Point of care tests for invasive fungal infections: a blueprint for increasing availability in Africa

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***Abstract*:** Invasive fungal infections (IFIs) such as cryptococcosis, disseminated histoplasmosis, and chronic pulmonary aspergillosis are significant causes of morbidity and mortality in Africa. Lack of laboratory infrastructure and laboratory personnel trained in diagnostic mycology hamper prompt detection and management of IFIs on the continent. Point-of-care tests (POCT) obviate the need for complex infrastructure, skilled technicians, and stable electricity and have had major impacts on the diagnosis of bacterial, viral, and parasitic infections in low- and middle-income countries. Over the last 10 years, POCTs for IFIs have become increasingly available and they have the potential to revolutionize the management of these infections if scaled up in Africa. At the beginning of 2021, the World Health Organization (WHO) Essential Diagnostic List (EDL) included a cryptococcal antigen test for the diagnosis of cryptococcosis, *Histoplasma* antigen test for the diagnosis of disseminated histoplasmosis, and *Aspergillus*-specific test for the diagnosis of chronic pulmonary aspergillosis. All of these are available in formats that may be used as POCTs and it is hoped that this will improve the diagnosis of these life-threatening IFIs, especially in low- and middle-income countries. This perspective review discusses commercially available POCTs and outlines strategies of a blueprint to achieve their roll-out in Africa. The strategies include raising awareness, conducting research that uncovers the exact burden of IFIs, increasing advocacy, integrating diagnosis of IFIs into existing public health programs, adoption of the WHO EDL at country levels, and improving logistics and supply chains.

Keywords: Africa, aspergillosis, cryptococcosis, histoplasmosis, invasive fungal infections, point-of-care tests

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Introduction

The burden of invasive fungal infections (IFIs) in Africa is substantial, driven mainly by the concomitantly high burdens of tuberculosis (TB), human immunodeficiency virus (HIV) infection, poverty, and the increasing incidence of cancers.[1] Cryptococcal meningitis (CM), for example, is now the leading cause of HIV-related meningitis among adults in sub-Saharan Africa, superseding TB meningitis and classic bacterial meningitis.[2] On the other hand, chronic pulmonary aspergillosis (CPA) complicates 5–10% of treated pulmonary TB and is frequently misdiagnosed as smear-negative TB.[3] Equally frequently misdiagnosed as TB is progressive acute disseminated histoplasmosis – an important opportunistic infection with multi-systemic involvement in advanced HIV disease.[4]

To make a rapid and accurate diagnosis of a fungal disease, a trained clinician must have a high index of suspicion, collect appropriate clinical specimens upon which appropriate tests must be conducted, using the right tools, by skilled laboratory personnel trained in diagnostic mycology. In general, conventional laboratory tests require

complex infrastructure, skilled technicians, stable electricity supply, all of which are in short supply in most low- and middle-income countries (LMICs).[5] Where available, the cost of these tests are prohibitive and they are often inaccessible to patients, many of whom lack health insurance coverage and must pay out-of-pocket.[6] To overcome these diagnostic challenges, in 2001, the Sexually Transmitted Diagnostics Initiative of the World Health Organization (WHO) developed a list of characteristics that make a diagnostic test suitable for LMICs: they should be Affordable, Sensitive, Specific, User-friendly, Rapid and Robust without need for special storage, Equipment-free, and Delivered to those who need it – the ASSURED criteria.[7]

Point-of-care (POC) testing has been defined in various ways in the medical literature.[7–10] All definitions emphasize the fact that POC testing leads to expedited clinical action, a *sine qua non* for improving outcomes in IFIs.[10] Point-of-care tests (POCTs) that consistently meet the WHO ASSURED criteria have become increasingly popular in LMICs. They have demonstrated significant impact on healthcare in these countries with respect to infectious diseases such as HIV, malaria, and TB.[5] Malaria rapid tests were instrumental in raising testing rates in Africa for suspected cases from below 5% in 2000 to 45% in 2010, while cost-effective nucleic acid-based testing for TB increased case detection rate by up to 50% and reduced treatment initiation delays by a factor of 10.[5] Over the last 10 years, POCTs for IFIs have become increasingly available.[11] These diagnostics can revolutionize the care of patients with IFIs in Africa and improve outcomes as has been achieved with the use of POCTs in bacterial, parasitic, and viral diseases. This review aims to highlight commercially available POC diagnostics for IFIs and present a blueprint for promoting uptake in Africa.

POCTs for IFIs

Currently, commercially available POCTs for IFIs are based on immunochromatographic technology (ICT) mostly in a lateral flow assay (LFA) format. They may be designed to detect antigen, e.g., cryptococcal antigen (CrAg), *Histoplasma* antigen, *Aspergillus*-specific antigen, and galactomannan (GM); or they may detect antibodies, e.g., *Aspergillus*-specific immunoglobulins (IgG, IgM, IgE, and IgA) and *Coccidioides immitis* antibodies (IgG and IgM). Potential molecular POCTs, such as the loop-mediated isothermal amplification (LAMP) assay for *Histoplasma capsulatum* and proximal ligation assay for *Aspergillus*, have been developed and evaluated but are not yet commercially available and will not be discussed further.[11]

POCTs targeting antigens

Cryptococcal antigen LFA. CM is the most common clinical manifestation of cryptococcosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. It is responsible for 15% of AIDS-related mortalities, amounting to approximately 181,100 deaths annually.[12] Although increased access to antiretroviral therapy (ART) has reduced the burden of AIDS-associated CM dramatically, the impact is much less in LMICs because of suboptimal access to ART and late presentations with advanced HIV disease.[13] The majority of cases of CM continue to occur in sub-Saharan Africa because, in addition to suboptimal ART access, diagnostic facilities, and access to optimal antifungal medications and intensive hospital-based treatments are limited.[14] To diagnose CM, cerebrospinal fluid (CSF) culture, India ink microscopy, or detection of CrAg is required.[15] Detection of CrAg is the most sensitive diagnostic tool for cryptococcosis with both sensitivity and specificity approaching 100%.[15] For over 35 years, latex agglutination (LA) and enzyme immunoassays (EIA) were the mainstay of CrAg detection, requiring skilled laboratory workers, steady electricity, heat inactivation, cold-chain shipping, and refrigeration of reagents; but this changed with the advent of a lateral flow immunochromatographic assay.[15] The CrAg LFA (IMMY, Norman, OK, USA), which has a shelf-life of 2 years and requires only 10 minutes to run, was the first POCT for IFIs that ticked all the boxes of WHO ASSURED criteria.[16] It has been validated extensively in Africa and many other LMICs.[17–20] The test has been demonstrated to have a limit of detection lower than EIA and LA tests,[17] and was superior to CSF culture, latex agglutination, and India ink microscopy for the diagnosis of CM in a multisite validation study in Uganda and South Africa.[20] Other commercially available products including CryptoPS (Biosynex, Strasbourg, France), Dynamiker (China), and StrongStep (Liming BIO, Nanjing, Jiangsu, China), were later introduced and equally show good performance with CSF (Table 1). However limited

Table 1. Commercially available POCTs for IFIs.

POCT	Target	IFI	Specimen type	Sensitivity, specificity	Manufacturer	Reference
CrAg LFA	Antigen	Cryptococcal meningitis, cryptococcosis	Whole blood, serum, plasma, CSF, urine	99.3%, 99.1% in CSF 100%, 98% in CSF 100%, 91% in CSF	IMMY, Norman, OK, USA StrongStep Liming Bio, Nanjing, Jiangsu, China Dynamiker, Tianjin, China	Boulware *et al.*[20] Mpoza *et al.*[22] Kwizera *et al.*[23]
Histoplasma antigen LFA	Antigen	Progressive disseminated histoplasmosis	Serum, urine	96%, 90% in serum	MiraVista, Indianapolis, IN, USA	Cáceres *et al.*[41]
Aspergillus GM LFA	Antigen	IPA	BAL fluid, serum	89%, 100% in BAL fluid; heme patients 91%, 87% in BAL fluid; Proven IPA heme patients 97%, 98% in serum; heme patients 49%, 95% in serum; heme patients 69%, 62% in BAL fluid; non-neutropenic patients 40%, 95% in BAL fluid; mixed patient cohort	IMMY, Norman, OK, USA	Jenks *et al.*[46] Mercier *et al.*[47] White *et al.*[48] Mercier *et al.*[49] Jenks *et al.*[50] Linder *et al.*[51]
Aspergillus specific LFD	Antigen	IPA	Bronchoalveolar lavage fluid, serum	82%, 87% in BAL fluid; Proven IPA in heme patients 62%, 63% in BAL fluid; non-neutropenic patients	OLM, Newcastle Upon Tyne, United Kingdom	Mercier *et al.*[47] Jenks *et al.*[50]
Aspergillus IgG LFA	Antibody	COA	Serum	88.9%, 96.3%; mixed CPA and ABPA cohort 91.6%, 98%; CPA patients 90.6%, 87.2%; ABPA	LD Bio Diagnostics, Lyon, France	Piarroux *et al.*[53] Hunter *et al.*[55] Hunter *et al.*[56]
Coccidioides antibody	Antibody	Coccidioidomycosis	Serum	31%, 92%	IMMY, Norman, OK, USA	Donovan *et al.*[61]

ABPA, allergic bronchopulmonary aspergillosis; BAL, Bronchoalveolar lavage; CPA, chronic pulmonary aspergillosis; CrAg, Cryptococcal antigen; CSF, cerebrospinal fluid; GM, galactomannan; heme, hematological; IPA, invasive pulmonary aspergillosis; IFI, invasive fungal infection; LFA, Lateral flow assay; LFD, lateral flow device; POCT, point-of-care-test, UK, United Kingdom.

validation data available for these newer products report less stellar performance in terms of specificity when other specimen types like plasma and serum are used.[21–23]

CrAg is detectable in the blood a median of 22 days prior to the onset of meningitis symptoms – a condition described as 'asymptomatic cryptococcal antigenaemia.'[24] This observation has driven widespread screening for and preemptive treatment of cryptococcal disease with widely available fluconazole; screen and treat programmes have significantly reduced the burden of CM in countries where they have been implemented. This screening is efficiently carried out with the LFA. Routine CrAg screening and treatment have been recommended by WHO since 2011 and have now been incorporated into HIV national guidelines in more than two dozen countries, 19 of which are in sub-Saharan Africa.[25,26] In 25–40% of people with asymptomatic cryptococcal antigenaemia, subclinical meningitis is present and the chances of mortality remain high despite preemptive fluconazole therapy.[27] Studies have shown that subclinical meningitis is strongly associated with CrAg titres of >1:160 and a Ugandan study showed increased mortality when titers are greater than or equal to 1:640.[28] These findings led to the development of semi-quantitative LFAs like CrAg SQ (IMMY) and Crypto PS (Biosynex).[29,30] When used, these assays help to stratify individuals in need of a lumbar puncture or more intensive anti-fungal therapy.[27]

Histoplasma *antigen LFA.* Histoplasmosis is a systemic mycosis acquired by inhaling the spores of the dimorphic fungus *Histoplasma capsulatum.*[31] Both varieties of this fungus, *Histoplasma capsulatum* var. capsulatum (Hcc) and *Histoplasma capsulatum* var. duboisii (Hcd), co-exist in sub-Saharan Africa.[4] Due to the preponderance of Hcc in the Ohio and Mississippi River valleys in the United States (US), histoplasmosis has traditionally been designated an endemic mycosis of the North Americas.[32] In recent years, however, the disease has been found to be more widespread in distribution, the truly global nature being made evident by the HIV/AIDS pandemic and the increased use of immunosuppressive agents.[33,34] Progressive disseminated histoplasmosis, caused primarily by Hcc, is a life-threatening illness and AIDS-defining opportunistic infection, included on the WHO stage 4/US Centers for Disease Control (CDC) category C events since 1987.[35] Approximately 100,000 people develop disseminated disease, with mortality rates, if treated, ranging between 30% and 50%, and 100% if not.[35] Disseminated histoplasmosis often resembles and can be misdiagnosed as pulmonary TB.[4] The neglected and hidden nature of the true burden of histoplasmosis in Latin America and Africa is due to lack of expertise and diagnostics.[4,36] Diagnosis was dependent on traditional means including microscopy, culture, and histopathology until the discovery of the *Histoplasma* antigen, which is excreted in the urine in progressive disseminated histoplasmosis.[37] The earliest test to detect *Histoplasm*a antigen was a radioimmunoassay. An enzyme immunoassay format was later developed as an alternative to circumvent hazardous exposure to radioactivity as well as other drawbacks of the former test.[38] While the development of an enzyme immunoassay to detect this antigen greatly improved the prospects of the test, this benefit was largely confined to North America because the test was laboratory developed and not commercially available.[39] The arrival of commercial assays further improved detection but availability and cost-effectiveness remained issues.[39,40] The game changer in the diagnosis of disseminated histoplasmosis globally may be in form of a recently commercialized lateral flow assay for the detection of *Histoplasma* antigen in both serum and urine samples (MiraVista Diagnostics, Indianapolis, IN, USA). This test showed a sensitivity and specificity of 96% and 90% respectively in serum samples and requires only 30 minutes to run.[41] However, testing with serum requires pretreatment with EDTA followed by boiling and centrifugation steps to dissociate immune complexes.[41] This prolongs the time for conducting the test, necessitates the use of additional equipment and may hinder point-of-care use. Consequently, the manufacturers abandoned the LFA for serum and developed one for urine that requires no pre-treatment or additional equipment.[42] With urine, the test showed sensitivity of 94% and specificity of 100%.[42]

GM LFA and Aspergillus *specific antigen LFD.* Invasive pulmonary aspergillosis (IPA) is an opportunistic infection with high mortality rates in patients with neutropenia or immunodeficiency.[43] Recently, it has been recognized as an emerging disease in non-neutropenic patients suffering from influenza or coronavirus disease 2019 (COVID-19).[44] Early diagnosis and prompt institution of treatment improves survival. Data on

IPA in Africa is scant, mainly due to non-availability of diagnostic tests. IPA may be diagnosed by detecting GM (an *Aspergillus* cell-wall polysaccharide released during fungal growth) or *Aspergillus*-specific antigen [an extracellular glycoprotein (mannoprotein) secreted by actively growing *Aspergillus* species] in serum and other body fluids, notably bronchoalveolar lavage (BAL) fluid.[43] POCTs for these two antigens have been commercialized recently: the sona *Aspergillus* galactomannan LFA, which detects GM, and the OLM *Aspergillus*-specific LFD (OLM Diagnostics, Newcastle upon Tyne, UK), an ICT that uses the JF5 monoclonal antibody to detect the *Aspergillus*-specific antigen. Both POCTs have been evaluated using BAL fluid and serum in patients with hematological malignancies and non-neutropenic patient groups alike (Table 1).[45–51] Generally, studies show better performance of the assays in hematological patients.[45] They also report better sensitivity and specificity when BAL fluid is used in any patient group, although one study reported high values of 97% and 98%, respectively, using serum in haematology patients.[48] Some researchers have reported better sensitivity with the *Aspergillus* GM LFA when compared with the *Aspergillus*-specific antigen LFD.[46,47] Automatic or digital read-out devices also improved performance of both assays in a couple of studies.[47] A drawback of the LFA is that it is not entirely equipment-free; thus, it requires vortexing, heating using a heat block and centrifugation of the sample before actual conduction of the test. These pre-analytical processes prolong test turn-around time from just 30 min to close to an hour, in contrast to the 15 min required to perform testing with the LFD.[45] Cross-reactivity with other fungi such as *Scedosporium* spp., *Fusarium* spp., *Saccharomyces cerevisiae*, and *Geotrichum* spp. has also been observed.[45]

POCTs targeting antibodies

Aspergillus *IgG LFA.* CPA, a life-threatening disease complicating other respiratory disorders such as tuberculosis, chronic obstructive pulmonary disease or sarcoidosis, affects at least 3 million people worldwide.[52] Contemporaneously, allergic bronchopulmonary aspergillosis (ABPA), a progressive lung disease caused by allergy to *Aspergillus* antigens affects approximately 4.8 million severe asthmatic patients globally.[53] *Aspergillus* IgG is the cornerstone for the diagnosis of these conditions.[54] However, the currently available anti-*Aspergillus* IgG detection assays are inappropriate for resource-poor laboratory settings, as they are expensive, rely on automated procedures, and require stable electricity.[53] A recently commercialized LFA (LDBio Diagnostics, Lyon, France) requires minimal laboratory equipment and can rapidly detect *Aspergillus* antibodies in less than 30 min. Recent evaluations of the POCT show good performance in CPA, ABPA, as well as mixed patient cohorts (Table 1).[53,55,56]

Coccidioides immitis *antibody LFA.* Coccidioidomycosis, caused by *Coccidioides immitis* and *Coccidioides posadasii*, is an endemic mycosis, also known as valley fever. The causative species are endemic to San Joaquin valley in California, Arizona and other parts of southwestern US and parts of Mexico, and Central and South America.[34] Although cases of coccidioidomycosis outside these locations are often imported, literature suggests the possibility of autochthonous disease in the African continent.[57–59] A veterinary study reported post mortem findings of coccidioidomycosis in chicken pullets in the Jos plateau of Nigeria, and cases have also been reported in patients with no history of travel outside Africa.[57–59] Additionally, a 70-year retrospective review of deep fungal infections diagnosed by histology in Uganda revealed four cases of coccidioidomycosis, although no indication was given about whether these were autochthonous.[60] Based on these reports, it is not out of place for African health personnel to be familiar with laboratory tests for coccidioidomycosis. Diagnosis is typically achieved using various serological means such as enzyme immunoassay, complement fixation, and immunodiffusion. These modalities, however, require expertise and equipment and are also time-consuming, requiring days to get results.[61] A *Coccidioides* antibody LFA (IMMY, Norman, OK, USA), with a test turn-around time of 30 min has been developed and commercialized. Although the assay has good specificity, a single evaluation study showed poor sensitivity of 31% (Table 1) when compared with standard enzyme immunoassay, making it the least promising of currently available POCTs for IFIs.[61]

Blueprint for IFI POCTs availability in Africa

Mere availability of rapid or simple tests does not automatically ensure their adoption or scale-up.[8] Despite the growing number of commercially available and excellently performing POCTs for

invasive fungal diseases (IFDs), these indispensable tools remain largely absent from the diagnostic armamentarium of laboratories across Africa. Barriers to the uptake of POCTs may be myriad, including economic reasons relating to cost; policy-related whereby the evidence base is not strong enough to back up policy recommendations; or even due to lack of awareness on the part of health-providers.[8] A random and informal survey conducted in 2019 to assess the availability and cost of non-culture diagnostics for IFDs in African health institutions showed that the only readily available POCT was the CrAg LFA, with variable cost ranging from $3 to $10 (IIO, unpublished data 2019). Even at that, in many facilities in sub-Saharan Africa, direct visualization using microscopy and India ink staining, which has a sensitivity of 80–85% in AIDS-related CM, is still the only available method to identify *Cryptococcus*[20]; while laboratories in Botswana, Malawi, Zimbabwe, Tanzania, and Uganda had CrAg LFA, the test was unavailable in Ghana and Gambia and available in only 3 of 18 Nigerian tertiary healthcare institutional laboratories (IIO, unpublished data 2019).

The authors present a blueprint comprising strategies for increasing the availability and accessibility of POCTs for IFIs in Africa. These strategies, rather than stand alone, go hand in hand and complement one another in the achievement of the desired goals- early diagnosis of IFIs and improved outcomes for infected patients.

Increasing awareness about IFIs and corresponding POCTs

Poor awareness about IFIs is a fundamental problem in nearly all African countries, and this has been substantiated by a few recent studies. In Nigeria, Oladele and colleagues revealed a low level of awareness about IFIs among resident doctors with only 0.002% having good knowledge.[62] Kwizera et al. from Uganda showed that of nearly 700 cases of histologically diagnosed IFIs, about 70% were clinically misdiagnosed.[60] Focused group discussions during a training programme conducted to improve health-care provider awareness and clinical skills in the management and prevention of CM revealed that many health care personnel were unaware that a test as simple as the CrAg LFA existed.[63] Bridging these knowledge gaps about IFIs and the corresponding POCTs will increase the demand for testing – a step towards making them readily available. This could be through continuous medical education, conferences, and by revamping undergraduate and post graduate curricula to emphasize medical mycology and also encourage student projects and theses in the area of fungal diseases.[63,64]

Research

Whilst there is some data on mucocutaneous fungal diseases, there is a relative paucity of epidemiological data on IFIs in Africa. It is noteworthy that CM, probably the most researched IFI on the continent, is also the disease whose POCT is most readily available. Even at that, there is a geographical disparity in data with the literature on CM in West Africa being sparse relative to East and Southern Africa.[65] Additionally, there are limited incidence cohort data on histoplasmosis despite the burden of HIV disease in sub-Saharan Africa, published studies on CPA are limited to two landmark papers and no data exist on the prevalence of IPA.[3,4,66] The scarcity of published research further compounds the knowledge gap contributing to low awareness. To raise both awareness and demand for fungal diagnostics in general, and POCTs specifically, well designed studies are needed to better define the burden, distribution, mortality, and socioeconomic consequences of IFIs on the continent. In addition, existing POCTs require rigorous evaluation studies relating to diagnostic accuracy, clinical impact, and cost in African settings. This will enable prudent decisions to be made concerning the adoption of these novel technologies.[11]

Integrating the diagnosis of IFDs into existing vertical disease programmes

IFIs are notorious for piggy-backing, often occurring against a backdrop of other debilitating diseases. For instance, CM and disseminated histoplasmosis affect people living with HIV, while CPA occurs in lungs already damaged by chronic lung disease, most especially pulmonary TB. Since greater public health priority is placed on HIV/AIDS and TB detection and control as well as antimicrobial resistance (AMR), Cole *et al.* have suggested a mainstreaming approach whereby the control of fungal diseases is integrated into existing health programmes for priority diseases and public health concerns.[67] POCTs for HIV-associated opportunistic mycoses should

thus be integrated within care and treatment programmes for HIV infection, tests for CPA integrated into TB or chronic respiratory disease programmes, while tests for IPA should be embedded in AMR and antimicrobial stewardship programmes.[67] The incorporation of IFI diagnostics into the respective priority areas will enable the achievement of set targets while concomitantly addressing the need for prompt identification and treatment of IFIs. For instance, efforts to reduce deaths from fungal diseases complicating HIV infection could reduce AIDS deaths by more than 30%: using recent global estimates of HIV-infected patients, annual AIDS deaths, existing data on incident rates of HIV related opportunistic infections and data on survival from these infections if diagnosed and treated, Denning projected that annual deaths for cryptococcal disease could fall by 70,000 and those for disseminated histoplasmosis by 48,000, with approximately 60% coverage of diagnostics and antifungal agents amounting to a total of >1,000,000 lives saved over 5 years.[68] *Aspergillus*-specific IgG LFAs if integrated will contribute to anti-pulmonary TB medication stewardship, reducing the unnecessary prescription of toxic antibiotics and possibly controlling development of antibiotic-resistant PTB.[63] Similarly, accurate diagnosis or exclusion of fungal infection such as invasive aspergillosis will have a substantial effect on antimicrobial drug usage and on the ability to limit AMR to bacteria, which is a major public health concern and threat to modern medicine.[69]

Country adoption of the WHO EDL

The WHO first released an EDL in 2018.[70] This list is a catalogue of tests needed to diagnose the most common conditions worldwide and diseases of global importance in both primary care and advanced settings. From just the CrAg assay in the first edition, rigorous and sustained efforts by the Global Action Fund for Fungal Infections (GAFFI) have led to a gradual increase in the number of fungal diagnostics included in the EDL to four tests in the latest edition released on 29 January 2021.[35,71,72] A few studies that assessed the availability of diagnostics in healthcare facilities in Africa against the EDL as a benchmark showed that many tests on the list are not available.[73,74] The EDL will translate to real access to the tests contained only when country governments adopt it, adapt it to the prevailing local disease prevalence, and implement it.[75] To date, only India has developed a national EDL, while a few other countries such as Nigeria, Bangladesh, and Pakistan have begun work with WHO to develop their national EDLs.[76] It is imperative that more African countries follow suit.

Advocacy

Advocacy is the act of taking a position on an issue and initiating actions in a deliberate attempt to influence private and public policy choices.[77] Pushing the agenda for the recognition of IFIs as a global health issue has taken tremendous advocacy efforts from bodies such as GAFFI and International Society for Human and Animal Mycology (ISHAM). For instance, GAFFI, which aims at universal access to essential diagnostics for serious fungal diseases regardless of income level by 2025,[78] in collaboration with an international team of experts strongly recommended CrAg, *Histoplasma* antigen, *Aspergillus* IgG, fungal culture, and direct microscopy/histopathology as essential tests in all laboratories in LMICs.[71] All these tests are now listed on the WHO EDL.[62] Similar moves must now be made at the continental and national levels to impress it upon the medical community and governments to adopt these diagnostics and make them available. Organizational structures for advocacy have gained ground in Africa, with the formation of medical mycology societies and a pan-African mycology working group.[79,80] While the WHO EDL and the GAFFI-enabled country burden estimates of serious fungal infections will come in handy as veritable tools in efforts at advocacy, these non-governmental organizations must fashion indigenous tools such as context-specific clinical guidelines that incorporate these POCTs.[81] They must begin cross fertilizing ideas with relevant professional stakeholders in the respiratory disease, infectious disease, and the HIV/AIDs treatment communities. Advocacy is also required in the areas of obtaining funding for research, raising awareness, and compelling government action.

Improve POC diagnostics logistics and supply chains

Most CrAg assays have been validated in Africa, given the high burden of CM on the continent. However, at the moment, none of the POCTs for cryptococcosis or any of the other IFIs are produced locally. It is invariable that costs will increase dramatically with the multilayered

distribution networks that are necessary for a manufacturer in a high-income country to reach the end users in countries where they have no presence.[39] In this situation, each additional intermediary level will amplify costs, whatever the manufacturers' initial cost. As a result, the tests remain relatively expensive to the population that requires it the most. One strategy that would potentially reduce the cost for end users is for manufacturers to set up production hubs in Africa. Another strategy is for manufacturers to target reference laboratories as this makes distribution easier and minimizes unnecessary cost hikes.

Limitations of the use of POCTs for diagnosing IFIs

Despite the undeniable advantages for LMICs, POCTs as a mainstay for diagnosis of IFIs has its drawbacks and limitations. While the use of these tests does not require laboratory personnel, performance may be linked to the experience of the operator and this may influence accuracy.[82] False negative tests could occur in the setting of high fungal burden leading to soluble immune complexes and lack of required agglutination reaction – the well-described prozone phenomenon.[83] Sole reliance on POCTs precludes obtaining isolates from culture, which may be useful for sensitivity testing and the detection of drug resistant isolates. Finally, users of POCT should be fully aware of the limitations of these tests and take into account the clinical setting and patient characteristics as these may affect performance.[82] For example, *Aspergillus* antigen is better detected in serum of hematological patients and may be falsely negative in non-neutropaenic patients.

Conclusions

Accurate, reliable, rapid and affordable POCTs for the diagnosis of IFIs have become popular in the last decade. They have the potential to revolutionize the management of IFIs in sub-Saharan Africa and other low resource settings, thereby improving prognosis and reducing mortality. However, these POCTs continue to be in short supply. Increasing awareness about fungal diseases through training, research, and concerted advocacy is required to encourage buy-in of clinicians and governments alike. This will improve demand and encourage manufacturers of the tests to optimize supply chains thereby reducing costs. The presence of most of these tests on the current WHO EDL will likely improve availability and accessibility, but governments have to adopt and adapt this list to local needs. Meanwhile, existing disease programmes for HIV/AIDS and TB present an opportunity for the seamless incorporation of POCT for IFIs into national testing algorithms since diseases like cryptococcosis, histoplasmosis, and CPA are notorious for piggybacking. Executing this blueprint requires all hands to be on deck.

Author contributions

IIO and FB were jointly responsible for conception, IIO performed literature search and initial manuscript drafting. IIO and FB made critical revisions for intellectual content and approved the final draft.

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