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EDITORIAL

Essential in vitro diagnostics for advanced HIV and serious fungal diseases: international experts' consensus recommendations

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Perspective

An accurate, timely diagnosis is the cornerstone of good medical practice. For opportunistic fungal infections in AIDS and other invasive mycoses, this is dependent on the availability of and accessibility to the relevant diagnostic tests. A call for a model List of Essential In Vitro Diagnostics (EDL)—"listed tests that should be reasonably available for people who need them, whether in the form of point-of-care tests in physicians' offices and pharmacies or as high-complexity tests in reference laboratories"—has been published [1]. In addition to better medical practice, other potential benefits of an EDL include clarification of priorities for policy makers, setting common goals for laboratory testing, improved healthcare delivery and overall better patient outcomes [1]. In the context of extensive antimicrobial resistance (AMR), a reduction in empiricism with more accurate diagnosis will play a key role in AMR control [2].

Globally, 37 million people are living with HIV [3], over a third of whom present or return to care after treatment interruption with advanced HIV disease defined as a CD4 cell count < 200 cells/mm^3 or a World Health Organization

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(WHO) clinical stage 3 or 4 event [4, 5]. The commonest causes of death in advanced AIDS are tuberculosis (TB), cryptococcosis, *Pneumocystis* pneumonia, bacterial pneumonia or sepsis, disseminated histoplasmosis (some regions), and cerebral toxoplasmosis [5]. There is a very close link between advanced HIV disease and serious fungal infections and TB [5, 6], and the greatest burden of these diseases is seen in low- and middle-income countries (LMICs). Annually, about 1 million people die of AIDS-related illnesses [3] and beyond HIV in total over 1.5 million people die from serious fungal infections [7]. This is comparable with the 1.7 million TB-related deaths in total [8]. Simple, fast, accurate and stable diagnostic tests are essential to improvement in targeted treatment and hence reducing deaths and suffering related to these diseases [9, 10]. Modelling by the Global Action Fund for Fungal Infections (GAFFI) suggests that making the key diagnostic tests for serious fungal diseases available for only 60% of patients in need, with treatments, could save over a million lives in the next 5 years [6], on top of the likely survival benefits of the ambitious 90-90-90 target, The 90-90-90 target is that by 2020, 90% of all people living with HIV will know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy (ART), and 90% of all people receiving ART will have viral suppression.

Led by GAFFI, which is focussed on ensuring universal access to diagnostics for serious fungal infections by 2025 [11], a workshop on essential diagnostics for serious fungal diseases, TB and other opportunistic infections in advanced HIV disease was convened in Kampala, Uganda, between 10th and 12th April 2018. The meeting was timed to precede and help inform the WHO's Strategic Advisory Group of Experts on In Vitro Diagnostics (SAGE-IVD) meeting from which the new WHO EDL emanated [12]. The overall aim of the workshop was to gather experts and review evidence on available diagnostics for HIV/AIDS-associated opportunistic infections and serious fungal diseases for inclusion in the WHO EDL, the first version of which has now been published [12]. The specific objectives of the meeting were, firstly, to review the evidence base for key tests to produce a consensus for recommendation at different strata of health care facility level (Tier) for inclusion in the WHO EDL. Secondly, the meeting aimed to provide the specific arguments and diagnostic performance criteria for the EDL applications to the WHO and thirdly to publish a summary report on the forum, highlighting strengths, weaknesses (gaps), opportunities and challenges with the commercially available diagnostic test portfolios.

Ninety-five participants including experienced clinicians, senior laboratory staff and public health practitioners contributed to the meeting. Participants were drawn from 27 countries, mainly LMICs, including Brazil, Cameroon, Egypt, Ethiopia, France, French Guiana, India, Ireland, Japan, Kenya, Malawi, Mozambique, Nigeria, Pakistan, Portugal, Senegal, Slovenia, South Africa, South Sudan, Spain, Swaziland, Tanzania, Uganda, Ukraine, the UK, the USA and Zambia. Contributors included representatives from WHO, UNITAID, Medecins Sans Frontieres, African Society for Laboratory Medicine, Clinton Health Access Initiative, Medical Access, WHO Collaborating Centers for Reference and Research on Fungi of Medical Importance/Antimicrobial Resistance (India/South Africa/USA), ministries of health, national reference laboratories, US Centers for Disease Control and Prevention, research institutes and both diagnostic and pharmaceutical companies with an interest in HIV/AIDS and/or fungal diseases.

The meeting included workshops on pre-selected key non-culture diagnostics, e.g. TB urinary antigen (LAM), cryptococcal antigen (CrAg), *Histoplasma* antigen, *Aspergillus* IgG, *Pneumocystis* PCR and *Toxoplasma* IgG/IgM and one on antifungal therapeutic monitoring. Shorter summaries of the clinical value of another 6 diagnostics, mainly, culture-based methods and other biomarker assays were also presented in the context of LMICs. The main question for each diagnostic method was—"is it essential?". Each presentation by an expert in the topic area reviewed the available evidence on diagnostic performance of each test, their ease of use and cost-effectiveness. The strength of recommendation was derived from the diagnostic performance, clinical value and suitability

Table 1 Categorisation of recommendation for non-culture based tests

Recommendation	Diagnostic performance	Clinical value	Suitability for LMICs
Very strong	Excellent (> 95% sensitivity/specificity)	Immediately life-saving	Yes
Strong	Excellent or good (> 90% sensitivity and > 90% specificity)	A critical diagnosis which changes treatment	Yes
Moderate	Excellent, good or quite good (> 80% sensitivity and > 80% specificity)	Allows specific therapy to be started or stopped, reducing diagnostic uncertainty	Partial
Low	Inadequate (sensitivity < 80% and specificity < 80%)	Allows specific therapy to be started or stopped, reducing diagnostic uncertainty	Not suitable
None	Test complex, variable performance, difficult to interpret or lack of specificity	Lack of survival benefit or clinical utility or more studies required in LMICs	Not suitable

Table 2 Consensus recommendation by experts on the essentiality of the different diagnostic modalities in low- and middle-income countries

Diagnostic assay	Recommendation	Setting	Comments
Cryptococcal antigen lateral flow assay	Very strong*	LMICs, AIDS-related cryptococcal meningitis	Four commercial assays[#], simple, fast (0–15 min), performance not equal between assays. Older CrAg latex agglutination is inferior to the FDA-approved CrAg LFA
Histoplasma antigen	Very strong	Areas of high incidence/prevalence, disseminated histoplasmosis in AIDS, otherwise as reference test	Urine or serum (1 ELISA)—significant improvement in diagnostic sensitivity, major regional variations in prevalence
Toxoplasma IgG	Strong Moderate	MICs with high toxoplasma prevalence LICs	5 good commercial assays, including one LFA and 3 ELISAs and 1 agglutination assay (laboratory based), LFA (20 min) could be bedside (detection of both IgG and IgM on whole blood). Allow stopping high-dose cotrimoxazole treatment if negative (reduce unnecessary toxicity and resistance development) and avoid misdiagnosis with TB or cryptococcosis. Could also prevent congenital toxoplasmosis in pregnant women. More data required on LMIC performance in AIDS and operational value
Aspergillus IgG	Strong	TB services in LMICs	7 commercial assays, automated assays (high quality/reference laboratory), ELISA moderate complexity
Cryptococcal antigen quantification/ titres	Strong	LMICs	4 tests commercially available—laboratory test, alternative to LFA LFA titres are not equivalent between manufacturers
Pneumocystis pneumonia PCR	Moderate	MICs only and not recommended in LICs	~ 8 commercial assays, superior to microscopy—laboratory with regular electricity, moderate complexity, appropriate for MICs and reference/research labs in LICs. Issue regarding result interpretation (colonization or PCP?)
Antifungal drug monitoring	Strong	MICs only and not recommended in LICs	Bioassay in LMICs. For the life-saving, essential medicines itraconazole, voriconazole and flucytosine, to ensure adequate levels in the blood, often a problem in neonates and patients with renal dysfunction (flucytosine), long-term therapy for aspergillosis (itraconazole), or in children, following dose changes, after a shift from intravenous to oral treatment or following a change in the patient's clinical condition (voriconazole)
TB LAM antigen	Very strong*	LMICs	1 assay commercially available—simple (20 min), probably best used at the bedside or community; turnaround time of < 2 h
Direct microscopy/ histopathology	Strong*	All laboratories of LMICs	Low cost, good turnaround time, variable sensitivity
1,3 Beta-D-glucan	None	–	Test complexity high, more studies required for opportunistic infections in advanced HIV and children with PCP, not able to identify the fungus
Blood culture	Strong*	All laboratories of LMICs	Bacterial, mycobacterial and fungal sepsis, allows optimal therapy, susceptibility testing and infection control, cost a major issue
Bacterial culture	Strong*	All laboratories of LMICs	Bacterial and mycobacterial infection
Fungal culture	Strong*	All laboratories of LMICs	Invasive fungal infections, scope of in vitro susceptibility testing, identify the agent helping in antifungal choice
Galactomannan	None	–	Test complex, more studies required in HIV and LMICs. Usually represents aspergillosis, but not able to identify the fungus Lateral flow assay now available

LMICs low- and middle-income countries, *MICs* middle-income countries, *LICs* low-income countries, *ELISA* enzyme-linked immunosorbent assays, *LFA* lateral flow assays, *PCR* polymerase chain reaction, *TB* tuberculosis, *LAM* lipoarabinomannan, *PCP Pneumocystis* pneumonia

*Included on the 2018 WHO List of Essential In Vitro Diagnostic. [#] 1 FDA approved and 2 CE marked

of the selected tests for LMICS (Table 1). Panel discussions then lead to a consensus recommendation for each diagnostic test (Table 2). For more broadly based tests such as direct microscopy, blood culture, and fungal culture, the meeting concluded that such tests were essential, although these were not reviewed in a systematic manner.

This workshop represents the first time that a consensus meeting to define essential diagnostics for advanced HIV and serious fungal diseases has been held. If implemented and made available in each country, these diagnostic tests will directly improve patient care and public health. TB LAM and CrAg tests both in the context of advanced HIV disease and blood culture for the diagnosis of bacterial and fungal bloodstream infections have been listed as general IVDs for health care facilities with clinical laboratories (Tier II) and direct microscopy for primary health care (Tier I) [12].

With the current knowledge that the WHO EDL will be expanded and be updated annually, several tests endorsed at the meeting will be the subject of future applications for inclusion on the EDL for LMICs. The highest priority tests for inclusion are *Histoplasma* antigen detection and therapeutic drug monitoring of itraconazole and voriconazole. More data are needed from LMICs on both *Aspergillus* IgG and toxoplasma serology performance and interpretation. *Pneumocystis* PCR is routine in Europe and has replaced microscopy, and the meeting endorsed its incorporation into the EDL for MICs but not LICs because of the need for frozen shipping, laboratory complexity, and reliable electricity. A simpler test would negate these difficulties. We also advocate for CrAg LFA to be added on the Tier I list to enable screening. Optimal usage of these tests will require CD4 counts (which have been included on the EDL), notably TB LAM (best performance in those with < 100 CD4 cells), and CrAg and *Histoplasma* antigen (best performance in those with < 200 CD4 counts).

Beyond advanced HIV disease, serious fungal infections, severe bacterial sepsis, TB and toxoplasmosis also occur in patients with cancers or who are on cancer treatment, transplant recipients, those critically ill and many other patients, so availability of diagnostics will be of universal benefit to humankind.

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