are enrolled on the basis of disease status. The sample size should be a minimum of 100 patients in each series. The control participants should be varied: healthy non-endemic, healthy endemic, and patients with potentially cross-reactive diseases.

Phase 3

Phase 3 studies are large-scale prospective studies validating the test in the target population. They need a sufficiently large (usually a minimum of 300 people) and representative sample of consecutively enrolled or randomly selected patients.

Phase 3 field studies

Phase 3 studies done in field conditions by representative health personnel, such as mid-level health-care workers in a clinic, rather than a specialised laboratory worker in a reference laboratory.

effectively no cross-reaction with sera from patients with other invasive fungal infections.[90]

Tuberculous meningitis

Tuberculous meningitis is difficult to diagnose[29,91] and research has been hampered by the different reference standards used.[64] The difficulties of diagnosis and development of a rapid diagnostic test for all forms of tuberculosis have been described elsewhere.[92] No rapid diagnostic test exists for tuberculous meningitis. However, the diagnostic accuracy of the Clearview TB-ELISA test (Alere, Waltham, MA, USA), which measures lipoarabinomannan tuberculosis antigen as a CSF biomarker, has been assessed.[93] This test provides a small incremental diagnostic yield when combined with CSF smear microscopy or a clinical prediction rule for diagnosis of HIV co-infected patients with a CD4 count of less than 200 cells per µL (sensitivity 63% [95% CI 47–68], specificity 93% [82–98]).

Clearview TB-ELISA is a laboratory-based test. The diagnostic accuracy of a new point-of-care lateral-flow urine test for lipoarabinomannan (Determine TB-LAM; Alere) has been assessed[65] for screening a cohort of patients in South Africa for active pulmonary tuberculosis before starting antiretroviral therapy. Positive cultures of *Mycobacterium tuberculosis* from sputum in liquid media were used as the reference standard. As with previous reports of laboratory-based lipoarabinomannan assays, the sensitivity of Determine TB-LAM when used alone is inversely proportional to CD4 count: 66·7% (95% CI 41·0–86·7) for patients with less than 50 cells per µL, 51·7% (32·5–70·6) for patients with less than 100 cells per µL, and 39·0% (26·5–52·6) for patients with less than 200 cells per µL, all with specificity greater than 98%. When Determine TB-LAM and sputum smear microscopy were combined, any positive result from either test yielded a sensitivity similar to a single-sample Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for patients with CD4 cell counts less than 50 cells per µL (72·2% for both) or less than 100 cells per µL (65·5% *vs* 75·9%).

Lipoarabinomannan detection seems to offer a small, but potentially useful increase in sensitivity for diagnosis of tuberculous meningitis in immunosuppressed individuals, and its high specificity makes it useful as a rule-in test. However, its performance and optimal use for CNS disease are still unclear. Adenosine deaminase-2—another CSF biomarker—has been proposed as a criterion to either confirm or exclude tuberculous meningitis in two systematic reviews.[94,95] The potential for adenosine deaminase-2 to be combined with interferon γ and lipoarabinomannan in an immunochromatographic diagnostic test is also being explored.

Bacterial meningitis

Several bacteria can cause bacterial meningitis, most of which can be treated with empirical antimicrobial regimens if third generation cephalosporins are available.[96] Thus, rapid diagnostic tests for bacterial meningitis—rather than for the particular species of bacteria that is causing disease—would be useful for non-epidemic bacterial meningitis. Conversely, rapid diagnostic tests detecting a single pathogen are useful in an outbreak, in which the pretest probability of detecting a given causal agent is already high and triage of many suspected bacterial causes is needed. In settings where supplies of cephalosporins are insufficient, identification of patients in whom alternative antimicrobial drugs can be used could also help to preserve limited supplies (eg, in children with meningitis caused by *Neisseria meningitidis*, compared with those with *Haemophilus influenzae*).

No CSF biomarker rapid diagnostic tests have been shown to have clinical utility. Unlike most other diseases discussed in this Review, untreated bacterial meningitis is often characterised by polymorphonuclear pleocytosis in the CSF.[96] A battery-operated device for measurement of white blood cell count in peripheral blood has been developed (HemoCue WBC DIFF; HemoCue AB, Ängelhom, Sweden) but its use for CSF is precluded by the lower thresholds for cell count (300 cells per µL) and differential cell analysis (1000 cells per µL). Good correlation between urine dipstick leucocyte esterase (which detects polymorphonuclear cells but not lymphocytes and monocytes) and bacterial meningitis has been described for grossly turbid CSF samples, but not for clear or haemorrhagic samples.[97,98] The detection limit of the leucocyte esterase patch on the dipsticks has been estimated at 175 leucocytes per µL, and CSF becomes cloudy on inspection at 200 leucocytes per µL.[99] Hand-held point-of-care tests to assess plasma glucose concentrations are not designed for CSF, and point-of-care tests to detect other promising biomarkers in CSF—eg, lactate—have not yet been studied.[100]

Accurate rapid diagnostic tests using immunochromatography to detect meningeal pathogens have been developed. The BinaxNOW *Streptoccocus pneumoniae* test (Alere) has excellent accuracy (sensitivity 95–100%, specificity 100%) when applied to CSF for diagnosis of pneumococcal meningitis.[66,67] In a multicentre diagnostic study, it showed a small increase in yield compared with culture and latex agglutination in Asia but not in Africa.[67] This study of 1173 patients suspected of having meningitis identified *S pneumoniae* by culture in only 69 (41%) of 169 patients with positive CSF cultures. This finding shows the need in non-outbreak settings for rapid diagnostic tests that detect all bacterial meningitis in addition to—or instead of—specific bacteria. An immunochromatographic dipstick combination for detection of *N meningitidis* serogroups A, C, Y, and W135 has been designed and validated by the Centre de Recherche Médicale et Sanitaire in Niger and the Institut Pasteur in France.[68] However, the dipsticks do not detect epidemic serogroup X or the more endemic serogroup B. Under reference laboratory conditions,[69] test performance was excellent (sensitivity of 93·8% and specificity of 100%). However, in a subsequent field evaluation[70] during an outbreak of *N meningitis* serogroup A in Burkina Faso, sensitivity was only 70%, leading the investigators to conclude that the dipsticks should not yet be introduced in peripheral health centres. The use of older latex agglutination tests has been largely abandoned.[96,101] In addition to variable accuracy, their limitations include the need for a cold chain, high cost, short shelf-life, and the need for a complex, multistep procdure done by trained staff.[67,70]

Neurosyphilis

Several rapid diagnostic tests detecting anti-treponemal antibodies have been assessed under laboratory[102] and field[103] conditions and are accurate, reliable, and easy to use, including using whole blood as a sample. Of these tests, the SD Bioline Syphilis 3.0 (Standard Diagnostics, Kyonggi-do, South Korea) was the most sensitive assay with whole blood in clinic conditions (sensitivity 86–100%, specificity 98–99%).[103]

Two rapid diagnostic tests designed for the simultaneous detection of anti-treponemal and anti-non-treponemal antibodies in the serum of patients with syphilis have been assessed in phase 2 diagnostic studies.[104,105] Compared with quantitative rapid plasma reagin test and the *Treponema pallidum* passive particle agglutination assay, sensitivity of both rapid diagnostic tests was greater than 96% and specificity exceeded 98%. These rapid tests are designed for simultaneous screening and confirmation of syphilis at the point-of-care in low-resource settings. However, validation with CSF samples is needed—particularly for the non-treponemal component—since neurological symptoms can be unrelated to syphilis and confirmation with the Venereal Disease Research Laboratory test in CSF is often unavailable in low-resource settings.

Herpes simplex virus type 1 encephalitis

No commercially available rapid diagnostic test has been developed for herpes simplex virus type 1 encephalitis. Development of such a test is unlikely because neither antigen-detection nor viral culture approach the sensitivity of nucleic acid amplification, and their use is strongly discouraged by experts and professional societies such as the Infectious Diseases Society of America.[106]

Co-infections

Co-infections can reduce the accuracy of rapid diagnostic tests, and this effect varies according to the test used. For example, human African trypanosomiasis is associated with severe polyclonal hypergammaglobulinaemia, which can cause false-positive serological test results. In a study[107] of several hundred patients with human African trypanosomiasis not known to be HIV-infected and with undetectable HIV viral loads, ELISA and rapid diagnostic tests detecting anti-HIV antibodies had decreased specificity, as low as 39% in some cases. Similarly, the specificity of most rapid diagnostic tests that detect malaria antigen is significantly decreased in patients with human African trypanosomiasis compared to trypanosomiasis-negative controls, with cross-reactions mostly affecting the HRP-2 and pan-pLDH test lines.[108] Other combinations of infections might also reduce the accuracy of immunoassay-based rapid diagnostic tests.

Nucleic acid amplification tests

For several key neurological pathogens, rapid diagnostic tests detecting antigens or antibodies are unlikely to be useful when used alone because of inherent limitations in sensitivity or specificity. Nucleic acid amplification tests can fill this gap in some cases, and recent advances have made their use in low-resource settings possible. They are not rapid diagnostic tests, and are more suitable for referral hospitals than for primary health clinics; however, even in rural Africa, many people with life-threatening infections are referred to such hospitals.

Loop-mediated isothermal amplification of DNA is a nucleic acid amplification test platform[109] that only needs a water bath or heating block maintained at 60–65°C for 30–40 min, and amplification products can be seen with the naked eye. A prototype loop-mediated isothermal amplification assay for detection of *T b gambiense* was introduced in July 2011 (Eiken Chemical Company, Japan) and is undergoing phase 2 trials for diagnosis of stage 1 human African trypanasomiasis.[110] Another loop-mediated isothermal amplification assay for tuberculosis is scheduled for WHO review (Eiken Chemical Company and Foundation for Innovative New Diagnostics, Geneva, Switzerland). The Xpert MTB/RIF assay is a fully automated PCR-based nucleic acid amplification test that has been validated for pulmonary tuberculosis,[111] but some studies have also assessed its potential for diagnosis of tuberculous meningitis. Sensitivity ranged from 29% to more than 80% using a composite reference standard, but specificity was very high (100%).[112–115]

No field-applicable nucleic acid amplification tests exist for herpes simplex virus type 1 encephalitis or *T gondii* infection, but both of these treatable and otherwise fatal infections are attractive targets for such a test, particularly in the absence of brain imaging. PCR has good diagnostic accuracy for herpes simplex virus type 1 encephalitis, with sensitivity of 96–98% and specificity of 95–99% in adults.[116] Moreover, herpes simplex virus DNA can be detected by PCR of CSF early in disease progress and remains detectable during the first week of treatment. Herpes simplex virus can also be detected with loop-mediated isothermal amplification assays.[117–119] Detection of *T gondii* by PCR in CSF can be used to rule-in disease because of its consistently high specificity (96–100%), but low sensitivity (50–77%).[120,121]

Research priorities and future directions

The ASSURED criteria for diagnostic tests were written almost 10 years ago, and the challenges of developing rapid diagnostic tests that meet the criteria have been well described.[122,123] Rapid diagnostic tests designed for use in low-resource settings must address the trade-off between maximising test performance on one hand and robustness under harsh conditions on the other. Despite these difficulties, progress has been made in the past decade towards making affordable high-quality tests available where they are most needed. In addition to continuing development of individual rapid diagnostic tests, their implementation and quality assurance also presents challenges.

The development of novel point-of-care diagnostic tests is an important advance for targeted screening for infections such as HIV and syphilis. However, with point-of-care tests, health workers assume responsibility for specimen collection and testing, as well as quality control and documentation. As more rapid diagnostic tests are developed, careful thought must be given to how and where they should be used, to avoid overwhelming clinical personnel. We have used the term rapid diagnostic test instead of point-of-care test to reflect this fact. As more rapid diagnostic tests become available, their desired point of execution will move from the bedside to near-care laboratories with minimal infrastructure, to minimise delays in treatment. This change will improve quality assurance, which is more challenging in point-of-care settings.[124] For immunoassay-based tests, the difficulties of user-interpretation,[125] documentation, and archiving of results might be dealt with by the introduction of battery-operated automated readers, which digitally photograph test strips, interpret the results, and archive standardised photos for subsequent quality assurance.[126–128] Some systems can read barcodes and interface directly with a computer or laboratory information system, reducing workload and transcription errors. Resources and effort should be invested in fostering a sustainable culture of quality assurance, since even low-skill rapid diagnostic tests can be used inappropriately, and inaccurate results can harm patients and undermine their confidence in local medical services.

A symptom-based approach to diagnosis of patients with suspected neurological infections is urgently needed that integrates relevant combinations of rapid diagnostic tests, to quickly and effectively treat patients and to reduce the burden of these infections. For detection of neurological infections in remote settings, highly sensitive rapid diagnostic tests might be most useful to safely rule out severe conditions and avert unnecessary costly referral to higher levels of care. However, the widespread adoption and effect of rapid diagnostic tests will depend on more than the availability of new tests. Effective adoption also needs: (1) comprehensive epidemiological studies using reference standard techniques to measure the prevalence of priority diseases (table 2); (2) validation of tests in field settings; and (3) validated evidence-based algorithms incorporating local epidemiological data and setting-specific information about how a given diagnostic test improves case management, which can vary substantially by locality.[107,129,130]

Diagnostic algorithms must be cost-effective to be sustainable, and the best diagnostic scheme will vary by setting (eg, using standard panels of tests in parallel *vs* sequential testing in a prespecified order). Rollout of rapid tests should be done in stages, and tailored to the epidemiology of each region. Several initiatives aim to refine the mapping of diseases such as schistosomiasis,[131] human African trypanosomiasis,[132–134] and malaria,[25,135] and these will be essential to the appropriate deployment of new diagnostic tests.

Treatment of neurological infections is often complex and might need to be modified on the basis of repeat diagnostic testing. Although test-of-cure is recommended for many of the diseases discussed in this review, such testing is usually based on culture or parasite detection. With the exception of non-treponemal tests for syphilis, no rapid diagnostic test based on antibody detection is useful for test-of-cure. Rapid diagnostic tests based on antigen detection (ie, for malaria, cryptococcal disease, and neuroschistosomiasis) provide gross information about disease activity, but their usefulness for testing whether disease has been cured should be thoroughly assessed.

Combination of new rapid diagnostic tests into a single device capable of detecting several analytes should be a priority. Such an approach would improve the feasibility of testing for multiple diseases. For example, the increasing popularity of Xpert MTB/RIF for diagnosis of tuberculosis could provide an opportunity for developing multiplexed CNS cassettes, targeting pathogens for which nucleic acid amplification tests in CSF are the best rapid diagnostic option—including toxoplasma, herpes simplex virus type 1, and possibly tuberculosis and human African trypanosomiasis. However, devices with a fixed combination of tests are often more costly, are less flexible, and are ill adapted to changes in local epidemiology than are individual devices. Furthermore, external quality assurance of such multiplexed tests is not made easier by their combination into a single device. The state of development of multiplexing technologies has been reviewed by Bissonnette and colleagues.[53]

The implementation of an increasing number of rapid diagnostic tests for neurological and other infections will need new approaches to diagnostic infrastructure, and care will have to be taken not to subvert those already in place. Some new tests might only offer added value when combined with old ones—eg, the Determine TB-LAM combined with smear microscopy.[65] Moreover, although microscopy (and bacterial culture, if available) can detect multiple pathologies at once—including unsuspected ones—most rapid diagnostic tests detect only a single pathogen.

Search strategy and selection criteria

We searched PubMed for articles published from Jan 1, 1990, to Dec 31, 2012, with the MeSH headings "Central Nervous System Infections", "Clinical Laboratory Techniques", "Point-of-Care Systems", "Serologic Tests", "Diagnosis", "Africa", "Developing Countries", and combining them with searches for the specific pathogens discussed in the text. We identified other reports of the epidemiology of neurological infections in central Africa by searching the Archives Collection of the Institute of Tropical Medicine, Antwerp, Belgium, and our personal files. Furthermore, relevant information from the review was discussed and validated at a 3-day expert workshop at the London School of Hygiene and Tropical Medicine (UK), held on Sept 20–23, 2011. We reviewed the reports identified from these searches. We included articles written in English, French, Spanish, Dutch, or German.

Conclusions

A pressing need exists for improved access in rural Africa to microbiological tests for severe and treatable neurological infections. There is reason for optimism, with advances in the past 10 years leading to promising rapid diagnostic tests for human African trypanosomiasis and cryptococcal meningoencephalitis that need little infrastructure or skill. For other diseases—eg, syphilis—highly accurate field-validated rapid diagnostic tests are available, but their role in diagnosis of disease with neurological involvement is yet to be established. For others still—eg, tuberculosis—advances in research have not yet yielded validated instruments for diagnosis of neurological disease. After decades of low prioritisation of diagnostic laboratories in health-care delivery structures for low-resource settings,[136] the renewed push for elimination or eradication of diseases such as malaria and schistosomiasis has put a new focus on diagnostics. For clinicians with patients suspected of having a neurological infection, these new diagnostic tests will need to be incorporated into validated diagnosis–treatment pathways based on evidence. Rapid diagnostic tests for malaria and HIV have revolutionised the care of these diseases, and the time has come for rapid diagnostic tests for other severe and treatable infections to be made available in the regions where they are most needed.

Contributors

CPY and EB conceived the Review, proposed the approach for selecting priority conditions, and took primary responsibility for the literature review and writing the first draft. MAM organised the 3-day expert workshop where information from the review was discussed and validated. All authors wrote sections or provided feedback and advice according to their areas of expertise, and extensively reviewed all drafts.

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Conflicts of interest

We declare that we have no conflicts of interest.

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