Published in final edited form as:

Expert Opin Med Diagn. 2012 May ; 6(3): . doi:10.1517/17530059.2012.681300.

CrAg Lateral Flow Assay for Cryptococcosis

Thomas R. Kozel¹ and Sean K. Bauman²

¹Department of Microbiology and Immunology, University of Nevada School of Medicine, Reno, NV 89557, USA

²Immuno-Mycolitics (IMMY), Norman, OK 73071, USA

Abstract

Importance of field—Cryptococcal meningitis is a leading cause of death globally among people with AIDS. In sub-Saharan Africa, cryptococcosis is estimated to kill more people than tuberculosis. Cryptococcosis is also an important infectious disease among immunosuppressed patients in countries with advanced medical care. Early diagnosis is the key to effective treatment, particularly in patients in resource-limited settings. A new lateral flow immunoassay (LFA) for cryptococcal antigen (CrAg) allows for rapid and inexpensive diagnosis of cryptococcosis at or near the point of patient contact.

Areas covered—This article reviews the need for improved diagnostics for cryptococcal meningitis and describes the features of an ideal diagnostic. The design of a new lateral flow immunoassay for CrAg is described as well as the results of initial clinical evaluation of the CrAg LFA.

Expert opinion—The CrAg LFA is recommended for use with serum, plasma or CSF for diagnosis of cryptococcal meningitis or non-meningeal cryptococcal disease in symptomatic patients. There is a need for further evaluation of LFA for screening of asymptomatic patients. However, the LFA is emerging as a valuable tool for screening of serum or plasma in ART-naïve adults with CD4 counts less than 100 cells/mm³ in geographic regions with a high prevalence of cryptococcal antigenemia. CrAg screening has the potential to identify patients with asymptomatic cryptococcal infection who should receive pre-emptive anti-fungal therapy.

Keywords

CrAg; lateral flow immunochromatographic assay; cryptococcosis

1. Introduction

Cryptococcosis is an infectious disease produced by the yeast *Cryptococcus neoformans*. Infection is believed to occur via inhalation of small desiccated yeast cells or spores from environmental sites. The yeast disseminates from the lungs to the central nervous system to produce cryptococcal meningitis. The vast majority of patients with symptomatic cryptococcosis are immunocompromised by T-cell suppression, especially patients with AIDS or those receiving prolonged immunosuppressive therapy. In developed countries, cryptococcosis is often associated with organ transplantation, prolonged use of corticosteroids, use of immune-modifying monoclonal antibodies, sarcoidosis, and diabetes[1].

In resource-limited countries, cryptococcal meningitis occurs most frequently in patients with HIV/AIDS, where cryptococcal disease may account for 13–44% of deaths in HIV-infected patients[2–4]. Park et al. estimated the global incidence of cryptococcal meningitis to be approximately 1 million cases per year[5]. The number of deaths due to cryptococcal meningitis has been estimated at more than 500,000 per year in sub-Saharan Africa alone (Fig. 1), more than the number of deaths attributed to tuberculosis.

The case-fatality rate in patients with HIV-related cryptococcal disease can be very high. For example, case-fatality rates of 35–65% have been reported in sub-Saharan Africa[6–8]. A recent report from the World Health Organization noted that the main reason for the unacceptably high case-fatality rate in resource-limited settings is a delay in presentation when meningitis is advanced and treatment is less effective. This delay in diagnosis is mainly due to limited access to lumbar puncture and the lack of rapid diagnostic assays[9]. Barriers to early diagnosis include poor sensitivity of culture and microscopy. Antigen detection assays can be sensitive and specific; however, existing assays are limited by i) requirements for refrigeration, ii) the need for technical expertise, iii) performance only in reference/diagnostic laboratories and iv) high cost[10]. As a consequence, there is a considerable need for a diagnostic test that meets the WHO ASSURED criteria for diagnostics for resource-limited countries (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, and Deliverable to those who need it)[11].

The ability of a diagnostic test to perform at the point of care (POC) offers particular promise for diagnosis of cryptococcosis in resource-limited settings. A test that can be done with minimally invasive samples for initial screening, e.g., fingerprick whole blood, plasma/serum or urine, would dramatically reduce the cost and other barriers associated with more invasive testing such as the lumbar puncture. Testing done at the POC would allow health care workers to begin treatment without the need for return visits and consequent loss to follow-up. Finally, instrument-free tests such as the lateral flow immunochromatographic assay (LFA) allow for testing at sites with little or no infrastructure. For example, only 28 percent of patients in sub-Saharan Africa have access to health care with moderate or advanced infrastructure[12].

2. *Cryptococcus* species and varieties – implications for diagnostics

Immunoassays for diagnosis of cryptococcosis detect cryptococcal capsular antigen (CrAg) in body fluids. Termed glucuronoxylomannan, CrAg occurs in four major serotypes (A, B, C and D). The species complex of pathogenic cryptococci includes *C. neoformans* and *C. gattii* (reviewed in Reference[13]). The species and varieties of *Cryptococcus* differ in the structure of their capsular polysaccharides, a factor that has consequences for development of diagnostic tests that detect CrAg. Cryptococcosis in most parts of the globe, particularly in patients with AIDS, is produced by *C. neoformans* (serotypes A and D). *C. neoformans* var. *grubii* (serotype A) has a global distribution and accounts for more than 90% of all cases of cryptococcosis[13]. *C. neoformans* var. *neoformans* (serotype D) also has a global distribution, but clinical cases are concentrated in Europe[14, 15].

C. gattii (serotypes B and C), has been recognized for many years, particularly in tropical areas of Australia, Asia, Africa and the Americas[15]. However, *C. gattii* has gained recent prominence as the cause of an ongoing outbreak that began in Vancouver Island, British Columbia. Since the initial reports from Vancouver Island, cryptococcosis caused by *C. gattii* has been found in animals and humans in other regions of southwestern Canada and the northwestern United States. A notable feature of the outbreak in the Pacific Northwest is the occurrence of disease in people without recognized immune deficiencies. Although there has not been an extensive serological characterization of isolates from the outbreak,

molecular typing has found that almost all isolates are genetic variants of the VGII class on the basis of PCR fingerprinting; isolates of VGII produce capsules of serotype B[13]. Serotype C has generally been regarded as rare. However, there are recent reports of serotype C in AIDS patients in regions of sub-Saharan Africa[16–18]. For example, 13% of isolates from AIDS patients in Botswana and Malawi were *C. gatti* serotype C[17].

The global variability in production of capsular polysaccharide of different serotypes places requirements on diagnostics development for assays that target CrAg. To be effective on a global basis, an immunoassay that targets the capsular polysaccharide must be sensitive to clinically relevant concentrations of CrAg of all serotypes.

3. Current and emerging approaches to diagnosis of cryptococcosis

The three pillars for diagnosis of cryptococcosis have been the India ink stain of body fluids for encapsulated yeasts, culture of body fluids, and immunoassay for CrAg. India ink staining lacks sensitivity, is often negative in patients, and requires experience on the part of laboratory personnel[1]. Culture may also have poor sensitivity, can take several days to a result, may require large specimen volumes and requires laboratory infrastructure[1, 10, 19]. Kambugu et al. reported results from CSF evaluation of 136 patients with cryptococcal meningitis; 90% had positive cryptococcal cultures, 97% had positive India ink stains, and 100% had positive CSF CrAg tests[7]. Given the limitations of culture and India ink staining, immunoassay for CrAg is central to diagnosis of disease[9, 10, 19]. CrAg is shed during infection into body fluids such as CSF, blood and urine (Fig. 2). Presence of CrAg in samples can be determined by latex agglutination[20], ELISA[21] and now, by lateral flow immunoassay[10, 22]. Introduced in 1966, latex agglutination for detection of CrAg is one of the earliest and most enduring of the immunoassays for diagnosis of infectious disease[20].

The World Health Organization (WHO) recently released guidelines for diagnosis and treatment of cryptococcal disease in HIV-infected patients[9]. These guidelines are based on the principle that early diagnosis and treatment are central to reducing mortality from cryptococcal disease. In settings with ready access to and no contraindication for a lumbar puncture (LP), a LP with rapid assay of CSF for CrAg is recommended. A rapid India ink examination of CSF is recommended as an alternative to CrAg testing if access to CrAg assay is either not available or rapid results are not assured. If there is not access to an immediate LP or the LP is contraindicated, the WHO Rapid Advice guidelines recommend testing of serum or plasma for CrAg. The WHO Guideline Development Group emphasized the value of a rapid CrAg test (either latex agglutination or LFA) of CSF or serum and noted that CrAg assays had a higher sensitivity and specificity, were easier to perform, and were less dependent on technician skill than the India ink test. A central tenet of the WHO Rapid Advice guidelines was time to result; results should be available at the time of the patient visit. Furthermore, the WHO Guideline Development Group emphasized that the CrAg LFA had several advantages over latex agglutination, including lower cost, rapid turnaround time, little training required, and can be performed with minimal laboratory infrastructure.

An emerging concept is that of screening ART-naïve patients with low CD4 counts for serum/plasma CrAg in regions where there is a high prevalence of cryptococcal antigenemia[9]. Such screening would identify patients at high risk for cryptococcal disease and allow for pre-emptive treatment with antifungal agents to prevent cryptococcal disease. This approach is based on the finding that CrAg is detectable in serum a median of 3 weeks prior to the onset of symptoms[2, 23]. A seminal study by Jarvis et al. examined blood samples that were available for retrospective evaluation from a large cohort of patients entering an ART program in Cape Town[24]. Screening of samples for CrAg was 100%

sensitive in predicting cryptococcal meningitis in the first year after starting ART and had a 100% negative predictive value for a negative screening result. The cost to prevent one death using a “screen and treat” strategy was estimated to range from \$77 to \$266 using latex agglutination as the CrAg assay[9, 25, 26]. A study of cost-effectiveness of a screen and treat strategy concluded that screening with LA and preemptive treatment with fluconazole is cost effective when the prevalence of CrAg asymptomatic antigenemia is 3%[25]. It is likely that the reduced cost of LFA and the ability to perform LFA at the POC could dramatically reduce the cost of CrAg screening, especially in settings that lack laboratory support. As a consequence, the WHO Rapid Advice report recommended prompt evaluation of LFA as a screening tool in asymptomatic patients across a range of prevalence settings.

4. How the CrAg LFA works

Immunoassay for CrAg is an antigen-capture test that detects free capsular antigen that has been released by the yeast into body fluids such as blood, BALF, CSF or urine (Fig. 2). The ideal immunoassay for diagnosis of cryptococcosis must meet two criteria. First, the test should be able to detect CrAg of all serotypes. This broad reactivity will allow for use of the test across the global spectrum of cryptococcal isolates. Second, the test needs to meet the needs for diagnosis of disease in countries or regions with both advanced and limited or no infrastructure. The majority of cases of cryptococcosis occur in countries with regions or clinical sites with very limited infrastructure. As a consequence, a test that addresses the needs of resource-limited countries must meet the WHO ASSURED criteria for diagnostic tests. At the same time, cryptococcosis continues to be an important opportunistic infection in countries where advanced medical treatment and immunosuppression associated with such treatment predisposes to opportunistic infections.

The IMMY CrAg LFA was designed to be a test that addresses the global spectrum of the species complex of pathogenic cryptococci. The IMMY CrAg LFA is constructed entirely from monoclonal antibodies (mAbs). Use of mAbs allows for consistent reagent quality and performance. The CrAg LFA uses a cocktail of two mAbs[22]. One mAb is highly reactive with CrAg of serotypes A, B and C; the second mAb is highly reactive with CrAg of serotypes A and D. Used together, the antibodies are highly reactive with CrAg across the range of cryptococcal serotypes[27]. Initial assessment of the serotype sensitivity of the CrAg LFA[28] shows a sensitivity that is greater than that of commercially available LA and EIA assays for CrAg of serotypes A, B, C and D[27].

The LFA for CrAg uses the lateral flow immunochromatographic assay platform (Fig. 3). Commonly referred to as a dipstick assay, the LFA is self contained and has clear endpoints. Importantly, the assay meets all of the WHO ASSURED criteria for the ideal diagnostic test. The test has a low cost (Affordable) and has a sensitivity that is equal to or better than latex agglutination (Sensitive). The LFA detects the same antigen that is detected by the widely accepted CrAg latex agglutination and ELISA assays and will, therefore, have the same specificity (Specific). Using a format similar to the home pregnancy test, the CrAg LFA can be used by personnel with minimal training (User-friendly) and produces a clear result within 10 min (Rapid). The test requires no electricity or advanced laboratory infrastructure (Equipment-free). Finally, the test is small, lightweight, requires no refrigeration and has a long shelf life (Deliverable to those who need it).

The CrAg LFA provides both qualitative and semi-quantitative, e.g. titer, results. The test requires five simple steps: (1) one drop of lateral flow (LF) specimen diluent is added to a tube, (2) patient specimen is added to the tube, (3) a CrAg LFA test strip is inserted into the tube, (4) the test is allowed to incubate for 10 min and (5) the strips are read visually. The

presence of two lines (test and control lines), regardless of the intensity of the test line, indicates a positive test result (Fig. 3). A single control line indicates a negative test result. If the control line does not appear, the results are invalid and the test should be repeated. For semi-quantitative results, the patient's titer is reported as the highest dilution that yields a positive test result.

The IMMY CrAg LFA is available either as a laboratory kit or as a field kit, which contains disposable pipettes, tubes and a kit box containing a tube holder. Both kit formats contain a CrAg positive control, LF specimen diluent, and CrAg LF test strips. Quality control can be performed by evaluating the CrAg positive control, which produces the presence of two lines (a positive result), and the LF specimen diluent (negative control), which produces a single control line (a negative result).

The CrAg LFA has been cleared by the U.S. FDA for use with serum and CSF specimens. In addition, the test has received the CE Mark for serum, plasma and CSF. The CE Mark indicates that the assay conforms to the essential requirements of the European Conformity Directives. Many governments around the world recognize either FDA clearance or the CE Mark for satisfying regulatory requirements in their respective countries.

5. Clinical evaluation

5.1 Pulmonary cryptococcosis

Cryptococcosis begins as a pulmonary infection, which may be asymptomatic or can progress to pneumonia (Fig. 2). Cryptococcal pneumonia is a common but often unrecognized problem[29–31]. Diagnosis of pulmonary cryptococcosis presents an opportunity to detect and treat the infection early when patients are more likely to have better outcomes[32]. The CrAg LFA was evaluated using stored sera from HIV patients hospitalized with acute respiratory illness in Thailand[10, 33]. Of 462 HIV patients with acute respiratory infection, 87 (19%) were identified as antigenemic by the CrAg LFA; 61% of the CrAg positive patients had no evidence of existing or past cryptococcal meningitis. The sensitivity of the CrAg LFA was 100% and 92% in sera and urine, respectively, when applied to 17 patients with positive blood cultures for *Cryptococcus*. The CrAg LFA had a positive percent agreement of 95.6% (87/91) and a negative percent agreement of 99.5% (369/371) when compared to the CrAg EIA.

5.2 Cryptococcal Meningitis

A WHO Rapid Advice report recently recommended the CrAg LFA for diagnosis of suspected cryptococcal meningitis[9]. In a retrospective study of culture-confirmed cryptococcal meningitis patients in South Africa, the CrAg LFA had a sensitivity of 100% when used with serum and plasma and 98% with urine[22]. Furthermore, CrAg LFA titers strongly correlated with CrAg concentration as determined by a quantitative ELISA. Despite several samples having CrAg concentrations greater than 10^5 ng/ml, no prozone effect was observed in the CrAg LFA. Prozone effect is a common cause of false-negative results in latex agglutination assays of specimens with high CrAg concentrations. Several prospective studies of the CrAg LFA are in progress. A preliminary report from a study done in Uganda was recently presented at the 2012 Conference on Retroviruses and Opportunistic Infections (CROI)[34]. The authors reported that the CrAg LFA was highly sensitive and specific relative to both culture and CrAg latex agglutination. CrAg latex agglutination and LFA had a high concordance in plasma and CSF samples (96% and 97%, respectively). Culture and India ink staining had a lower concordance with LFA (85% and 87%, respectively). Importantly, a low concordance was also found between culture and India ink when compared with CrAg latex agglutination, suggesting that the positive LFA results were

not false positives, but more sensitive in detecting cryptococcal infection than culture or India ink.

6. Expert opinion

6.1. CrAg LFA for diagnosis of cryptococcosis in symptomatic patients

Despite the early and widespread use of HAART in HIV/AIDS in developed countries, cryptococcosis remains a significant risk in patients who are immunosuppressed through advanced medical intervention or undiagnosed AIDS. The CrAg LFA meets all of the needs of laboratorians and clinicians in countries or sites with advanced laboratory infrastructure. Specifically, the CrAg LFA is highly sensitive and specific, is easy to perform, and provides rapid and robust results. The high sensitivity of the CrAg LFA may be particularly important in early diagnosis of *C. gattii* infection which is responsible for the outbreak that is occurring in the Pacific Northwest.

The WHO Rapid Advice Guideline Development Group recommended use of CrAg assay by LA or LFA as the preferred diagnostic approach to diagnosis of suspected cryptococcal meningitis in resource-limited settings[9]. EIA was not recommended at such sites due to high cost [9, 19]. Unlike LA and EIA, the CrAg LFA is the only commercially available CrAg assay that meets all of the WHO ASSURED criteria, which enables reliable use in resource-limited settings. The CrAg LFA is quick, simple, affordable and effective. Because the CrAg LFA performs well with a variety of specimen types it could be used as a POC test that would reduce the time to diagnosis, facilitate early treatment, and ultimately reduce mortality[10].

6.2. CrAg LFA to screen of patients at high risk for cryptococcosis in regions with high prevalence

The WHO Rapid Advice Guideline Development Group conditionally recommended routine screening of serum or plasma for CrAg in ART-naïve adults. CrAg-positive patients would be given pre-emptive anti-fungal therapy to reduce the development of cryptococcal disease. Such screening is recommended in patients with CD4 counts less than 100 cells/mm³ and where this population has a high prevalence of cryptococcal antigenemia (> 3%)[9]. The high sensitivity of the CrAg LFA makes the test ideally suited for screening patients at high risk for cryptococcosis. The CrAg LFA is suitable for use with plasma which is readily available from samples routinely taken for HIV testing and CD4 cell-count monitoring. The CrAg LFA can be performed on a small number of patient samples or it can be easily performed on a large number of patient samples. Moreover, the overall cost of testing by LFA is lower than that of either LA or EIA; cost is a key consideration for implementing widespread screening[19, 25]. There is a critical need for further evaluation of the CrAg LFA as a critical component of a “screen and treat” strategy for prevention of cryptococcal meningitis.

6.3. A look to the future

Diagnosis of cryptococcosis using finger prick whole blood or urine are currently undergoing evaluation and could be very useful in resource-limited settings. Finger prick samples or urine would allow use of the CrAg LFA at sites where blood processing is difficult or the lumbar puncture is not easily performed or accepted. The further down the healthcare chain (e.g. advanced to moderate to minimal or no infrastructure) that the CrAg LFA can be performed, the more patients that can be diagnosed and treated, resulting in improved mortality. The CrAg LFA is central to the U.S. Centers for Disease Control and Prevention’s call to action for equipping one-half of all HIV clinics in Africa and Asia to perform *Cryptococcus* testing and treatment by 2015[35]. Implementation of such a strategy was estimated to save 50,000 2013; 100,000 lives every year.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Perfect, JR. *Cryptococcus neoformans*. In: Mandell, GL.; Bennett, JE.; Dolin, R., editors. Principles and practice of infectious diseases. 7th ed.. Philadelphia, PA: Churchill Livingstone Elsevier; 2010. p. 3287-3303.
2. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS*. 2002; 16:1031–1038. [PubMed: 11953469]
3. Okongo M, Morgan D, Mayanja B, et al. Causes of death in a rural, population-based human immunodeficiency virus type 1 (HIV-1) natural history cohort in Uganda. *Int J Epidemiol*. 1998; 27:698–702. [PubMed: 9758128]
4. Churchyard GJ, Kleinschmidt I, Corbett EL, et al. Factors associated with an increased case-fatality rate in HIV-infected and non-infected South African gold miners with pulmonary tuberculosis. *Int J Tuberc Lung Dis*. 2000; 4:705–712. [PubMed: 10949321]
5. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009; 23:525–530. [PubMed: 19182676] •• A key report that calculated the global impact of cryptococcal meningitis.
6. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naïve or antiretroviral-experienced patients treated with amphotericin B or fluconazole. *Clin Infect Dis*. 2007; 45:76–80. [PubMed: 17554704]
7. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis*. 2008; 46:1694–1701. [PubMed: 18433339]
8. Lessells RJ, Mutevedzi PC, Heller T, Newell ML. Poor long-term outcomes for cryptococcal meningitis in rural South Africa. *S Afr Med J*. 2011; 101:251–252. [PubMed: 21786729]
9. WHO Cryptococcal Guideline Working Group. Rapid advice: Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. 2011 WHO Press, World Health Organization Available at: http://whqlibdoc.who.int/publications/2011-9789241502979_eng.pdf •• This report provides detailed recommendations for diagnosis and treatment of cryptococcal meningitis in resource-limited settings.
10. Lindsley MD, Mekha N, Baggett HC, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis*. 2011; 53:321–325. [PubMed: 21810743]
11. UNICEF/UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. World Health Organization; 2009. Sexually Transmitted Diseases Diagnostic Initiative (SDI). Available at: www.who.int/std_diagnostics
12. Girosi F, Olmsted SS, Keeler E, et al. Developing and interpreting models to improve diagnostics in developing countries. *Nature*. 2006; 444(Suppl. 1):3–8. [PubMed: 17159889]
13. Litvintseva, AP.; Xu, J.; Mitchell, TG. Population structure and ecology of *Cryptococcus neoformans* and *Cryptococcus gattii*. In: Heitman, J.; Kozel, TR.; Kwon-Chung, KJ.; Perfect, JR.; Casadevall, A., editors. *Cryptococcus: from human pathogen to model yeast*. Washington, DC: ASM Press; 2011. p. 97-111.
14. Dromer F, Mathoulin S, Dupont B, et al. Individual and environmental factors associated with infection due to *Cryptococcus neoformans* serotype D. French Cryptococcosis Study Group. *Clin Infect Dis*. 1996; 23:91–96. [PubMed: 8816135]
15. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *Am J Epidemiol*. 1984; 120:123–130. [PubMed: 6377880]
16. Karstaedt AS, Crewe-Brown HH, Dromer F. Cryptococcal meningitis caused by *Cryptococcus neoformans* var. *gattii*, serotype C, in AIDS patients in Soweto, South Africa. *Med Mycol*. 2002; 40:7–11. [PubMed: 11860015]
17. Litvintseva AP, Thakur R, Reller LB, Mitchell TG. Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDS in Sub-Saharan Africa. *J Infect Dis*. 2005; 192:888–892. [PubMed: 16088839]

18. Thakur, R.; Steele, KT.; Nthobatsang, R., et al. Prevalence of *C. neoformans* and *C. gattii* among patients with cryptococcal meningitis at a tertiary hospital in Gaborone, Botswana: March 2005/February 2007, abstr. 1048. Abst 47th Annual Meeting IDSA; Philadelphia, PA. 2009.
19. Vanselow M, Brandt ME, Park BJ. Diagnosis and management of cryptococcal disease in resource-limited settings. *Curr Fungal Infect Rep.* 2012; 6:35–40.
20. Gordon MA, Vedder DK. Serologic tests in diagnosis and prognosis of cryptococcosis. *Am Med Assoc.* 1966; 197:961–967.
21. Gade W, Hinnefeld SW, Babcock LS, et al. Comparison of the PREMIER cryptococcal antigen enzyme immunoassay and the latex agglutination assay for detection of cryptococcal antigens. *J Clin Microbiol.* 1991; 29:1616–1619. [PubMed: 1761681]
22. Jarvis JN, Percival A, Bauman S, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin Infect Dis.* 2011; 53:1019–1023. [PubMed: 21940419] • This is a retrospective study of the CrAg LFA in patients with cryptococcal meningitis.
23. Micol R, Lortholary O, Sar B, et al. Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. *J Acquir Immune Defic Syndr.* 2007; 45:555–559. [PubMed: 17577124]
24. Jarvis JN, Lawn SD, Vogt M, et al. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis.* 2009; 48:856–862. [PubMed: 19222372] • This study provides the conceptual foundation for a "screen and treat" strategy to prevent cryptococcal meningitis.
25. Meya DB, Manabe YC, Castelnovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count <100 cells/ml who start HIV therapy in resource-limited settings. *Clin Infect Dis.* 2010; 51:448–455. [PubMed: 20597693] • This study was one of the first to demonstrate the clinical and economic impact of early diagnosis and treatment of cryptococcal meningitis.
26. Micol R, Tajahmady A, Lortholary O, et al. Cost-effectiveness of primary prophylaxis of AIDS associated cryptococcosis in Cambodia. *PLoS One.* 2010; 5:e13856. [PubMed: 21085478]
27. Kozel TR, Thorkildson P, Percival A. Monoclonal antibodies specific for immunorecessive epitopes of glucuronoxylomannan, the major capsular polysaccharide of *Cryptococcus neoformans* reduce serotype bias in an immunoassay for cryptococcal antigen. *Clin Vaccine Immunol.* 2011; 18:1292–1296. [PubMed: 21697342]
28. Kozel, T.; Bauman, S. New developments in diagnosis: toward a point of care test. Presented at: 8th International Conference on Cryptococcus and Cryptococcosis; Charleston, SC. 2011.
29. Yoo SD, Worodria W, Med M, et al. The prevalence and clinical course of HIV-associated pulmonary cryptococcosis in Uganda. *J Acquir Immune Defic Syndr.* 2010; 54:269–274. [PubMed: 20150818]
30. Jarvis JN, Wainwright H, Harrison TS, et al. Pulmonary cryptococcosis misdiagnosed as smear-negative pulmonary tuberculosis with fatal consequences. *Int J Infect Dis.* 2010; 14S:e310–e312. [PubMed: 20932485]
31. Brizendine KD, Baddley JW, Pappas PG. Pulmonary cryptococcosis. *Semin Respir Crit Care Med.* 2011; 32:727–734. [PubMed: 22167400]
32. Driver JA, Saunders CA, Heinze-Lacey B, Sugar AM. Cryptococcal pneumonia in AIDS: is cryptococcal meningitis preceded by clinically recognizable pneumonia? *J Acquir Immune Defic Syndr Hum Retrovirol.* 1995; 9:168–171. [PubMed: 7749794]
33. Harris JR, Lindsley MD, Hanchaichon S, et al. High prevalence of cryptococcal infection among HIV-infected patients hospitalized with pneumonia in Thailand. *Clin Infect Dis.* 2011
34. Rolfes, M.; Butler, E.; von Hohenberg, M., et al. Paper #953 - Evaluation of a novel point-of-care lateral flow assay to detect cryptococcal antigen in plasma and CSF; 19th Conference on Retroviruses and Opportunistic Infections (CROI); 2012. Available at <http://www.retroconference.org/2012b/Abstracts/44439.htm>
35. Centers for Disease Control and Prevention. Fighting a deadly fungus: A new strategy to reduce deaths due to *Cryptococcus*. National Center for Emerging & Zoonotic Infectious Diseases. 2011.

Available at: http://www.cdc.gov/ncezid/dfwed/factsheets/factsheet_cryptococcus_revised%205-17-2011.pdf

36. WHO. Revised Global Burden of Disease (GBD) 2002 estimates. WHO Health Statistics and Health Information Systems. 2002. Available at http://www.who.int/healthinfo/global_burden_disease/estimates_regional_2002_revised/en/

Article highlights

- Cryptococcal meningitis is a major cause of death among AIDS patients in resource-limited countries.
- Cryptococcosis continues to be a serious infection in developed countries among patients with deficiencies in T-cell immunity.
- Detection of cryptococcal antigen (CrAg) by immunoassay of serum and cerebral spinal fluid has emerged as a reliable means for diagnosis of cryptococcosis.
- A new lateral flow assay (LFA) for detection of CrAg offers many advantages for diagnosis of cryptococcosis in both resource-limited and developed countries.

This box summarizes key points contained in the article.

Comparison of LFA with alternative technologies

Lateral flow assay

- Requires no pretreatment of sample
- High sensitivity for CrAg of all serotypes
- Suitable for use in settings with no, minimal or advanced infrastructure
- Rapid results – 10 min
- Low overall cost

Latex agglutination

- Serum samples pretreated with Pronase
- Reduced sensitivity for CrAg of serotype C
- Not suitable for use in settings with no or minimal infrastructure
- Rapid results – 10–30 min (depending on specimen type)
- Intermediate overall cost

Enzyme immunoassay

- Requires no pretreatment of sample
- Reduced sensitivity for CrAg of serotypes C and D
- Not suitable for use in settings with no or minimal infrastructure
- Longer time to result – 35–45 min
- Higher overall cost

Fig. 1.
Estimated causes of death from infectious disease in sub-Saharan Africa, excluding HIV, 2009 (Adapted from Park, et al., 2009[5] and WHO statistics[36]).

Fig. 2.
Schematic of temporal sequence of CrAg concentration in body fluids during progression of disease from pulmonary infection to meningitis.

Fig. 3. Panel A - Schematic showing operation of lateral flow immunochromatographic assay (LFA) for detection of cryptococcal antigen (CrAg). LFA is constructed from monoclonal antibodies (mAbs) specific for CrAg. Panel B – Images of positive and negative LFA.