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Expert Review of Anti-infective Therapy

ISSN: 1478-7210 (Print) 1744-8336 (Online) Journal homepage: www.tandfonline.com/journals/ierz20

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To cite this article: Jonathan Falconer, Tshiamo M. Mmotsa, Nelesh P. Govender & Joseph N. Jarvis (2025) Diagnosis of cryptococcal meningitis in people living with HIV in low-income countries: barriers and strategies, Expert Review of Anti-infective Therapy, 23:10, 893-905, DOI: 10.1080/14787210.2025.2554999

To link to this article: https://doi.org/10.1080/14787210.2025.2554999

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Published online: 29 Sep 2025.

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EXPERT REVIEW OF ANTI-INFECTIVE THERAPY
2025, VOL. 23, NO. 10, 893–905
https://doi.org/10.1080/14787210.2025.2554999

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SPECIAL REPORT

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Diagnosis of cryptococcal meningitis in people living with HIV in low-income countries: barriers and strategies

Jonathan Falconer[a,b*], Tshiamo M. Mmotsa[a*], Nelesh P. Govender[a] and Joseph N. Jarvis[c,d]

[a]School of Pathology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa; [b]South African Medical Unit, Medecins Sans Frontieres, Cape Town, South Africa; [c]Clinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene Tropical & Medicine, London, UK; [d]Botswana Harvard Partnership, Gaborone, Botswana

ABSTRACT

Introduction: Cryptococcal meningitis (CM) is the second leading cause of AIDS-related mortality where the burden of advanced HIV disease is concentrated. Advances in diagnostics and treatment, including cryptococcal antigen (CrAg) screening and short-course antifungal regimens, have improved clinical outcomes in trials, but replicating these same benefits in routine care has proven more difficult.

Areas covered: This review outlines the biological rationale for CrAg screening and examines the major operational barriers to effective CM diagnosis in low-income countries. An exploratory literature review identified peer-reviewed articles published before May 2025. We assess challenges for CD4 testing, CrAg screening, lumbar puncture (LP) performance, and routine surveillance. Novel approaches, including risk stratification with semi-quantitative CrAg testing are also described.

Expert opinion: The impact of recent diagnostic and treatment advances for cryptococcal disease has been constrained by gaps in implementation. Closing the diagnostic gap requires strengthening decentralized CD4 testing; expanding reflex and point-of-care CrAg, including use of semi-quantitative CrAg assays to prioritize those at highest risk for urgent LP and/or enhanced antifungal treatment; strengthening healthcare provider training, referral systems, LP access, and enhancing community engagement. Integration of these measures into national HIV programs alongside operational research could reduce mortality for patients and costs for health systems.

ARTICLE HISTORY
Received 23 June 2025
Accepted 27 August 2025

KEYWORDS
Cryptococcal meningitis; advanced HIV disease; cryptococcal antigen; lumbar puncture; cryptococcal disease diagnosis

1. Introduction

Cryptococcal meningitis (CM), an opportunistic fungal infection associated with advanced HIV disease (AHD), has a near 100% mortality if left untreated [1,2]. It is the most common cause of adult meningitis in Africa and the most significant contributor to AHD-related mortality after tuberculosis (TB) [3], accounting for over 19% of all AHD-related mortality and over 110,000 deaths per year [2]. Whilst there is a small but important burden of cryptococcal disease in people not living with HIV, the majority of cases are seen in association with AHD meaning the burden of CM globally is closely linked to that of AHD [4]. Despite progress in antiretroviral therapy (ART) rollout, the burden of AHD remains high [5–7]. A recent rapid review showed that 44.3% of inpatients and 33.5% of outpatients among people living with HIV (PLWHIV) presenting to care have AHD, and this is concentrated in lower resource settings in low-income countries (LICs) [8,9]. Modeling of the impact of funding changes after the reduction in USAID and PEPFAR funding in February 2025 predict an increase in HIV-related deaths and AHD burden in the years ahead, with associated increases in the burden of CM [10,11].

Cryptococcal antigen (CrAg) testing has transformed diagnosis of HIV-associated CM, and shorter induction regimens with amphotericin B-based treatments in combination with flucytosine and fluconazole have resulted in a substantial survival benefit [12–16]. Translating these research advances into improved diagnoses and outcome benefits remains a challenge in low-resource settings and there is a significant gap between the numbers of cryptococcal disease cases predicted by detailed modeling and the number of cases actually detected [2]. The Lancet Commission published in 2021 reported that closing the diagnostic gap – defined as the proportion of patients with a disease that remain undiagnosed – across a range of diagnostics in HIV care including cryptococcal disease could save over 250,000 lives per year. The reasons for this diagnostic gap are multifactorial and are underpinned by challenges at patient level, health unit level and health system level in low resource settings [17]. This review discusses the current barriers and strategies to the diagnosis of CM programs in low-resource settings.

2. Search methods

We conducted an exploratory literature search on PubMed.gov, Scopus, Google Scholar, and ClinicalTrials.gov. Variations of search terms such as 'cryptococcal disease,' 'cryptococcal meningitis,' 'advanced HIV disease,' 'low-resource settings,' and 'diagnostics' were used to search these databases. Peer-reviewed articles, conference abstracts, project reports, international and

CONTACT Jonathan Falconer Johnathanfalconer@gmail.com University of Witwatersrand, 1 Jan Smuts Avenue, Braamfontein, Johannesburg 2000, South Africa
*These authors contributed equally.

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Article highlights

- Cryptococcal meningitis continues to be a significant contributor to mortality in people living with advanced HIV disease in low-resource settings.
- Challenges to CM diagnosis in low-resource settings include limited access to CD4 testing, cryptococcal antigen screening, lumbar puncture and the lack of antifungal treatments.
- These challenges are multilayered and include patient-level barriers around stigma and community mistrust of lumbar puncture procedures, health unit-level barriers to provider training and knowledge and health system-level barriers around availability of consumables & interrupted referral pathways from primary care.
- Strategies to close the diagnostic gap and reduce mortality must address these challenges at multiple levels: patient-level interventions such as health education and community engagement; provider-level measures including targeted training at health facilities; and system-level improvements focusing on effective supply chain management and streamlined referral pathways.
- Novel approaches such as reflex serum CrAg screening and stratification of patients with semi-quantitative CrAg testing provide opportunities to increase case detection and streamline care.

national guidance, and ongoing trial descriptions published before 30 May 2025 were included in our narrative review.

3. Natural history of cryptococcal disease

Cryptococcosis, primarily caused by *Cryptococcus neoformans* and *Cryptococcus gattii* species-complexes, is an invasive fungal infection that is usually only clinically apparent among immunocompromised populations, particularly individuals with AHD. Following environmental exposure, typically through inhalation of desiccated yeast cells or spores from contaminated soil or avian droppings, *Cryptococcus spp*. establishes a pulmonary infection that may remain latent [3,18]. Reactivation of latent pulmonary infection during immunosuppression can then disseminate into the blood (detected as antigenaemia or cryptococcaemia on blood culture) that can spread to other organs, notably the central nervous system (CNS), resulting in life-threatening meningoencephalitis [19].

Historically, diagnosis of cryptococcal disease relied on India ink microscopy, culture, and latex agglutination assays – methods with limitations in sensitivity, turnaround time, and technical complexity, particularly in resource-limited settings [20–22] Table 1. The introduction of the CrAg lateral flow assays (LFA) has enabled rapid, equipment-free detection in serum, plasma, and cerebrospinal fluid at a cost of $2–3 per test. The LFA's high sensitivity and specificity compared to culture make it suitable for use in low-resource settings. Detection of cryptococcal antigenaemia using the CrAg LFA enables preemptive fluconazole treatment to prevent progression to CM and facilitates earlier diagnosis by fast-tracking patients for lumbar puncture (LP). CrAg screening has been associated with a 28% (95% CI 10–43) reduction in mortality in a clinical trial setting, though this has not been confirmed in implementation programs [23]. This offers the opportunity for quality diagnostics for cryptococcal disease to be offered across all levels of laboratory infrastructure levels defined by the World Health Organization's (WHO) tiering system of laboratory access [24]. The integration of CrAg testing into the WHO's package of care for advanced HIV disease has made it a cornerstone of current cryptococcal disease screening and management strategies [25].

Despite their technical simplicity and proven diagnostic accuracy, effective implementation of CrAg testing remains challenging in low-resource settings. WHO guidelines outline a diagnostic cascade for cryptococcal disease beginning with CD4 testing, either prompted by clinical symptoms of HIV or conducted routinely. Individuals with a CD4 count < 200 cells/μL should undergo serum CrAg screening. Those testing positive require an LP to assess for CNS involvement. Patients with a positive cerebrospinal fluid (CSF) CrAg result should be treated for CM, while those with a negative result should receive preemptive fluconazole to prevent progression to CM.

In practice, this cascade is frequently disrupted. As the burden of CM becomes increasingly concentrated in low-resource health systems, diagnosis is shaped by multiple factors beyond test availability. These include patient-level delays in presentation, facility-level constraints such as limited access to diagnostics and LP consumables and broader system-level barriers including weak referral mechanisms and inconsistent supply chains. Consequently, diagnostic efforts are often fragmented, with patients entering the care pathway at different stages of illness or not at all. Figure 1 illustrates both the idealized diagnostic sequence and the common nonstandard entry points observed in routine practice.

Effective diagnosis therefore requires not only the availability of appropriate tools but their integration into functioning clinical pathways supported by trained personnel, robust infrastructure, and patient-centered referral systems. Barriers across the diagnostic cascade contribute to missed or delayed

Table 1. Summary of different diagnostic tests for cryptococcal meningitis (CM) with different test performance. CSF: cerebrospinal fluid, LFA: lateral flow assay, CrAg: cryptococcal antigen.

Diagnostic Test	Specimen Type	Sensitivity (%)	Specificity (%)	Comments
India Ink Microscopy	CSF	42–86	~100	Rapid, low-cost, but poor sensitivity in low fungal burden or early disease [20,22].
Culture	CSF, blood, sputum	90–95 (compared to aggregate of other diagnostics including CrAg)	~100	Gold standard but slow (up to 7 days); requires lab infrastructure [21,22].
Latex Agglutination Test	Serum, CSF	93–99	93–98	Historically used; affected by high dose hook effect and requires lab processing [22].
Cryptococcal Antigen LFA	Serum, plasma, CSF, urine	95–100	97–100	Rapid, highly sensitive; suitable for point-of-care and decentralized use. Test performance can vary at extremes of antigen burden [21–23].

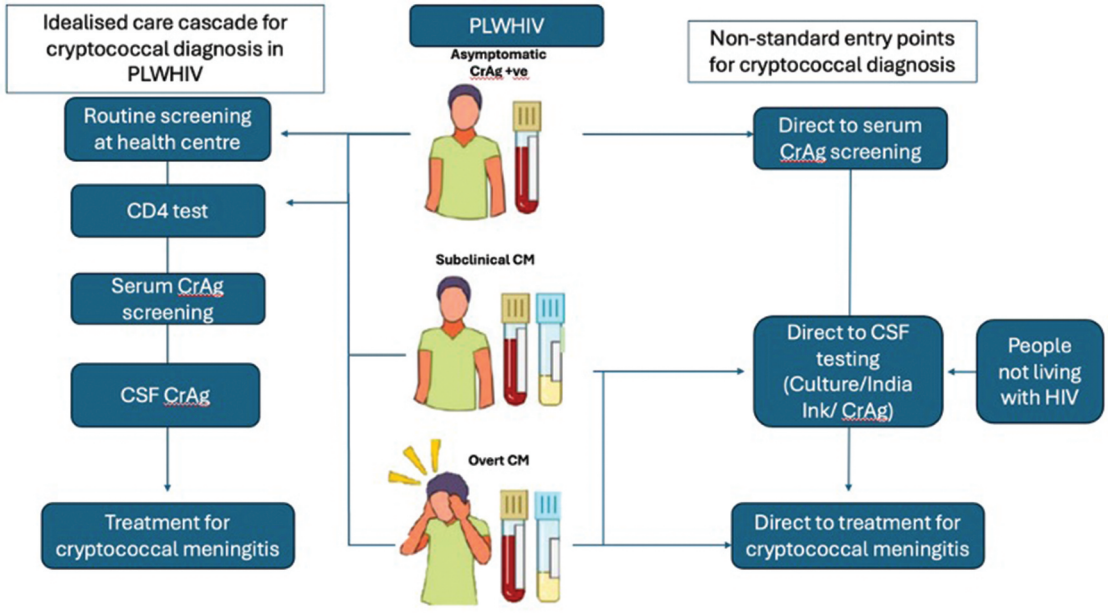


Figure 1. Possible pathways to diagnosis for a person living with cryptococcal meningitis. Adapted from R. Wake et al [26] with permission. PLWHIV: person living with HIV, CrAg: cryptococcal antigen, CSF: cerebrospinal fluid, CM: cryptococcal meningitis.

diagnoses and suboptimal outcomes. This review will examine each stage of the cryptococcal diagnostic cascade, outlining key implementation challenges and identifying

4. CD4 testing: a key precursor to cryptococcal diagnostics

The WHO defines AHD in adults and adolescents as those with a CD4 cell count below 200 cells/μL or WHO clinical stage 3 and 4 disease [25]. Identifying a CD4 count below 200 cells/μL determines eligibility for the evidence-based recommended package for AHD including screening for cryptococcal disease. CD4 testing is therefore a key step in the diagnostic pathway to screening for cryptococcal disease. Since the adoption of the 'test-and-treat' approach for HIV in 2014, HIV programs have shifted focus toward viral load testing to monitor treatment response, resulting in a decline in CD4 testing coverage. A large multi-cohort study involving over 500,000 PLWHIV in Southern Africa revealed that 78% of those eligible received CD4 testing in 2008 and in 2017 this figure had fallen to 38% [27]. A continent-wide survey conducted in 2022 found that 64% of African countries reported comprehensive access to CD4 testing, while the remaining countries had either limited or minimal access [28].

This situation has been exacerbated by the discontinuation of the two principal near-patient CD4 flow cytometry platforms: the Abbott PIMA and BD FACS Presto machines. Both were withdrawn from the market, leaving a gap in decentralized CD4 testing capabilities [29].

The reduction in CD4 availability has been identified as a significant concern in the WHO 2024 assessment of the HIV diagnostic landscape [30]. One proposed solution has been the novel semi-quantitative point-of-care (POC) CD4 lateral flow assay (VISITEC), indicating whether a patient's CD4 count is below 200 cells/μL [29,31]. These assays can be performed at POC and provide results within 40 min. However, challenges remain regarding the test performance and feasibility in POC settings. The sensitivity to detect advanced HIV disease is consistently above 90% but specificity varied significantly based on the testing environment. Specificity ranged from 87.7% when using venous samples in a controlled laboratory setting to 61.4% when using finger-prick samples at busy clinic points of care [32–35].

Cost-effective CD4 tests with secure supply chains are needed and challenges identified around test performance in the new lateral flow assays has been identified as a key area for focus by stakeholders [29,35]. This includes developing dedicated lay cadres [36], creating 'lab-like' settings [37] near the patient and making use of diagnostic adjuncts such as cube readers [38]. Beyond this, new diagnostic platforms that might change the way and sample type that is used for CD4 testing are under development [39].

5. CrAg screening: overcoming operational challenges

5.1. The basis for screening

CrAg screening enables the early identification of individuals at high risk of CM. Its rationale was first demonstrated in a Ugandan natural history study, nested within a pneumococcal vaccine trial conducted during the pre-ART era, which showed that CrAg becomes detectable a median of 22 days before the onset of meningitis symptoms, supporting the potential for early diagnosis and preemptive treatment [40]. Subsequent work in South Africa demonstrated that individuals who tested positive for serum CrAg at the time of ART initiation had a significantly higher risk of developing CM, while CrAg-negative patients had a 100% negative predictive value for the disease [41]. Prevalence studies among people living with AHD indicate a CrAg positivity rate

ranging from 2% to 15% in both Asia and Africa, increasing as CD4 counts decline [28,42–46].

In response to this evidence, the WHO incorporated CrAg screening into its 2011 rapid advice for managing cryptococcal disease and more recently updated-recommending screening for individuals with CD4 counts below 100 cells/μL and provision of preemptive antifungal therapy [47–49]. The REMSTART trial validated the efficacy of this approach. Among individuals with AHD and positive CrAg results, a bundled intervention of screening, social support, and fluconazole preemptive treatment reduced mortality by 28% (absolute risk reduction from 18% to 13%, 95% CI: 10–43%) [50]. Furthermore, in South Africa and Uganda, patients with a previous CrAg test had greater survival from CM than those without a CrAg test [51,52]. Cost-effectiveness modeling estimated the cost per daily adjusted life years (DALY) averted at just $21, supporting widespread programmatic rollout across Africa [53].

CrAg screening enables the identification of patients who would benefit from preemptive therapy or LP to diagnose meningitis early. Prioritizing high-risk individuals in this way allows for more efficient resource use, particularly in settings with limited diagnostic capacity.

5.2. Access to diagnostics

Despite its inclusion in WHO guidelines and HIV management protocols in over 19 African countries [54], access to CrAg screening remains limited. A 2022 cross-sectional survey across 14 countries found that only 25% of patients had frequent access to CrAg testing, while more than 40% had no access at all [28]. By comparison, access to India ink for meningitis diagnosis was reported at 33.5% and LP at 74.0% [28]. As the HIV epidemic accelerates outside of Africa modeling suggests that most countries outside of Africa do not have national CrAg screening programs with a few exceptions, e.g. Guatemala [2]. In some low resource settings such as in Chile, there is still no access to CrAg at all so screening is not available and India Ink remains the only available diagnostic for cryptococcal meningitis [55].

Resource disparities between tertiary and primary care facilities contribute to these gaps. Tertiary hospitals are better stocked and more likely to have consistent test kit availability, while peripheral facilities often experience stock-outs and limited procurement capacity [56].

Efforts to close this access gap include advocating for CrAg screening as an essential diagnostic strategy by WHO, and integrating CrAg into national HIV guidelines [57] as well as mapping by organizations like GAFFI to identify diagnostic gaps [58,59]. The Global Fund's inclusion of CrAg tests on its procurement list has further strengthened diagnostic availability by enabling bulk purchasing and supply chain support for LICs.

Progress in access is confronting a new barrier in funding. The 2025 announcement of the abrupt termination of most PEPFAR-funded programs has created uncertainty around diagnostic availability. At the time of writing, the American government's contribution to the Global Fund's next replenishment – previously 33% of its funding – is unresolved [60]. Together, these developments present major risks to CrAg screening continuity across high-burden settings.

5.3. Implementation challenges

Beyond supply and policy-level barriers, programmatic implementation faces additional obstacles. Limited awareness of cryptococcal disease including CrAg testing guidelines among healthcare providers can result in delayed diagnosis and care [61–63]. Reflexive CrAg screening offers one solution to reduce provider dependency. In South Africa, residual blood from patients with CD4 < 100 cells/μL is automatically tested for CrAg in the laboratory without requiring clinician initiation. This approach followed a 3-year pilot comparing reflexive versus provider-initiated testing, which showed improved coverage (from 27% to 95%) and greater cost-effectiveness [64–66]. Tanzania transitioned to reflexive laboratory screening after early evaluations showed poor uptake and limited implementation of preemptive antifungal treatment under clinician-initiated models. In rural hospitals, reflexive testing eliminated the excess mortality previously linked to missed cryptococcal disease [67].

Despite improvements, reflexive CrAg screening programs continue to face operational challenges. Only a minority of CrAg-positive individuals are linked to care or initiated on antifungal therapy [68,69]. The reasons for this are multifactorial: healthcare providers not reviewing patients for clinical evidence of meningitis despite a positive serum CrAg, LPs often only being available at secondary care facilities and weak referral systems meaning those recommended for an LP at primary care often fail to have it. A recent review of the South African national CrAg screening program found that as few as 9% of people with a positive serum CrAg who required an LP were confirmed to have had one [66]. The success of the REMSTART trial was partly due to structured follow-up and adherence support, components often lacking in routine implementation. In South Africa, the CAST-NET study found that while reflexive testing achieved >99% CrAg coverage among samples with CD4 < 100 cells/μL, only 32% of CrAg-positive patients received appropriate preemptive fluconazole, and just 39% had documented clinical review for meningitis symptoms [70] suggesting that effective implementation remains a significant barrier to realizing the benefits of CrAg screening.

5.4. Point-of-care testing and quality assurance

As an alternative to centralized reflexive testing, some programs have pursued decentralized, POC CrAg screening using lateral flow assays. Programs in Malawi and Lesotho demonstrated that introducing POC kits at peripheral health centers significantly increased screening coverage and enabled earlier intervention [71,72].

However, POC implementation raises concerns about test fidelity. In South Africa's laboratory-based program, external quality assurance identified only 0.7% of tests as discordant [73]. In contrast, field evaluations of POC use revealed substantial drops in sensitivity – down to 20% in some cases –

when finger-prick samples were applied directly to the test strip without adequate procedural training [26]. These findings underscore the need for robust quality assurance mechanisms. In Malawi, a national 'hub and spoke' model was introduced, assigning nine regional laboratories to oversee the quality of POC testing across the country [74]. Training, procedural standardization, and remote supervision were critical components of this effort.

5.5. Data management and integration

Operational issues are not unique to either reflexive or POC models. In Eswatini, a hybrid system using both approaches encountered significant challenges in data harmonization. Challenges in record linking between laboratory and clinic files hindered patient tracking and continuity of care, complicating program evaluation and weakening real-time monitoring systems [75,76].

CrAg screening has proven effective in reducing CM-related mortality in a clinical trial however, its impact is limited by ongoing barriers in access, provider training, diagnostic quality, and patient follow-up. Strengthening health system capacity, standardizing implementation practices, and ensuring stable funding mechanisms are essential for translating diagnostic access into patient survival gains

6. Diagnosis of cryptococcal meningitis: the importance of lumbar punctures

In people living with HIV, symptoms of meningitis or a positive serum CrAg should prompt consideration of CM. Confirmatory diagnosis requires CSF analysis via an LP. However, multiple barriers can delay or prevent this step. At the patient level, delayed care-seeking, cultural barriers and reliance on informal providers such as traditional healers or over-the-counter remedies are common [77–79]. Patients also reported that they often ignored symptoms such as headaches which meant that they delayed seeking care [80]. At the provider level, limited recognition of CM contributes to misdiagnosis – as demonstrated in a multicentre study where CM was frequently mistaken for influenza or malaria [80]. Even when suspected, provider hesitancy or lack of capacity to perform LPs remains a bottleneck to timely diagnosis. Even in countries such as Chile, where there were no cultural barriers or refusal of LPs in patients, LP uptake was low [55]. This may be an indication of poor implementation of the WHO guidelines in this setting.

In addition to timely diagnosis, LPs are critical for the management of raised intracranial pressure (ICP), a common complication of cryptococcal meningitis. Raised ICP is associated with a high fungal burden in the CSF and poor clinical outcomes. However, effective management of raised ICP in routine practice, especially in low-resource settings, is challenging. Barriers to the effective management of raised ICP in low-resource settings are largely driven by challenges in performing LPs when clinically indicated as well as access to key consumables like spinal manometers. Apart from a few centers, mainly in South Africa, spinal manometers are often unavailable due to perceived challenges with cost and access. In the absence of manometers, intracranial pressure frequently goes unmeasured. Alternative options for assessing CSF pressure include estimating pressure based on the CSF drip rate from the LP needle, or using intravenous (IV) infusion lines attached to the spinal needle as a makeshift alternative [81,82]. Performing LPs, both diagnostic and therapeutic, is a common bottleneck in care leading to the description of 'the tap gap.'

6.1. Lumbar punctures: closing the tap gap

An LP is required to make a definitive diagnosis of CM. This is often only available at higher level health facilities and encounters substantial operational and structural barriers. Health system barriers have been reported in low-resource settings with limited access to LPs. A study in Zambia in both urban and rural hospitals reported that in most of these hospitals, there was a shortage of consumables such as LP kits, sterile needles, and the basics; sterile gloves and iodine, and the laboratories were often outdated [83,84]. The lack of LP kits was also reported in an evaluation study conducted in Nigeria [85]. These gaps in the availability of resources and infrastructure have resulted in hesitation from healthcare workers to refer patients for an LP even when it is prescribed [86]. Their main concerns were related to whether the patient can afford care, whether there are resources to perform the LP and treatment availability in case CM was confirmed. Doctors from Zambia reported being hesitant to refer patients for LPs due to the high cost of both diagnostics and treatment, and treatment shortages [84]. Other provider-related factors include healthcare worker incompetence, fears of performing an LP, and time constraints for already overworked healthcare workers [84,87].

Furthermore, patients and their caregivers (in unconscious patients), presented some barriers to uptake of LPs, in one Tanzanian setting only 24% of people with a positive cryptococcal antigen result consented to an LP [50]. These barriers included limited knowledge on LPs including benefits and myths, mistrust in the system due to death of a community member shortly after an LP was performed, and fear of being stigmatized (LPs being associated with HIV) [83–85]. Elafros [83] and colleagues also reported that caregivers of unconscious patients did not want to be responsible for making decisions and opted to consult family or other community members, and this may result in delayed diagnosis and ultimately death. In ambulatory patients in South Africa it was observed that some patients may opt to sign a refusal of care due to their fears and time constraints from work commitments (unpublished observations).

Ultimately, successful implementation of diagnostics for cryptococcal disease in resource-limited settings requires a combination of decentralized testing, workforce training, robust data management, policy integration, and international funding. By addressing these key areas, programs can increase screening coverage and improve outcomes for patients with cryptococcal disease.

7. Novel diagnostics in cryptococcal disease: a focus for low-resource settings

In recent years, diagnostic innovations have focused on sub-stratifying patients with cryptococcal disease according to their risk of CNS involvement and clinical outcomes. Quantitative serum CrAg titers are a key tool in this approach, as they correlate with both the likelihood of CM and patient prognosis. Although titer thresholds vary between diagnostic platforms, plasma titers <1:80 on the IMMY CrAg assay are associated with a low probability of meningitis, whereas titers ≥1:160 indicate an increased risk [67,88,89]. In a South African study, elevated blood CrAg levels were significantly associated with CM even among asymptomatic individuals. An optimal threshold of >1:160 (sensitivity 88.2%, specificity 82.1%) was identified to detect CM. This threshold enabled detection of the 34% of CM cases that were otherwise asymptomatic [90]. Titers ≥1:1280 are strongly predictive of CNS involvement and associated with high fungal burdens which are a risk factor for raised intracranial pressure in need of therapeutic LPs. The implementation of semi-quantitative CrAg testing as a triage mechanism offers the potential to prioritize LP and intensive antifungal therapy for those at highest risk, thereby reducing diagnostic delays and optimizing resource use. In Uganda, a pilot program integrating this testing into routine HIV care demonstrated that patients with CrAg titers above a defined threshold had a significantly higher incidence of CM, supporting its utility for targeted LP referral [22]. These findings have been reinforced by recent data from the ACACIA trial which showed that high CrAg titers correlate well with the need for LP and enhanced antifungal prophylaxis. Patients with low titers had a low risk of CNS disease, suggesting that stratification may allow safer deferral of invasive procedures in select cases [91]. Semi-quantitative CrAg stratification, coupled with novel therapeutic approaches including more intensive oral treatment regimens for individuals with high antigen titers – represent advances in tailoring cryptococcal disease management to patient risk profiles [92,93]. This approach is being tested in the EFFECT trial [93] as a secondary outcome and is especially relevant in low-resource or remote settings, where access to LPs and inpatient care may be limited. Sub-stratification enables allocation of limited resources toward patients most likely to benefit, while reducing unnecessary procedures and care costs for those at lower risk.

8. Antifungal resistance as an emerging concern

Alongside these novel approaches to stratifying risk, a new concern is emerging: antifungal resistance in *Cryptococcus* species, particularly to fluconazole. Secondary resistance is rare if combination antifungal therapy including amphotericin B and flucytosine is used in the induction phase. However access to these compounds [12,14] is limited in most countries, especially in LICs with a high burden of CM [94,95]. In the absence of combination therapy, fluconazole monotherapy is often the only treatment option, liable to encourage secondary resistance [96]. This was shown in both a study in Tanzania and a surveillance study in South Africa in the era of fluconazole monotherapy where 67% of subsequent relapses demonstrated reduced susceptibility to fluconazole [97]. A systematic review found a mean fluconazole resistance prevalence of 12.1% across all isolates ($N = 4995$) identified between 1988 and 2017, with higher resistance (24.1%) in relapse cases [98] although the data is limited by differences in susceptibility testing methods as well as variable thresholds used to define resistance.

In addition to secondary resistance emerging during treatment, there is a theoretical risk of primary resistance, where resistant *Cryptococcus* strains are acquired directly from the environment. As *Cryptococcus* is an environmental pathogen, exposure to azole or azole-like compounds (for example, agricultural fungicides) could in theory select for resistant strains prior to human infection [99,100]. While this phenomenon is well-documented for *Aspergillus* spp., it has not been clearly demonstrated for *Cryptococcus* in clinical settings, and evidence remains limited. Fluconazole resistance, whether primary or secondary, can result in delayed fungal clearance and is associated with increased incidence of relapse [101,102].

Antifungal susceptibility testing (AST) requires standardized methods, sophisticated lab infrastructure and remains unavailable in most low-resource settings. Interpretation of AST results is also limited because no clinical breakpoints have been developed for *Cryptococcus* species by either the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI). Even if resistance is identified, the consequent need to switch to other treatments is often unachievable in LICs due to high cost and limited availability of second-line antifungals such as itraconazole, voriconazole or posaconazole [94] and difficulty in administering weekly amphotericin B as maintenance treatment. Emerging resistance has been identified as an area of concern in the recent WHO report into invasive fungal disease and developing context-appropriate tools to perform AST will be important as resistance levels increase [90]. In the absence of diagnostics, the most significant intervention will be to ensure access to combined antifungal treatments to avoid the development of secondary resistance likely with fluconazole monotherapy treatment. Diagnostic and treatment approaches will also need to be adopted for the rising risk of antifungal resistance, particularly in relapse cases.

9. Implementing effective diagnostic strategies and clinical care pathways

Complex health systems are increasingly recognized as barriers to diagnosis and effective care [1,103,104]. Part of the continued advocacy from the most recent large clinical trial into CM, the AMBITION-CM trial, has been ongoing implementation of effective diagnosis and treatment through the Single High Dose Ambisome to Reduce Excess Mortality from Cryptococcal Meningitis (SHARE-CM) [105] and AmbiOne projects.

The Driving Reduced AIDS-associated Meningoencephalitis Mortality (DREAMM) study in Cameroon, Malawi, and Tanzania provided a model for integrating effective CNS infection

diagnostic strategies and clinical care pathways and found a significant mortality benefit from effective implementation of CM care in routine care settings [106]. Key components included routine CrAg screening among patients with CD4 counts < 100 cells/μL, rapid LP for CrAg-positive patients, and the use of a short-course amphotericin B-based regimens. Multidisciplinary training to build local capacity in CM management as well as continuous mentorship and feedback to maintain high standards of care were suggested as key components.

However, sustaining these improvements outside of trial settings remains a critical challenge. Many of the supportive structures such as mentorship, supply chains, and training were reliant on research funding and external technical assistance. Achieving long-term sustainability will require integration of these practices into national HIV programs, along with committed domestic and international support [107].

10. Measurement drives action: the importance of reporting on cryptococcal meningitis diagnoses

Effective management of CM in low-resource settings is dependent on accurate and consistent reporting. Measurement drives action and improved reporting on CM diagnoses can enhance programmatic responses and inform targeted interventions [108].

National initiatives across Africa have illustrated the impact of robust surveillance systems. In South Africa, the National Institute for Communicable Diseases (NICD) operates a comprehensive cryptococcal screening program embedded within the national HIV care framework as well as an established long-term CM surveillance system [109]. The screening program includes active reporting of CrAg-positive cases, allowing for dynamic adjustments in service delivery and treatment protocols. Similarly, in Botswana, CM case reporting has been integrated into routine HIV program monitoring, generating epidemiological insights and identifying gaps in patient management [110].

Despite these advances, such models remain rare. A substantial gap persists between the estimated and reported number of CM cases in most African countries. For example, in Guinea, Médecins Sans Frontières (MSF) has been delegated responsibility for the diagnosis and treatment of AHD by the Ministry of Health and remains the sole provider of cryptococcal diagnostics and treatment. In 2024, MSF reported only 40 cases of cryptococcal disease. This is in contrast to modeled estimates, which suggest an annual burden of 500–1,000 cases in the country [1,111].

This discrepancy highlights a surveillance gap. Without reliable data on CM case numbers, national programs are unable to assess service coverage, identify geographic or programmatic gaps, or advocate effectively for resources. Strengthening health information systems – particularly those that integrate laboratory diagnostics, patient-level data, and facility-based reporting – is important for improving case detection and closing the diagnostic gap. Furthermore, greater community engagement and involvement in diagnostic processes may enhance early care-seeking and improve reporting completeness.

11. Community engagement: the involvement of those with lived experiences

Gaps in awareness around CM care are present at the level of healthcare providers, patients and their caregivers and has been implicated in worse outcomes. Community engagement efforts are important for awareness and capacity building [112]. Community engagement and social support efforts in patients, caregivers, and healthcare workers from the Cryptococcal Optimal ART Timing (COAT) trial and Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis (ASTRO-CM) study resulted in improved rates of screening and referrals, adherence to treatment and follow-up visits, acceptance of LPs and data completeness.

Through SHARE-CM, healthcare professionals and HIV advocates, in collaboration with patients from the AMBITION-CM trial, have been working together to raise AHD-associated CM in both patients and the community. This includes multiple workshops that have led to the creation of posters, pamphlets, and videos on the early recognition of CM symptoms, including headaches and nausea, and the importance of LPs.

In South Africa, Johannesburg, an Expert Patient Group (EPG) was convened in 2024 and aims to develop and raise awareness of asymptomatic cryptococcal disease in the context of an HIV diagnosis. These community engagement efforts are in collaboration with the past EFFECT trial participants [93], include dialogue about patients' lived experiences and information sharing from researchers on AHD, asymptomatic cryptococcal disease, and other opportunistic infections. Through these dialogues between researchers and patients, we hope to develop patient-facing materials that encourage health-seeking behavior, including HIV and cryptococcal disease screening and adherence to follow-up visits in languages that the patients and the community will understand.

12. Conclusion

CM continues to represent a significant cause of morbidity and mortality among individuals with advanced HIV disease in low-resource settings. This review highlights persistent gaps in the screening, diagnostic, and treatment pathway, despite the availability of effective tools and WHO-recommended protocols.

CD4 testing, the critical entry point for CrAg screening, has experienced reduced uptake due to shifting HIV programmatic priorities and the withdrawal of key near-patient diagnostic platforms. Newer lateral flow – based CD4 assays offer a promising alternative, though operational and performance challenges remain. CrAg screening, supported by strong evidence of cost-effectiveness and mortality benefit, remains underutilized in many settings. Barriers include limited availability of test kits at peripheral levels, inconsistent implementation of reflexive testing, and gaps in linkage to confirmatory diagnosis and treatment. Decentralized point-of-care strategies and reflex laboratory-based models have improved coverage in select programs, although data quality, treatment

initiation, and patient follow-up remain suboptimal. LP remains the definitive diagnostic tool for CM and essential for managing raised intracranial pressure. However, consistent barriers including limited access to consumables, inadequate provider training, and procedural hesitancy – limit its use in many facilities.

Emerging approaches, including semi-quantitative CrAg testing to guide risk stratification and targeted interventions, offer a potential solution to diagnostic bottlenecks. Robust surveillance systems for cryptococcal disease are lacking across most high-burden countries. Experiences from South Africa and Botswana demonstrate the feasibility and value of integrated CrAg screening and CM reporting systems. However, most programs continue to report far fewer cases than modeled estimates suggest. To reduce mortality from CM, implementation fidelity, decentralized access, surveillance, and integration of novel diagnostics must be prioritized within national HIV care frameworks.

13. Expert opinion

Advances in prevention, diagnosis, and treatment of HIV-associated CM, particularly the use of CrAg LFAs and short-course liposomal amphotericin B regimens, have created opportunities to reduce mortality. However, their impact remains limited by implementation challenges in low-resource settings with regional variations in cryptococcal disease burden and access to treatment and diagnostics. A health systems-level approach is essential to close the prevention, diagnostic, and treatment gap. This includes restoring decentralized CD4 testing to diagnose AHD, expanding reflex and POC CrAg screening for early cryptococcal disease detection, ensuring timely and safe access to LP and antifungal therapy, and embedding community engagement and involvement throughout the continuum of care. The barriers and facilitators of effective care have been highlighted in Table 2.

The clinical pathway for cryptococcal disease is complex, with barriers at the patient, provider, and facility levels. While CrAg screening is a critical gateway to prevention of CM, it must be linked to clear and efficient diagnostic and treatment pathways to ensure effectiveness. For patients who test CrAg-positive, the next critical step is an LP to exclude CNS involvement and guide appropriate treatment. However, LP implementation remains a major bottleneck. Challenges include healthcare provider hesitancy due to inadequate training or misconceptions, lack of sterile kits or manometers, limited patient understanding and consent, and referral delays in facilities lacking capacity for the procedure.

Improving LP access requires coordinated interventions: healthcare worker capacity building, ensuring consistent availability of LP kits and manometers, establishing protocols for prompt LP following a positive CrAg result, and addressing community and patient concerns through education and counseling. When an LP cannot be done on-site, robust referral systems with transport support and clear feedback loops must be in place. Monitoring LP uptake and turnaround times should be part of routine quality improvement.

Screening approaches such as reflexive laboratory-based CrAg testing or POC testing can be adapted based on local capacity, the former more appropriate for settings with a robust laboratory infrastructure and the latter suitable for places with fewer lab resources. Semi-quantitative CrAg testing offers additional value by identifying high-risk patients who most urgently need LP and may benefit from meningitis treatment. Prioritizing these high-risk patients could allow limited resources to be directed efficiently.

Table 2. Summary of barriers and facilitators to effective care.

Category	Barrier	Strategy
CD4 Testing	Decline in CD4 testing due to focus on viral load and withdrawal of key CD4 testing platforms.	Reintroduce cost-effective, point-of-care CD4 tests and develop accessible, quality CD4 tests for decentralized use.
CrAg Screening	**Access Challenges**	
	Limited access to CrAg screening in primary, rural care, and resource disparity between tertiary and primary care.	Expand access through policy inclusion, equip primary care centers, and reduce stock-outs.
	Funding gaps due to changes in international funding and delays in screening initiation.	Secure funding through global partnerships and implement reflexive CrAg testing in labs for better coverage.
	Operational Challenges	
	Low provider awareness, poor patient adherence, and reliance on informal care.	Educate providers, promote health-seeking behavior, and improve follow-up care using models like REMSTART.
	Inaccurate point-of-care testing, data management issues, and barriers in follow-up care.	Improve test accuracy with training, integrate data management, and ensure consistent follow-up care.
Lumbar Puncture	**Access challenges**	
	Lack of LP kits, sterile gloves, and iodine.	Ensure adequate supply of LP kits and consumables.
	Hesitance to refer patients for LP due to costs, treatment availability, and resource constraints.	Provide LP kits, address cost concerns, and ensure treatment availability.
	Operational Challenges	
	Patient and caregiver barriers due to fear or mistrust of the procedure.	Educate patients and caregivers to alleviate fears and improve understanding.
	Hesitancy from healthcare workers due to lack of training and time constraints.	Train healthcare workers and address time and resource pressures.
Diagnostic Strategies	**Effective Implementation**	
	Complex health systems hindering effective diagnostics.	Implement integrated care pathways with multidisciplinary training.
	Sub-stratification of Cryptococcal Disease	
	Lack of targeted diagnostic strategies for high-risk patients.	Use semi-quantitative CrAg testing to identify high-risk patients.
	Limited access to novel therapies in low-resource settings.	Consider novel therapies in low-resource settings for severe cases.
Data Management	Inconsistent reporting of CM cases, leading to under-estimation of the true burden.	Strengthen surveillance and reporting to close the care gap.

Broader health systems support is also crucial. Effective implementation relies on coordinated collaboration between ministries of health, donor funders, implementing partner organizations, and communities. These collaborations should support procurement of affordable diagnostics and consumables, ongoing health system strengthening, healthcare worker training and supervision, community education and involvement, and reliable data collection and monitoring. Cryptococcal disease care should not be delivered in isolation but integrated into AHD care, including ART adherence support, access to CD4 testing, and screening and management of other opportunistic infections such as TB.

Programmatic monitoring and evaluation are vital. Lessons from HIV/TB integration efforts and national cryptococcal disease surveillance in countries such as South Africa and Botswana provide useful models for scale-up. These approaches should be adapted and extended not only to TB and CM but also to other opportunistic infections to reduce AHD-related mortality.

Despite recent gains in funding for cryptococcal disease research, resources for implementation in HIV programs remain limited and are vulnerable to international funding cuts. Sustained progress will depend on coordinated partnerships and national leadership to drive policy implementation and resource mobilization. National programs must ensure that updated guidelines translate into operational improvements at the facility level.

Importantly, effective implementation can be cost-saving. CrAg screening prevents progression to CM, which is more expensive to manage due to hospitalization, costly antifungal regimens, repeated LPs, and associated loss of income for patients and caregivers. In many settings, patients also face direct catastrophic out-of-pocket costs for care. Preventing CM-related mortality and morbidity thus reduces both government expenditure and patient financial burden.

A simple, low-cost, highly sensitive, and specific diagnostic test, the CrAg LFA, has been available for more than a decade. The key antifungal medicines, amphotericin B, flucytosine, and fluconazole, are becoming more widely available and cheaper with generic formulations. The challenge now lies in ensuring access to these diagnostics and treatments with implementation of evidence-based interventions across the cascade of care and robust monitoring. Continued investment in operational research, including health economics and qualitative-methods studies, is critical to drive innovation, inform local adaptations, and overcome remaining barriers in the diagnostic and treatment pathway for CM.

Funding

Authors J. Falconer, T.M. Mmotsa, and N.P Govender are funded by the National Institute for Health Research (NIHR) through a Global Health Research Professorship to NPG [award number NIHR303140] using UK aid from the UK Government to support global health research. The views expressed in this publication are those of the authors and not necessarily those of the NIHR or the UK Department of Health and Social Care.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

Author contributions

The first draft of the manuscript was written by J. Falconer and T.M. Mmotsa, while the critical review and revision of the first draft were conducted by J.N. Jarvis and N.P Govender. All authors approved the final version of the manuscript.

Confirm AI policy

ChatGPT version 4.0 was used for simple grammar and copy-editing review.

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