BY 2025, ensure that **95%** of people with serious fungal disease are diagnosed and **95%** treated. **95-95**

GLOBAL FUNGAL INFECTION FORUM III
ESSENTIAL DIAGNOSTICS FOR ADVANCED HIV DISEASE AND SERIOUS FUNGAL INFECTIONS

Kampala Uganda / 10-12th April 2018

95/95 BY 2025

gaffi GLOBAL ACTION FUND FOR FUNGAL INFECTIONS

GAFFI has now held 3 successful Global Fungal Infection Forums

Defining the future of fungal disease management, together.

GFIF 1
(Seattle, February 2015), 10 year GAFFI Roadmap, which resulted in the 95/95 by 2025 logo and plan: 95% of the world's population having access to fungal disease diagnostics and antifungals by 2025.
https://www.gaffi.org/global-fungal-infection-forum/about-global-fungal-infection-forum/

GFIF 2
(Liverpool, October 2016), consensus definition of chronic pulmonary aspergillosis for low and middle income countries.
https://www.gaffi.org/global-fungal-infection-forum-2-in-liverpool/

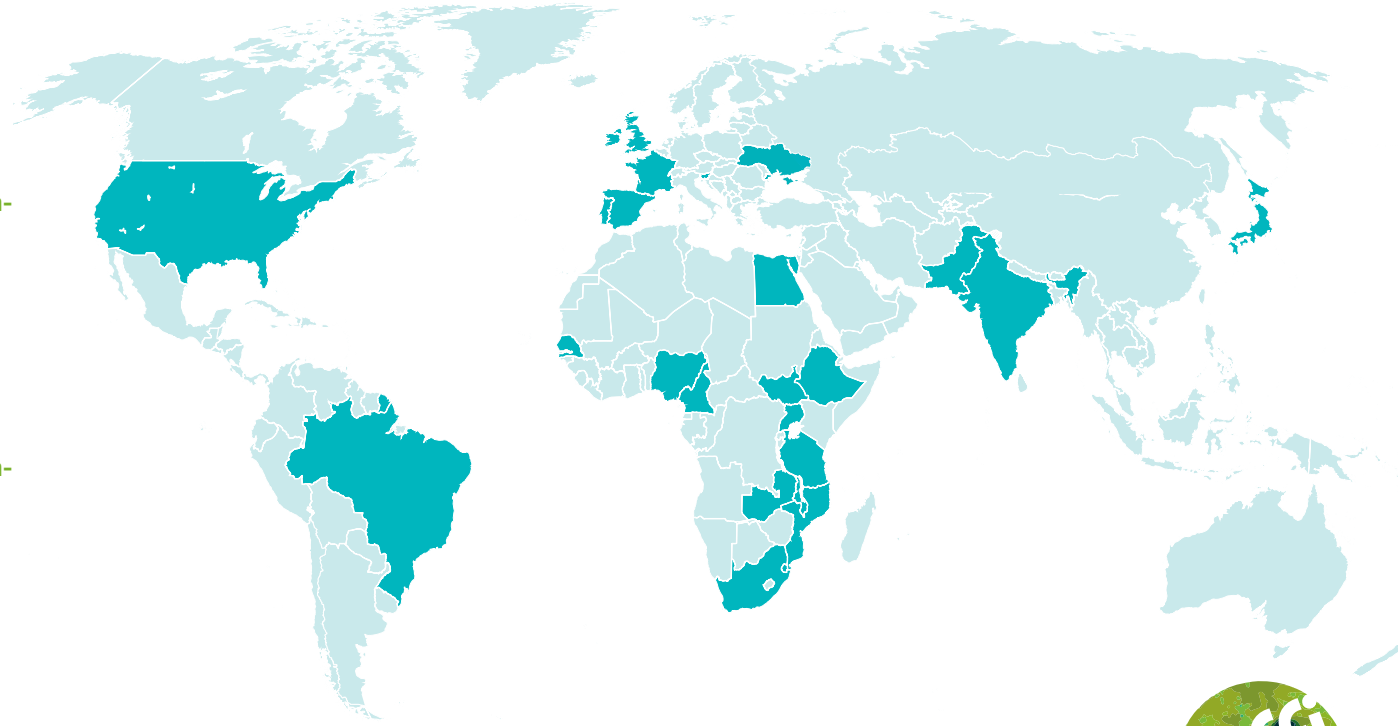
GFIF 3
(Kampala, April 2018), developing consensus on which diagnostics for AIDS and serious fungal diseases should be included on the WHO Essential Diagnostic List.
https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

GAFFI has Ambassadors in 50 countries.
https://www.gaffi.org/who/our-ambassadors/

The burden of serious fungal disease has been estimated in >75 countries so far, covering >80% of the global population.
https://www.gaffi.org/media/academic-papers/

Attendees in Kampala came from the following countries
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, United Kingdom, USA and Zambia.

The participants at the meeting are listed at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/



List of abbreviations

AIDS Acquired immunodeficiency syndrome
IFI Invasive fungal infection
ART Antiretroviral therapy
BAL Bronchoalveolar lavage
BG Beta -1,3-D-glucan
CDC Centers for Disease Control and Prevention, Atlanta, GA, USA
COPD Chronic obstructive pulmonary disease
CPA Chronic pulmonary aspergillosis
CrAg Cryptococcal antigen tests
CSF Cerebrospinal fluid
EDL Model List of Essential In Vitro Diagnostics
EIA Enzyme immunoassay
ELISA Enzyme linked immunosorbent assay
EQA External Quality Assurance
GAFFI Global Action Fund for Fungal Infections
GM Galactomannan
HIV Human immunodeficiency virus
HHV Human herpes virus
HPLC High performance liquid chromatography
HPV Human papilloma virus
IA Invasive aspergillosis
IRIS Immune inflammatory response syndrome
JCV John Cunningham virus
LAM Lipoarabinomannan
LFA Lateral flow assay
LFD Lateral flow device
LICs Low income countries
LIMS Laboratory information management systems
LMICs Low and middle income countries
MAC *Mycobacteria avium* complex
MAI *Mycobacteria avium-intracellulare* complex
MALDI-ToF Matrix assisted laser desorption ionization-time of flight mass spectrometry
MICs Middle income countries
NPV Negative predictive value
OD Optical density
PCP *Pneumocystis* pneumonia (see PJP)
PCR Polymerase chain reaction
PJP *Pneumocystis jirovecii* pneumonia (see PCP)
PLWHIV people living HIV infection
SSA sub-Saharan Africa
TB Tuberculosis
TDM Therapeutic drug monitoring
USA United States of America
WHO World Health Organisation

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Executive summary

Rapid and accurate diagnosis of disease is key to excellent healthcare.

The last 30 years of the HIV pandemic has taught us that the only means of saving these lives is with high quality, rapid testing, coupled with timely treatment initiation. However, it's alarming that 1 in 3 of people living with HIV still present to care with advanced disease and many die from opportunistic infections. People with advanced HIV infection and others with life-threatening fungal disease look deceptively well even when seriously ill. Yet when life-threatening infection is treated early and appropriately it is curable, and these, usually young people can go on and live normal lives.

In Kampala, 95 experts in HIV disease, fungal disease, public health and laboratory medicine convened to develop consensus on which tests should be deemed 'Essential', with a primary focus on low and middle income countries, and non-culture based tests. Key considerations were diagnostic test performance, simplicity of use and clinical impact.

Very strong recommendations for inclusion onto the EDL included the:

- Cryptococcal antigen lateral flow assay to screen for and diagnose cryptococcal meningitis on whole blood, serum and cerebrospinal fluid
- *Mycobacterium tuberculosis* antigen (lipoarabinomannan) in urine to diagnose disseminated TB in AIDS in those with CD4 counts under 100 cells per µl
- *Histoplasma* antigen in serum or urine to diagnose disseminated histoplasmosis in AIDS in countries where the disease is common

The first 2 of these were included in the first WHO EDL issued May 2018, along with direct microscopy and histopathology, blood culture and bacterial, mycobacterial and fungal cultures.

Strong recommendations for EDL inclusion were for:

- *Aspergillus* IgG detection to diagnose chronic pulmonary aspergillosis which is often misdiagnosed as TB
- Cryptococcal antigen quantification (and as an alternative to a lateral flow assay) to determine the likelihood of meningitis based on antigen amount in serum (titre of >1:160)
- *Pneumocystis* PCR (strong recommendation for middle income countries and reference laboratories in low income countries).
- Therapeutic monitoring of itraconazole and voriconazole using bioassay to ensure enough antifungal is present for efficacy and not in excess causing toxicity. Low levels increase the risk of antifungal resistance

These tests and *Histoplasma* antigen will be the subject of future applications to the WHO for inclusion onto the EDL.

Strong/Moderate recommendation for EDL inclusion:

Included *Toxoplasma* IgG serology (moderate recommendation to allow discontinuation of toxic empirical therapy of brain disease, but a strong recommendation in middle income countries with a high sero-prevalence.

Not recommended for EDL inclusion

Beta-1,3-D-glucan (not recommended because of test complexity, likelihood of false positives from blood tubes and a lack of data in HIV associated infection) and galactomannan (not recommended as value outside neutropenic leukaemia patients (serum) and bronchoscopy samples not clear).

Need for country adoption to improve patient survival

Adoption of diagnostic tests included on the WHO EDL by hospitals, states and countries is the next critical step to realize the benefits of inclusion for patients. In addition to advanced HIV disease, patients with severe lung disease, those having cancer treatment, organ transplant recipients and many other patients develop serious fungal infections, so easily available diagnostics will be of universal benefit to humankind.

GAFFI convened experts to develop consensus and initiate the application process for high performance tests to diagnose life-threatening fungal infections.

General research needs

During the meeting the lack of data in many settings became apparent. Presenters were asked to identify major research priorities for their topic. Many research needs are generic. These are summarised here:

1. Direct comparisons of commercially available tests to evaluate relative diagnostic performance.
2. Operational research evaluation of overall utility of different approaches of integration of point of care tests into clinical care settings. Such evaluations would include the sequence of testing (i.e. CD4 count before TB LAM or CrAg or not), how best to document positive and negative results, and the personnel effort to do the tests in clinic or in the laboratory, and the implications (explanation to patients, immediate or delayed admission to hospital, survival etc.).
3. Documentation of country and locality frequency of endemic fungal disease, notably histoplasmosis in most of Africa and *Pneumocystis* pneumonia, as incidence appears to be quite variable.
4. The frequency in different centres and countries of dual and triple infections, to inform testing for multiple infections.
5. The clinical and economic value of tests with high negative predictive values which allow potentially damaging therapy to be stopped, notably anti-tuberculous therapy, corticosteroids for *Pneumocystis* pneumonia and high dose cotrimoxazole for *Pneumocystis* pneumonia and cerebral toxoplasmosis.
6. Feasibility and clinical value of combined HIV and opportunistic fungal infection point of care diagnostics e.g. a combined HIV and CrAg LFD.
7. Comparison of visual and machine interpretations of ‘point of care tests’, especially faint lines and negative results in a patient with high clinical suspicion of an opportunistic infection.
8. Cost-effectiveness studies are required for most tests in different medical settings, recognizing that surviving patients always consume more resource than patients who die.

Major research needs for individual tests are described in each section.

Session	Diagnostic method	Recommendation	Setting	Comments
Breakout session **One**	Cryptococcal antigen lateral flow assay	Very strong $	LMICs, AIDS-related cryptococcal meningitis	See page **14**
	Histoplasma antigen	Very strong	Ares of high incidence/prevalence, disseminated histoplasmosis in AIDS, otherwise as reference test	See page **25**
	Toxoplasma IgG	Strong Moderate	MICs with high sero-prevalence LMICs	See page **35**
	Aspergillus IgG	Strong	TB services in LMICs	See page **33**
Breakout session **Two**	Cryptococcal antigen quantification/titres	Strong	LMICs	See page **15**
	Pneumocystis pneumonia PCR	Strong	MICs and reference laboratories in LICs	See page **39**
	Antifungal drug monitoring	Strong	MICs only and not recommended in LICs	See page **41**
	TB LAM antigen	Very strong $	LMICs	See page **21**
Expert presentations and discussions	Direct microscopy/histopathology	Strong $	All laboratories of LMICs	See page **43**
	Beta-D-Glucan	None	-	See page **31**
	Blood cultures	Strong $	All laboratories of LMICs	See page **43**
	Bacterial cultures	Strong $	All laboratories of LMICs	See page **43**
	Fungal cultures	Strong $	All laboratories of LMICs	See page **43**
	Galactomannan	None/strong	In limited circumstances in MICs only	See page **29**

Table 1. Recommendation by experts on the essentiality of the different diagnostic modalities in low and middle income countries.
$ included on the 2018 WHO List of Essential In Vitro Diagnostic. Abbreviations - See P4.

Introduction

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) remains one of the world's most important public health challenges, particularly in low and middle-income countries (LMICs).

Late presentation to care, re-presenting to health facilities with advanced HIV after a period of antiretroviral therapy (ART) interruption, use of an ART regimen that is no longer effective due to development of drug resistance, and non-availability of regular viral load testing to optimize treatment regimens is responsible for the high mortality rates in LMICs (Nimkar et al. 2018; Frigati et al. 2018). Globally, about 36.7 [30.8-42.9] million people were estimated to be living with HIV in 2016 (UNAIDS 2017). Adolescents and young adults disproportionately constitute a growing share of new HIV infections and of people living with HIV (PLWHIV) (UNAIDS 2015; Frigati et al. 2018).

Despite ART scale-up, recent data show that a considerable proportion of HIV-infected, treatment experienced patients are admitted or enter into care with advanced diseases (defined as CD4 <200 cells/mm3 or a World Health Organization (WHO) stage 3/4 illness and all children <5 years), usually with very low CD4 counts from not being in continuous care or having failed first-line ART with poor virologic suppression (Ousley et al. 2018; Carmona et al. 2018; Osler et al. 2018). Too many people still die of AIDS – an estimated 1,000,000 annually and nearly half are attributable to fungal diseases. Fungal diseases are also an all too common terminal event in patients with cancer, in critical care and those with serious lung diseases, with another 1,000,000 or more deaths annually (**Table 2**).

Over 35 million [29 to 42 million] people are estimated to have died from AIDS-related illnesses since the start of the epidemic. However, with expanding access to ART over the last 10 years, AIDS-related deaths have declined substantially by nearly 50% (UNAIDS 2017; World Health Organization 2017b). Tuberculosis (TB), severe bacterial infections, *Pneumocystis* pneumonia (PCP) and cryptococcal meningitis are major causes of death (Walker et al. 2012). Despite an over 30% decline in TB-related deaths among PLWHIV between 2005 and 2015, TB remains the leading cause of deaths among these individuals, with an estimated 1.2 million cases resulting in 374,000 deaths in 2016 (UNAIDS 2017; World Health Organization 2017a).

Although the use of PCP prophylaxis and ART have lowered the frequency of PCP in HIV infected individuals, AIDS-related PCP is among the leading AIDS defining diagnoses, with over 400,000 cases estimated every year in those patients that present late to care, fall out or are not in care in large urban settings (Brown 2012; Guarner 2017; Denning 2016). Cryptococcal meningitis is the leading cause of adult meningitis in Sub-Saharan Africa, accounting for 15%-20% of AIDS-attributable mortality (Rajasingham et al. 2017; Jarvis & Harrison 2007). Other important opportunistic infections include mucocutaneous candidiasis, toxoplasmosis and cytomegalovirus infections (Anon 2017; Masur et al. 2014; Limper et al. 2017) (**Table 3**). In some localities, histoplasmosis or talaromycosis are among the most frequent potentially lethal infections (Adenis et al. 2014; Chastain et al. 2017b).

References available online at: **https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/**

Fungal infections	Tuberculosis (2016)	HIV (2016)	Malaria (2016)
~1,700,000	~1,700,000	~1,000,000	~445,000

Table 2.
Estimated annual deaths from fungal diseases,tuberculosis, HIV and malaria.
Some duplication as patients may die of 2 or 3 of these conditions together.

CD4 level	Aetiology				
	Mycobacterial	**Viral**	**Bacterial**	**Fungal**	**Parasitic**
Any CD4 count	Tuberculosis	Kaposi sarcoma (HHV8) Hepatitis B Hepatitis C Herpes simplex infections GB virus C infection	Syphilis Bartonellosis	Onychomycosis	Visceral leishmaniasis
CD4 count below 300 cells/mm³		HIV wasting syndrome		Oral and cutaneous candidiasis Seborrheic dermatitis Chronic intestinal microsporidiosis	Chronic intestinal cryptosporidiosis Chronic intestinal cystoisosporiasis#
CD4 count below 200 cells/mm³		Lymphoma Invasive cervical/anal cancer (HPV)	Recurrent bacterial respiratory diseases Severe bacterial sepsis *Salmonella* septicaemia, recurrent	*Pneumocystis* pneumonia	Toxoplasmosis
CD4 count below 100 cells/mm³	Non-tuberculous Mycobacterial (NTM) infections MAC/MAI (common) Other NTMs	HIV-encephalopathy		Cryptococcal disease (especially meningitis) Oesophageal candidiasis Invasive aspergillosis Disseminated histoplasmosis# Coccidiodomycosis# Talaromycosis#	
CD4 count below 50 cells/mm³		Cytomegalovirus infection Progressive multifocal leukoencephalopathy (JCV)			

Table 3.
AIDS-related opportunistic infections by CD4 counts, including some geographically restricted cases (#) and rare infections **(green).**

Data from Centres for Disease Control and Prevention (Anon 2017), AIDSinfo (Masur et al. 2014) and WHO (World Health Organization (WHO). 2005). Any disseminated/ invasive mycoses is an HIV-infected person is a WHO stage 4 disease. Abbreviations - See P4.

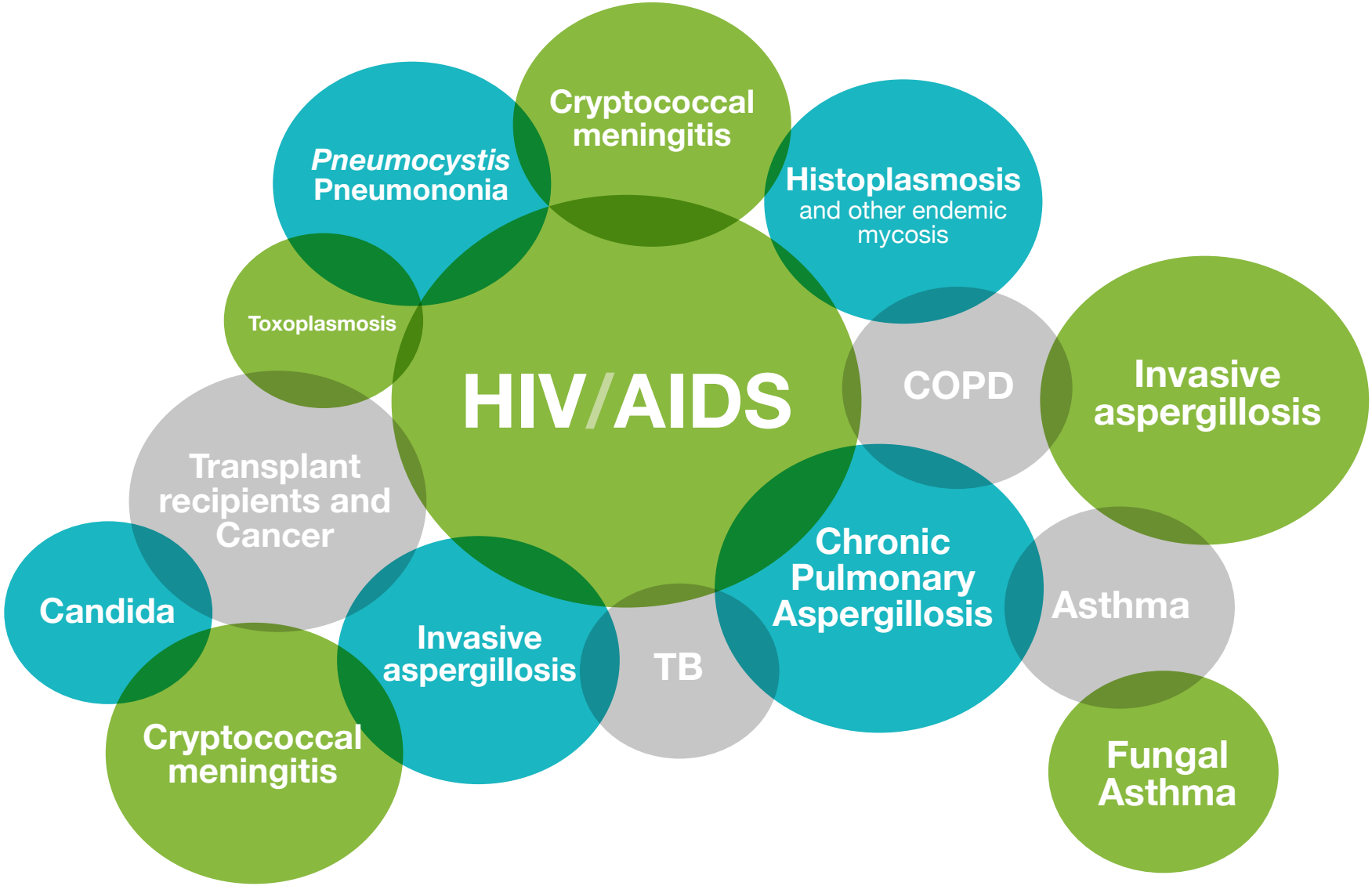

Figure 1.
Interaction between AIDS, fungal diseases, TB and other medical conditions.
Note, the size of each circle doesn't reflect the overall burden of that disease. Abbreviations - See P4.

Interaction between AIDS, serious fungal diseases, TB and other medical conditions

Meeting objectives.

Recently, the WHO issued clinical guidelines on advanced HIV management. The focus of these guidelines is to 'provide an enhanced package of prophylactic, diagnostic, and therapeutic interventions for those initiating ART' with advanced disease, as well as providing recommendations on rapid initiation of ART (World Health Organization 2017b). The advanced HIV disease recommendations are based on the leading causes of morbidity and mortality, mainly TB, severe bacterial infections and cryptococcal meningitis but also toxoplasmosis, and PCP (Ford et al. 2018). The WHO at present recommends cryptococcal antigen (CrAg) screening HIV-infected adults with CD4 cell count ≤100 cells/mm3 and urine lateral flow urine lipoarabinomannan assay (LF-LAM) for TB diagnosis in patients with symptoms and signs of TB with CD4 cell count ≤100 cells/mm3 or at any CD4 cell count value if seriously ill. In addition, WHO also recommends sputum Xpert MTB/RIF as first test for TB diagnosis in symptomatic patients irrespective of CD4 cell count (World Health Organization 2017b).

Invasive fungal diseases (IFD) caused by both true-pathogenic (primary) and opportunistic pathogens have increased markedly in the past few decades (Brown 2012). The key factors driving the increased prevalence being major advances in healthcare and the increasing number of individuals at risk including persons with HIV/AIDS, transplant recipients, cancer patients and people on immunosuppressive therapy (Vallabhaneni et al. 2016; Arendrup 2010; Denning 1998; Pagano et al. 2006). IFDs are associated with significant morbidity and mortality in immunocompromised patients despite the availability of effective therapies (Brown 2012). The big four fungal genera implicated in IFD globally are *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis jirovecii*, (Hope et al. 2005; Ascioglu et al. 2002; Chastain et al. 2017a). Timely and accurate diagnosis is essential but challenging because of nonspecific clinical and radiographic findings, underlying patient debilitation that precludes potentially definitive diagnostic procedures, and low yield of cultures (Clancy & Nguyen 2013; Hope et al. 2005). Culture-independent serum antigen detection tests, such as the (1>3) β-d-glucan (BD) assay and galactomannan (GM) index may allow earlier diagnosis of IFD than is otherwise feasible with traditional methods (Yeo & Wong 2002; Hoenigl et al. 2014; Lahmer et al. 2016; Moura et al. 2018).

The Global Action Fund for Fungal Infections (GAFFI) with partners, organised a two-and-a-half day workshop on the 'Essential Diagnostics for Advanced HIV disease and Serious Fungal Infections' in Kampala Uganda with the following objectives:-

Overall objective

To gather experts and review evidence on available in vitro diagnostics for opportunistic infections for inclusion on the WHO Model List of Essential In Vitro Diagnostics (EDL) with the focus on LMICs and non-culture tests.

Specific objectives

1. To overview the evidence base for key tests to produce a consensus for recommendation for inclusion in the WHO EDL.
2. To provide the specific arguments and diagnostic performance criteria supporting applications to the WHO for inclusion in the EDL.
3. To publish a summary report on the forum, highlighting key gaps in our diagnostic test portfolio.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

Methods-Pre-meeting

The meeting was timed to precede and help inform the WHO's Strategic Advisory Group of Experts on In Vitro Diagnostic (SAGE-IVD) meeting.

An initial selection was made to focus on key non-culture diagnostics, i.e. TB urinary antigen (LAM), cryptococcal antigen, *Histoplasma* antigen, *Aspergillus* IgG, *Pneumocystis* PCR and *Toxoplasma* IgG/IgM and on antifungal therapeutic monitoring. Summary arguments were also presented for culture based diagnostics and direct microscopy. Experts in HIV, Microbiology, Mycology, Parasitology, Public Health and Laboratory Sciences were invited from LMICs, global organisations, research institutes, academic institutions and diagnostic and antifungal marketing companies globally. Open invitations were made available on social media (LinkedIn, Twitter etc.). Diagnostic companies with studies in progress were invited to submit any data for consideration at the meeting to ensure the most up to date information was available to the participants.

Diagnostic tests not considered

Several diagnostic and monitoring tests were not considered (**Table 4**) after initial screening based on:

- Not commercially available or likely to be in the near future
- Low burden of infection
- Prior exposure screening using skin tests
- Any tests for fungal allergy
- Not yet adaptable to low and/or middle income countries
- Of limited clinical value

Test	Purpose	Reason for exclusion
Coccidioides antibody	To diagnose coccidioidomycosis	Geographically limited, some high performance tests not commercially available. Other means of making most diagnoses.
Paracoccidioides antibody	To diagnose paracoccidioidomycosis	Very geographically limited, no tests commercially available. Other means of making most diagnoses
Talaromyces marneffei PCR	To diagnose *T. marneffei* infection, usually in AIDS	Very geographically limited, no tests commercially available.
MALDI-TOF MS identification of fungi	Rapid identification of yeasts and moulds	Complex equipment and incomplete identification database currently.
Sequence-based identification of fungi	Definitive identification of yeasts and moulds, including cryptic species	Complex process for most laboratories and transport to a sequencing center expensive in many locations; interpretation requires a high level of skill.
Antifungal susceptibility testing	To detect antifungal resistance in fungi	Skill required is not widespread, pure antifungal compound and other key equipment required.
Candida albicans germ tube antibody	To diagnose or rule out invasive candidiasis caused by *C. albicans*	Increasing trend in non-*albicans Candida* species which are multi-antifungal resistant

Table 4.
The tests not considered at the workshop.
Abbreviations - See P4.

The Meeting

Ninety-five participants comprising 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, UK, Ukraine, USA and Zambia. Contributors were from the 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 including the US Centres for Disease Control and Prevention, research institutes and both diagnostic and pharmaceutical companies with an interest in AIDS and/or fungal diseases, as well as GAFFI itself.

Most of the meeting was plenary, with 2 breakout sessions, each consisting of 4 discussion groups led by an expert. Each presentation by an expert in the topic area reviewed the available evidence on diagnostic performance of each test, their ease of use and direct test cost. The strength of recommendation was derived from the diagnostic performance, clinical value and suitability of the selected tests for LMICS (**Table 5**). A summary recommendation for each test was developed using the approach as shown in (**Table 1**).

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 5.
Categorisation of recommendation.
Abbreviations - See P4.

‘There are 4 observational cohorts in Uganda, Ethiopia, Tanzania, and South Africa demonstrating that cryptococcal antigen lateral flow assay (CrAg LFA by Immy (Norman, Oklahoma)) titers >=1:160 are associated with prediction of disseminated disease and 3-fold higher failure of fluconazole pre-emptive therapy. Thus cryptococcal antigen quantification can play an important role in risk stratification of those requiring enhanced antifungal therapy.’

Dr. David R. Boulware, MD, MPH
University of Minnesota, USA

Cryptococcal lateral flow assay and cryptococcal antigen quantification

***Cryptococcus neoformans* remains a major cause of meningitis in adults living with HIV/AIDS.**

Cryptococcal meningitis is a leading cause of death in AIDS in LMICs and especially in sub-Saharan Africa. (Jarvis et al. 2010; Thinyane et al. 2015; Durski et al. 2013). An updated analysis of the global burden of cryptococcal meningitis estimated ~ 223,100 incident cases globally in 2014, 73% of which were estimated to occur in SSA (Rajasingham et al. 2017). Recent data suggests a plateau in deaths from AIDS-associated cryptococcosis in the past 10 years (Osler et al. 2018). In March 2018, the WHO updated the guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children (WHO, 2018).

Cryptococcus spp. shed their polysaccharide capsular antigen (CrAg) very early into the bloodstream during dissemination with a median of 3 weeks before the development of clinical symptoms (French et al. 2002). CrAg detection has revolutionised diagnosis of cryptococcal meningitis and asymptomatic cryptococcal antigenaemia. CrAg lateral flow assay (LFA) performed on cerebrospinal fluid or blood (serum, plasma, or whole blood) samples yields a very high sensitivity (>95%) and specificity (~99%) compared to urine samples (sensitivity ~85%) (Boulware et al. 2014). Performance of the CrAg test is superior to both India ink preparation (sensitivity ~70-80%) in CSF and culture of *Cryptococcus* (sensitivity ~90% (in CSF sample). In isolated pulmonary cryptococcosis, bronchoalveolar lavage (BAL) samples have a better yield compared to serum sample (sensitivity of ~83% vs ~74%, respectively) (Oshima et al. 2018). A CrAg titre of >1:8 in BAL samples of HIV-infected patients results in a sensitivity of 100% and a specificity of ~98% (Baughman et al. 1992; Kralovic & Rhodes 1998). Latex agglutination allows CrAg quantification with overall sensitivities of 93% to 100% and specificities of 93% to 98%. However, patients with very early disease and those with low fungal burden are often missed (false-negative) with the latex agglutination technique (Maziarz & Perfect 2016). Screening and pre-emptive treatment for cryptococcal infection combined with a short initial period of adherence support after initiation of ART has been shown to save lives (Mfinanga et al. 2015). (**Figure 2**). CrAg testing is also effective in the hands of lay cadres (Rick et al, 2017).

Validation studies have been done for 4 CrAg LFAs (IMMY, Biosynex CryptoPS, StrongStep and FungiXpert) and 1 assay (Dynamiker) is currently under validation. Sensitivities vary between 96 and 99% with corresponding sensitivities of between 90 and 99% in serum and sensitivities of 98-100% and specificities of 98-100% in CSF. Specificity in blood is more variable (90-99%). The consensus recommendation from our meeting was that a minimum diagnostic performance of a commercially available CrAg LFAs should be >98% sensitivity and 99% specificity (Kozel & Bauman 2012; Mpoza et al. 2018; Temfack et al. 2018).

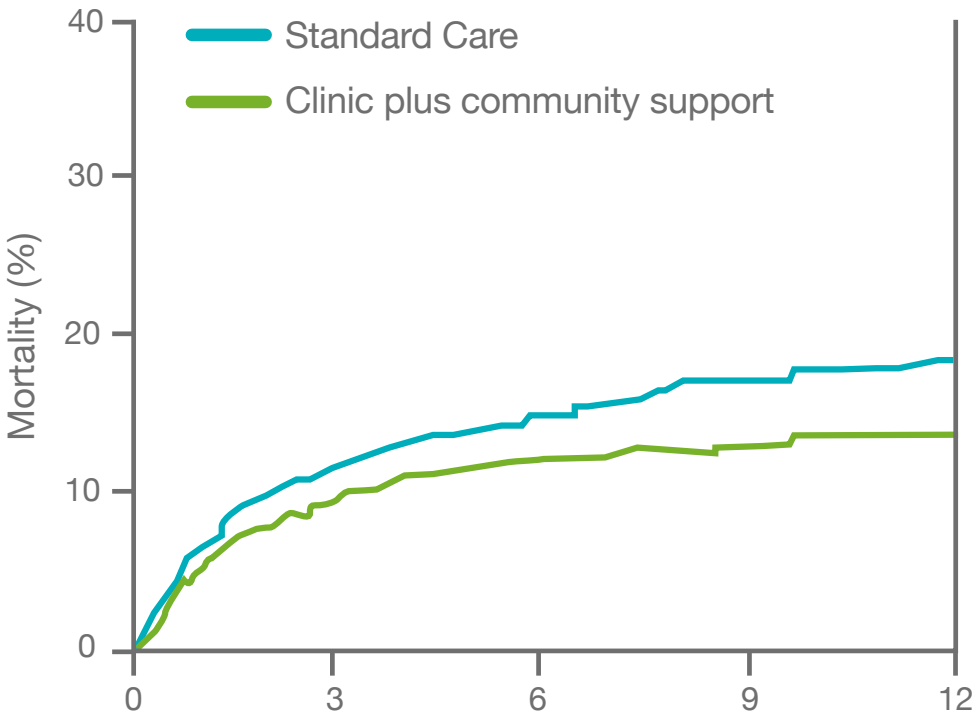

Figure 2.
2000 patient study in Tanzania and Zambia. Mortality was 28% lower at 12 months in those with CrAg screening and ART support (p=0.004).' Graph from Mfinanga S et al. Lancet 2015; 385:2173 Abbreviations - See P4.

References available online at:
https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

Strength	Weaknesses	Opportunities	Challenges
For diagnosis and screening	Qualitative	Semi-quantification of all test kits to include extra band of the optimal titre to distinguish meningitis from pre-meningitis	Difference in performance of the available commercial kits
Low cost	Dual antigen titre testing not yet available at optimal titre to distinguish meningitis from pre-meningitis	More kits in development	
No or very minimal infrastructural requirement	Visual reading subject to error at low titres with faint bands		
Time to results <20 minutes	Lower sensitivity and specificity in urine and saliva		
Shipping and storage at room temperature	Possibility of 'lost' results if performed at bedside or in clinic		
Can be used at all levels of healthcare system	False negative results in extremely high fungal burden (prozone effect)		
High diagnostic performance			
Multiple sample types (CSF, serum plasma, urine) can be used in small volumes (~40µL)			
Minimal sample handling			
No routine maintenance required			
Cost-effective			
Survival benefit			
Multi-site validation in 1000's of patients in multiple countries and multiple sample types (one of the commercially available assays)			

Table 6.
Considerations for lateral flow assay point-of-care devices, with or without a second positive CrAg band at a recommended titre of >1:160 that distinguishes meningitis from those with disseminated cryptococcal disease without meningitis with a reported sensitivity of 88.2% and specificity of 82.1%. Abbreviations - See P4.

Right
Alexander Jordan and Gregory Greene
(Both from the CDC, USA)

Far Right
Dr Conrad Muzoora meets
Dr William Worodria, witnessing is
Dr Juvenal Nkeramahame
(Uganda all)

Strength	Weaknesses	Opportunities	Challenges
Diagnostic and screening tool	Dilution series with latex test is time consuming and requires highly precise pipetting and calibrated pipettes for accuracy	Dual band LFD will reduce cost and time for a dilution series, with 3 dilution steps: 1:5, 1:25, 1:500.	Requires laboratory and ELISA reader and reliable electricity for EIA
Accurate quantification	Limited data on the test's validation in samples other than blood and CSF.	EIA does not require a dilution series	Correlation between EIA OD and latex titres not well described
Minimal laboratory equipment for latex agglutination – ELISA reader for EIA	Inconsistency of some titres when repeated,	May provide a better understanding of burden of infection, with implications for management	Use of the CrAg LFA at the point-of-care requires regular re-training and supervision
Dilution series not require for EIA	Not a longitudinal marker for measuring response to successful therapy.	May be the best test to diagnose pulmonary cryptococcosis using BAL	
Time to results <60 minutes, after sample received in laboratory	Dilution series done with LFA relatively expensive (depends on the titre)	Mortality benefits or cost-effectiveness compared to the standard CrAg LFAs to be assessed	
Shipping and storage at room temperature		Use of whole blood allows decentralization of CrAg testing at the primary healthcare level	
High diagnostic performance			
Multiple sample types (CSF, serum plasma, whole blood, urine, BAL) can be used in small volumes (~40µL)			
Minimal sample handling			
No routine maintenance required except for ELISA reader			
Latex agglutination widely accepted and approved for use in almost every country			
Result recorded in LIMS			

Table 7.
Summary of the strengths, weaknesses, opportunities and challenges (SWOC) analysis of CrAg quantification to titre as an essential in vitro diagnostic.
Abbreviations - See P4.

Quantification and titres of cryptococcal antigen in serum and CSF

Generating the most useful quantitative data of CrAg is not yet standardized. Dilution steps of greater than 5-fold tend to introduce inaccuracy as do pipettes that are not properly calibrated. Minimising cost and laboratory effort is a key goal. This table illustrates some options for dilution series (Table 3). A dual concentration LFD set at 1:160 further minimizes the need for dilutions.

Baseline CrAg titres in serum and CSF has been shown to correlate with fungal burden. A blood titre of 1:160 correlates with disseminated cryptococcal disease (Beyene et al. 2017) or cryptococcal meningitis (Wake et al. 2018) (**Figure 3**) , and a CSF titre of 1:1024 is associated with worse prognosis (Kabanda et al. 2014). One of the commercially available LFDs has a second band at a CrAg titre of 1:1024 (Temfack et al. 2018). The clinical utility of CrAg quantification in monitoring treatment response is unclear.

Further research is required to establish the cost-effectiveness or mortality benefit of quantitative compared to the standard CrAg LFAs. Additionally, utility of CrAg quantification in distinguishing clinical failure from relapse and immune reconstitution inflammatory syndrome in patients on therapy should be assessed. At the present, it's unclear whether CrAg quantification would provide a better understanding of burden of infection, and the implications of this for patients' management remains an area of future research interest.

IN CONCLUSION

Based on the available evidence, there was a very strong recommendation of CrAg LFA for both diagnosis of CM and screening for asymptomatic cryptococcal antigenaemia. CrAg antigen quantification guides clinical decisions especially in patients with asymptomatic antigenaemia. In the near future, with more studies, quantitative/semi-quantitative LFAs may replace the classic CrAg LFAs.

Conventional 2-fold	Breakpoint test for 1:160 2 x 4-fold, 1x 10-fold	Immy recommended dilution series
1:2	1:4	1:5
1:4	1:16	1:10
1:8	1:160	1:20
1:16		1:40
1:32		1:80
1:64		1:160
1:128		1:320
1:256		1:640
1:512		1:1280
1:1024		1:2560
1:2048		

Table 8.
Dilution series for cryptococcal antigen detection.
Abbreviations - See P4.

Figure 3.
Blood cryptococcal antigen (CrAg) titers in asymptomatic CrAg-positive patients (n = 37) (Upper), CrAg-positive patients with headache only (n = 25) (lower), with or without concurrent cryptococcal meningitis. CSF, cerebrospinal fluid; NPV, negative predictive value. Adapted from Figure 2A and 2B in Wake et al (Wake et al. 2018).

‘There are 2 randomised controlled trials confirming a survival benefit associated with the use of LAM which is stronger evidence than for almost any other test on the EDL.’

Dr Tom H Boyles
University of the Witwatersrand, South Africa

Tuberculosis lateral flow urine lipoarabinomannan assay

Tuberculosis (TB) is the leading cause of death in patients with advanced HIV disease. (World Health Organization 2017b).

In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374,000 deaths among HIV-positive people (World Health Organization 2017a).

Most deaths from TB could be prevented with early diagnosis and appropriate treatment. In HIV-TB co-infection, disease is more likely to be extra-pulmonary, patients with pulmonary disease have lower sputum bacillary load making conventional sputum examination less sensitive (World Health Organization 2015). Tests based on the detection of mycobacterial lipoarabinomannan (LAM) antigen in urine have emerged as potential point-of-care tests for TB. LAM is a lipopolysaccharide component of the *Mycobacterium tuberculosis* cell wall, detectable in urine of individuals with tuberculosis, including pulmonary, extra-pulmonary, and disseminated forms of tuberculosis and appears to be present only in people with active TB disease. It is shown only low cross-reactivity with nontuberculous mycobacterial infections (Qvist et al. 2014; World Health Organization 2015).

Sensitivity of the currently available assays increases with decreasing CD4 counts. In a recent Cochrane Database Systematic Review and Meta-analysis of 12 studies, pooled sensitivity and specificity of LF-LAM were 56% (41% to 70%) and 90% (81% to 95%) in participants with a CD4 count of less than or equal to 100 cells per µL versus 26% (16% to 46%) and 92% (78% to 97%) in participants with a CD4 count greater than 100 cells per µL (Shah et al. 2016). LAM is not a useful diagnostic test in HIV negative patients (MacLean & Pai 2018).

The superiority of urine-based testing over sputum-based testing is in its ease of collection and storage, and negates the infection risks associated with sputum collection. Some scholars argue that the presence of LAM in the urine is a marker of higher mycobacterial load and greater disease dissemination and severity, and rapid treatment of such patients is life-saving (MacLean & Pai 2018). Data from hospitalized HIV-infected children has shown that mortality is 4.9-fold higher among LAM-positives (LaCourse et al. 2018), and an earlier systematic review found detectable LAM in the urine an independent risk factor for mortality among patients receiving treatment for HIV-TB in sub-Saharan Africa (Gupta-Wright et al. 2016). Two randomised clinical trials have shown that bedside LAM-guided initiation of anti-tuberculosis treatment in HIV-positive hospitalised patients with suspected tuberculosis was associated with reduced 8-week mortality (Peter et al. 2016; Gupta-Wright et al. 2018).

References available online at: **https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/**

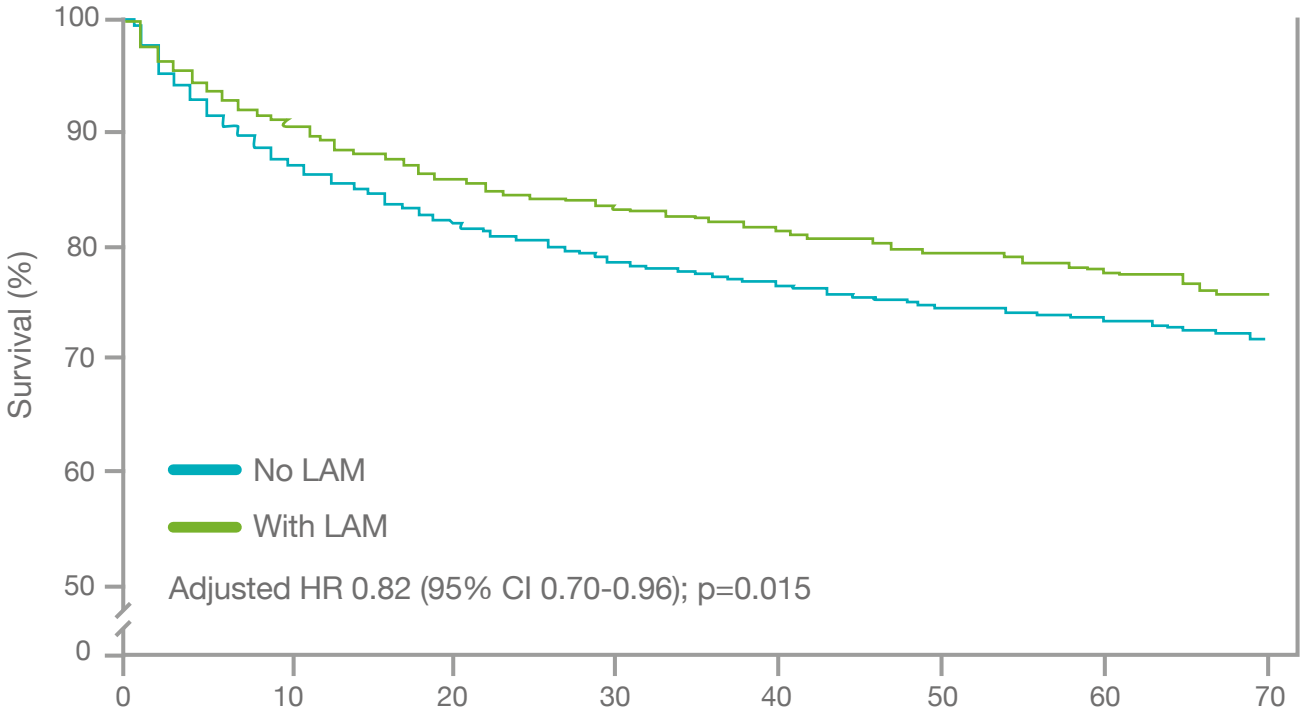

Figure 4.
2500 patient study in Zimbabwe, Tanzania, South Africa and Zambia. Mortality at 8 weeks was 4% lower in those screened with TB LAM (p=0.012). Graph from Peter JG et al; Lancet 2016;387:1187
Abbreviations - See P4.

Tuberculosis lateral flow urine lipoarabinomannan assay

In the advanced HIV disease guidelines, WHO recommends the use of LAM (for diagnosis of TB) in adults, adolescence and children with signs and symptoms of TB with CD4 counts of less than or equals to 100 cells per µL or in seriously ill patients regardless of their CD4 counts (World Health Organization 2017b).

Two priority research areas are: 1) development and validation of clinical algorithms for diagnosing TB in seriously ill adults and children with HIV and 2) development of a higher-sensitivity LAM-based test to increase the proportion of positives compared with the current WHO endorsed LAM test. When the new tests are launched, direct comparisons will be required, especially as the current marketed test has a sensitivity of 58%.

IN CONCLUSION

There was a very strong recommendation of urine LAM test as an essential diagnostic tests for people living with HIV with CD4 <100 cells/µl.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis and screening of TB	Limited data on cost-effectiveness	More kits in development	Possible cross-reactivity with NTM
No sample processing is required	Low sensitivity in patients who are not seriously ill	Routine use in clinical practice without a laboratory	Limited data in children
Cheap and cost saving	Only useful in HIV positive patients	Selection criteria for testing in primary care not yet established	Quality assurance
Time to results ~20 mins at the point of care	Most useful in patients with lowest CD4 count, which may not be known when patients present		
Semi-quantitative	Frequency of testing unclear		
Several validation studies involving many patient samples			
Predicts mortality and guides treatment initiation by identifying sick patients with TB			
Mortality benefit			
Strong correlation between mycobacterial burden (disease severity) and LAM positivity			

Table 9.
Strengths, weaknesses, opportunities and challenges (SWOC) analysis of lateral flow urine lipoarabinomannan assay as an essential in vitro diagnostic.

Abbreviations - See P4.

Dr Tafese B Tufa Arsi University, Ethiopia
Expressing his opinion on what should constitute an EDL

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‘Disseminated histoplasmosis is a frequent, treatable, but mostly undiagnosed cause of death in patients with advanced HIV disease. There is therefore a critical need to provide Histoplasma antigen detection tests in low and middle income countries in order to diagnose and treat histoplamosis to reduce the burden of disease and death.’

Professor Mathieu Nacher
The University of French Guiana, Cayenne, French Guiana

Histoplasma antigen detection

Histoplasmosis, caused by the thermally dimorphic fungus *Histoplasma capsulatum* (worldwide distribution) and *Histoplasma duboisii* (endemic in Africa), is the most common endemic human mycosis (Kauffman 2009).

Histoplasma is transmitted by way of the respiratory tract, but once inhaled into the alveoli; the organism readily spreads throughout the body, causing a wide spectrum of manifestations that range from subclinical infections to progressive disseminated disease, affecting both immunocompetent and immunosuppressed individuals (Wheat 2006). Large and small outbreaks have been attributed to histoplasmosis, but most infections are sporadic (Kauffman 2009).

Progressive disseminated histoplasmosis is an increasingly commonly recognised cause of infection in patients with advanced HIV disease from areas endemic for histoplasmosis (Johnson et al. 1986; A. A. Adenis et al. 2014). It is one of the major AIDS-defining infections and a major killer of HIV-infected patients in South and Central America, where it kills ~5,000 - 10,000 people per year of the estimated 24,000 deaths from AIDS there (Nacher et al. 2011; Daher et al. 2007; Nacher for The neglected histoplasmosis in Latin America Group 2016). On the other hand, in the USA, the incidence of AIDS-associated histoplasmosis has declined significantly in the past few years with ARV therapy (Richer et al. 2016; Kauffman 2009).

In the immunocompromised person, *Histoplasma* polysaccharide antigen detection tests allow rapid diagnosis of disseminated histoplasmosis in urine, serum, bronchoalveolar lavage (BAL), and cerebrospinal fluid (CSF) samples before positive cultures can be identified (Hage et al. 2011). Antigen concentration is greatest in urine and can be used to monitor response to antifungal therapy and to identify relapsing patients (Richardson & Warnock 2012). In AIDS patients with disseminated disease, Histoplasma antigen has been detected in the urine of 95-100% and in the serum of 80% of the patients (Wheat et al. 2016; Scheel et al. 2009; Cáceres et al. 2018).

The availability of a simple, rapid method to detect *H. capsulatum* infection in LMICs would dramatically decrease the time to diagnosis and treatment and deaths in patients with AIDS-related disseminated histoplasmosis (**Figure 5**). At the present, however, only one ELISA test is commercially available and none of the LFAs in development are have been commercialised.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

IN CONCLUSION

The panel of experts very strongly recommended *Histoplasma* antigen detection as an essential diagnostic in endemic areas, or in non-endemic areas as a reference test for imported cases of histoplasmosis.

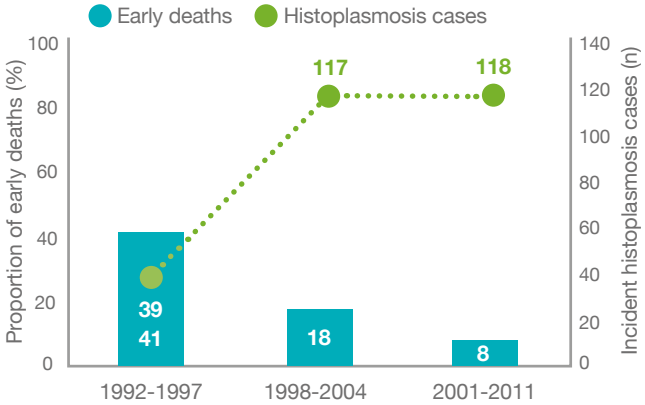

Figure 5.
Benefits of screening for histoplasmosis. Mycology Reference Laboratory in Cayenne, French Guiana transforms the outcome of disseminated histoplasmosis in AIDS with rapid testing – needs replicating with urinary antigen. Adapted from Adenis et al (Adenis et al. 2014)

Abbreviations - See P4.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis Low cost Minimal infrastructural requirement Sensitive Urine or blood samples can be used (not both in the same patient at the same time) Cost-effective Multi-site validation in in endemic areas	Cross reactivity with *Blastomyces, Talaromyces, Paracoccidioides* and rarely, *Aspergillus* (less specific) Less sensitive in subacute and chronic histoplasmosis	More kits in development, especially the LFDs	Only one commercially available kit Quality assurance

Table 10.
Summarises the strengths, weaknesses, opportunities and challenges (SWOC) analysis of *Histoplasma* antigen detection as an essential in vitro diagnostic.

Abbreviations - See P4.

Dr. Felix Bongomin
Global Action Fund for Fungal Infections,
Geneva, Switzerland

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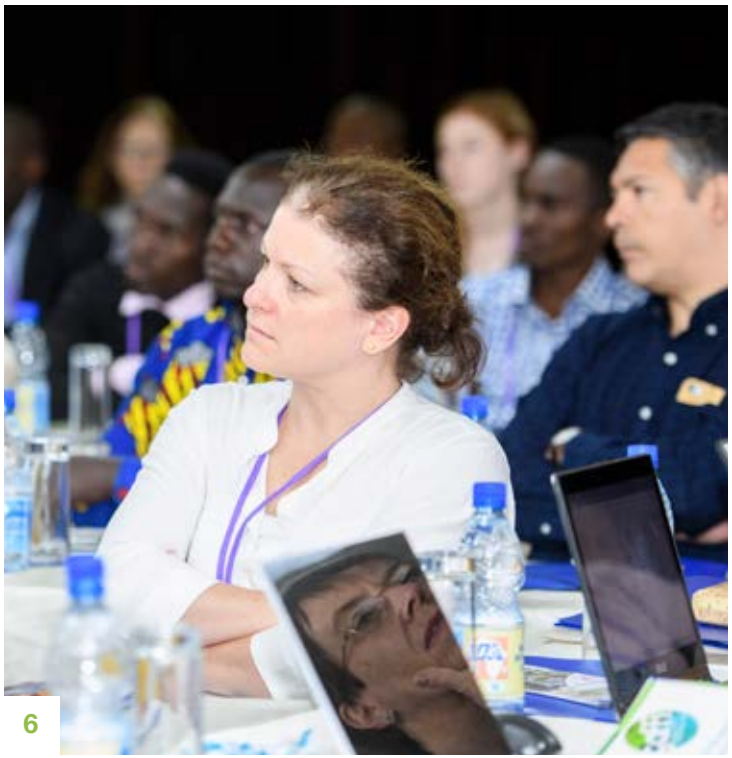
GLOBAL FUNGAL INFECTION FORUM III

Kampala Uganda / 10-12th April 2018

1. **Mr Richard Kwizera** Kampala, Uganda
2. **Associate Professor Nelesh Govender** NICD, South Africa
3. **Prof Malcolm Richardson** Mycology Reference Centre, Manchester, UK
4. **Dr Samson Haumba** Swaziland
5. **Sean Bauman** IMMY, USA
6. **Edith Fortier** Medecins Sans Frontieres, Kampala, Uganda



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1. **Dr Ana Alastruey-Izquierdo** Madrid, Spain
2. **Dr Tom M Chiller** (CDC) **and Dr Samuel Fayemiwo** Ibadan, Nigeria
3. **Prof Florence Robert-Gangneux** Rennes, France
4. **Prof David Denning** (GAFFI) **and Dr Christine Mendengue** Cameroon
5. **Dr David Mukasa and Dr Enock Kagimu** Mulago Hospital, Uganda

2

3

4

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‘*Aspergillus* antigen testing has revolutionised the diagnosis of invasive aspergillosis, but there too few data in AIDS, intensive care, COPD and in LMICs currently to support a strong recommendation for EDL inclusion.’

Professor David W Denning

President of GAFFI

The University of Manchester, Manchester, UK

Aspergillus antigen detection

Invasive aspergillosis (IA) remains a major cause of morbidity and mortality in a wide range of immunocompromised patients.

Over 10 million patients worldwide are at risk annually of developing IA. It is highly likely that over 300,000 individuals develop IA annually with mortality rates ranging between 30% and 95% despite recent advances in diagnosis and treatment (Brown 2012; Bongomin et al. 2017). Recent papers have re-emphasised that advanced HIV disease is a significant risk factor for the development of IA (Kaur et al. 2017; Denis et al. 2015).

The diagnosis of IA can be challenging: host risk factors, clinical symptoms and radiological findings must be put into context. Demonstration of morphological features consistent with *Aspergillus* species or recovery of *Aspergillus* from a tissue sample is required to define a positive (definitive) case. Obtaining tissue samples is often problematic in these multi-morbid patients and the yield of fungi in culture is low, difficult to interpret and prior antifungal prophylaxis further reduces its sensitivity (de Pauw et al. 2008; Horvath & Dummer 1996). In the past few years, new, non-culture based diagnostic methods have emerged in attempts to further reduce the unacceptably high mortality associated with IA. The detection of galactomannan or galactomannan-like biomarkers by ELISA or immune-chromatographic technology (lateral flow devices) contributes to this diagnostic armamentarium (Moura et al. 2018).

Galactomannan (GM) is a component of the *Aspergillus* cell wall. The sensitivity of serum testing is variable, ~22% to 90% and specificity ranges between 80% and 90% (Pfeiffer et al. 2006). Sensitivity in serum is much higher in severely neutropenic (e.g. haematological malignancies) not taking mould-active prophylaxis than in non-neutropenic patients (Leeflang et al. 2015; Verweij & Mennink-Kersten 2006; Nguyen et al. 2007). GM induces antibody formation, so more slowly evolving cases of IA are more likely to be negative, probably because of antibody binding.

Bronchoalveolar lavage GM outperforms serum GM in almost all patient groups, however, sensitivity is lower in patients on anti-mould prophylaxis and those on empiric antifungal therapy (Duarte et al. 2014; Maertens et al. 2009). In IA patients with detectable GM in serum, a combination of baseline GM and 1-week decay has been shown to be predictive of all-cause mortality (Koo et al. 2010). Contamination from diet and drugs or infection with other fungal pathogens (*Histoplasma* and *Fusarium*) can cause ‘false-positive’ results (Zandijk et al. 2008). Sputum galactomannan assay is not useful for the diagnosis or monitoring of treatment of either chronic pulmonary aspergillosis or allergic bronchopulmonary aspergillosis (Fayemiwo et al. 2017).

The diagnostic performance of GM assay in IA is comparable to that of *Aspergillus* PCR, moreover with a better specificity (White et al. 2015). GM assay is recommended for the diagnosis of IA and screening for IA in high risk patients (Ullmann et al. 2018; Patterson et al. 2016; Nguyen et al. 2007).

There are 2 commercial ELISA assays available for the detection of GM (Bio-Rad and Dynamiker). Two newly commercialised point of care *Aspergillus* antigen tests in an LFD format could significantly alter how and where these tests are done, especially in LMICs.

There was a strong recommendation by the consensus committee of GM assay in MICs under the following circumstances:

1. For bronchoscopy fluids (BAL and bronchial aspirate) in middle income countries when aspergillosis is considered - ICU, transplant recipients, AIDS (probably), possible CPA based on radiology. Turnaround time should be <72 hours.
2. For serum in acute leukaemia, neutropenic patients not receiving voriconazole or posaconazole prophylaxis, and with voriconazole or amphotericin B available for treatment and the turnaround time is <7 days.
3. To diagnose disseminated histoplasmosis or *Talaromyces marneffei* infection in AIDS, in the absence of specific diagnostic tools.

IN CONCLUSION

A key biomarker for invasive aspergillosis, galactomannan detection using ELISA requires a high level of technical skill and is most useful in bronchoscopy samples and serum in leukaemia patients.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis, screening and prognostication	Variable diagnostic performance, partly related to prophylactic and empirical antifungal therapy	Evaluation of galactomannan utility in monitoring of treatment	Different cut-off values for the different sample types
Relatively cheap	ELISA requires equipment, uninterrupted electricity and a well-established laboratory	More studies required to validate the test in a wide variety of hosts, especially advanced HIV disease	Not able to identify the fungus
Time to results 15 minutes to 3 hours	Routine maintenance required		Bronchoscopy is required to obtain BAL
Minimal sample handling, some platforms are automated	Requires trained personnel to perform the test and read results		Quality assurance
Quantitative and qualitative	No data on survival benefits and cost-effectiveness		Assessing performance and overall clinical utility of the new LFDs
Often the only positive test in cases of invasive aspergillosis	False positivity with diet, drugs and other fungi		
Can contribute to the diagnosis of all forms of aspergillosis and disseminated histoplasmosis	False negativity in the solid organ recipients, haemolysed specimens, hyperbilirubinemia, and in the presence of anti-aspergillus antibodies		
LFDs require minimal equipment	Different performance in neutropenic and non-neutropenic patients		

Table 11.
Strengths, weaknesses, opportunities and challenges (SWOC) analysis of galactomannan detection by ELISA or a lateral flow device (LFD) as an essential in vitro diagnostic. Abbreviations - See P4.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

‘Beta-1,3-D-glucan test is particularly sensitive, nearly 100%, for *Pneumocystis jirovecii* pneumonia in AIDS patients and will be instrumental for its early detection in an HIV population.’

Taminori Obayashi, MD, PhD
Higashi Saitama General Hospital, Saitama, Japan

Beta-1,3-D-glucan detection

Beta-1,3-D-glucan (BG) assay is a major polysaccharide cell wall component ubiquitously found in most of the fungal species except *Histoplasma* and agents of mucormycosis (Obayashi et al. 1995; Odabasi et al. 2004).

Being a ‘pan-fungal’ diagnostic test, detection of BG in serum is a non-specific indicator of various invasive fungal infections notably *Candida* spp. *Aspergillus* spp. and *Pneumocystis* pneumonia (PCP) (Dobias et al. 2018). Other ubiquitous environmental fungi (*Penicillium* spp. and *Paecilomyces* spp) and a few bacteria (*Pseudomonas aeruginosa*) also synthesise BG which can cause contamination of the sample, hence a false positive (De Vlieger et al. 2011). Accordingly, a negative BG glucan in the presence of suggestive clinical and radiological picture consistent with IFI may point towards other pathogens, especially mucormycosis (Marty & Koo 2009). In cryptococcal meningitis, detection of cerebrospinal fluid BG has been shown to have an important prognostic value (Rhein et al. 2014). The risk of false positive results (e.g. after major surgery, administration of beta lactam antibiotic, immunoglobulin administration, blood products, consumption of seaweeds etc.) are thought to be possibly lower in real life than in theory (Giacobbe et al. 2017).

BG has good diagnostic accuracy for distinguishing proven or probable invasive fungal infections (IFIs) from non-IFIs. A meta-analysis analysing 16 studies with a total of 2979 patients (594 with proven or probable IFIs), demonstrated a pooled sensitivity of 76.8% (95% confidence interval [CI], 67.1%-84.3%), and a specificity of 85.3% (95% CI, 79.6%-89.7%) (Karageorgopoulos et al. 2011). The true-positive rates of BG are uniform across patient groups at risk of IFDs, including AIDS, transplant recipients and other risk groups, making it superior to other tests such as galactomannan assay (McCarty & Pappas 2016). In proven invasive candidiasis, a sensitivity of 65% to 90% and specificity of 80% has been reported (Pappas et al. 2018). For invasive aspergillosis in critically ill patients, sensitivity and specificity of BG has been reported between 52% to 90% and 69% to 75% respectively (Obayashi et al. 1995; De Vlieger et al. 2011; Presterl et al. 2009; Dobias et al. 2018; Talento et al. 2017). For PCP, a sensitivity and specificity of 96% to ~100% and 84% respectively are documented with a negative predictive value of 96.3% (Onishi et al. 2012; Pazos et al. 2006; Son et al. 2017).

The excellent (>95%) negative predictive value of BG has generated interest in its use as a screening test in high-risk patients (Obayashi et al. 1995). Important to note is that the Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) turbidimetric assay has a lower sensitivity and negative predictive value compared with other commercial assays (Yoshida et al. 2011).

References available online at: **https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/**

For the detection of BG, there are currently three commercially available kits, with differing cut-off values. These kits are the Fungitell assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA), Fungitec-G test MKII (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), and β-Glucan test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). β-Glucan test Maruha (Maruha Nichiro Corporation, Tokyo, Japan) is no longer commercially available.

Given the test complexity, costs, inability to identify the fungus and the need for more studies in opportunistic fungal infections in advanced HIV patients, BD glucan assay was not recommended as an essential diagnostic test in LMICs.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis and screening for invasive fungal diseases	Non-specific (may also be positive in certain bacterial sepsis)	Evaluation of BD assay utility in monitoring of treatment	If positive, does not distinguish which fungus is implicated
Good sensitivity	Requires equipment, uninterrupted electricity and a well-established laboratory	Prognostic implications of BD	Quality assurance
Time to results ~2 hours	Ideally glucan-free blood tubes required to minimise false positives.	Performance of BD assay in patients with advanced HIV disease	Limited data in paediatrics
Minimal sample handling, some platforms are automated	Routine maintenance required	Performance for subacute invasive aspergillosis in non-neutropenic patients unknown	Unlikely to be able to be made into a point of care or highly simplified assay
Quantitative	Requires trained personnel to perform the test and read-off the results	Diagnostic performance for PCP in children	
High negative predictive value allows IFDs to be ruled out and contributes to antifungal stewardship	No data on survival benefits and cost-effectiveness		
True-positive rate is consistent across risk populations	False positivity with immunoglobulin therapy, blood products and foods (seaweed)		
	Not useful in evaluating response to therapy in every case		

Table 12.
Strengths, weaknesses, opportunities and challenges (SWOC) analysis of Beta-1,3-D-glucan detection tests as an essential in vitro diagnostic.

Abbreviations - See P4.

‘Better diagnosis of chronic pulmonary aspergillosis is critical in the battle to improve outcomes by promoting access to specific Aspergillus-specific IgG testing in areas of high TB prevalence.’

Professor Malcolm D Richardson
Mycology Reference Centre, Manchester, UK

Aspergillus IgG test

Detection of *Aspergillus*-specific antibodies provides key diagnostic evidence of chronic forms of aspergillosis, specifically in chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) (Denning et al. 2003; Agarwal et al. 2016).

Aspergillus-specific IgG antibody levels play a critical role in the serological diagnosis, monitoring of treatment and exclusion of CPA (McCarthy & Pepys 1973; Page et al. 2015). The presence of *Aspergillus*-specific IgE antibody indicates sensitisation to *Aspergillus*, which is common in more severe forms of asthma, notably ABPA.

CPA is estimated to affect 3 million persons worldwide, but likely an underestimate, in part because CPA occurs in patients with active PTB, as a sequela of prior PTB or as a complication of other pulmonary disorders with similar symptoms to PTB and are incorrectly diagnosed and treated as PTB (The Fungal Infection Trust 2017; Denning et al. 2018). CPA usually occurs in patients with underlying pulmonary disease, mainly (20-80%) previously treated pulmonary tuberculosis and usually progresses silently in the initial months. Therefore, it is not uncommon for patients to present with life-threatening haemoptysis and/or respiratory failure. CPA is considered to be one of the most refractory pulmonary infectious diseases with mortality as high as 85% over 5 years (Nakamoto et al. 2013; Lowes et al. 2017).

Enzyme-linked immunosorbent assay (ELISA) platforms for the detection of *Aspergillus fumigatus* IgG have been in use for decades (Richardson et al. 1982). It is more sensitive and faster than the precipitin test.

Recently developed commercial ELISAs provide a promising alternative method with reported sensitivity and specificity range from 75% to 96% and 97% to 99%, respectively in patients with CPA (Page et al. 2016; Denning et al. 2018).

Guidelines strongly recommend *Aspergillus* IgG as an essential test for the diagnosis of CPA (Ullmann et al. 2018; Patterson et al. 2016; Denning et al. 2018). Several commercially available kits exist for the detection and quantification of *Aspergillus* IgG with variable diagnostic performances. Two automated platforms and 6 ELISA kits are commercially available and 2 lateral flow assays (LFAs) are currently being commercialised. The most recent and first of its kind analysis of these ELISA kits has shown that cut-off values differ significantly from manufacturers’ guidance and from patients from different ethnic backgrounds (Page et al. 2018).

Research areas include evaluation of the new LFAs, cost effectiveness and mortality benefits of these assays, particularly in LMICs. A key question remains whether integration of CPA diagnosis and care into TB services would improve CPA outcome and reduce toxicities and cost associated with empiric anti-TB therapy.

References available online at: **https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/**

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis and monitoring therapy of chronic pulmonary aspergillosis and other forms of aspergillosis	Requires equipment, uninterrupted electricity and a well-established laboratory (unless LFD)	More kits in development	Difference in performance of the available commercial kits
Relatively cheap	Routine maintenance required	LFDs in development	Different optimal cut-offs for different kits and possibly in different (ethnic) populations
Time to results <4 hours	Requires trained personnel to perform the test and read-off the results	As CPA is a chronic disorder, centralisation of testing is feasible with good sample transport	Uncertainty about performance for non-*fumigatus* Aspergillus infections
Good diagnostic performance	No data on survival benefits and cost-effectiveness		Cannot be used at the point of care at present
Minimal sample handling, some platforms are automated			Quality assurance
Quantitative			
Several validation studies involving samples from several countries			

Table 13.
Strengths, weaknesses, opportunities and challenges (SWOC) analysis of *Aspergillus* IgG detection as an essential in vitro diagnostic. Abbreviations - See P4.

IN CONCLUSION **There was a strong consensus recommendation for *Aspergillus* IgG test to be incorporated into TB services in LMICs.**

‘Serologic screening of toxoplasmosis would allow better recognition of the burden of cerebral toxoplasmosis in LMIC, which is currently either underdiagnosed or blindly overtreated, and could be as prevalent as cryptococcosis.’

Professor Florence Robert-Gangneux
Université Rennes 1 et Centre Hospitalier Universitaire de Rennes, Rennes, France

Toxoplasmosis IgG detection

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite that infects up to a third (1-2 billion) of the world’s population (Guy 2014).

Infection is mainly acquired by ingestion of food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts (Montoya & Liesenfeld 2004; Bodaghi et al. 2012). High seroprevalence (50%-85%) of toxoplasmosis exists in Latin America, parts of Eastern/ Central Europe, the Middle East, parts of south-east Asia and sub-Saharan Africa (Pappas et al. 2009). More than 13 million HIV-infected people were *Toxoplasma*-seropositive (with 87% in sub-Saharan Africa), thus at risk for cerebral toxoplasmosis (Wang et al. 2017).

Most immunocompetent individuals with primary infection are usually asymptomatic but some patients present with nonspecific flu-like symptoms and lymphadenopathy (Saadatnia & Golkar 2012). Congenital toxoplasmosis results from trans-placental transmission of the parasite when primary infection is acquired during pregnancy (with higher frequency in later stages of pregnancy) and may cause severe damage to the foetus including cardiac, ocular and cerebral anomalies with seizures and mental retardation or visual impairment (Hampton 2015). In immunocompromised patients, reactivation of latent infection can cause retinochoroiditis, as well as life-threatening encephalitis or disseminated disease (Weiss & Dubey 2009). Toxoplasmic encephalitis is an AIDS-defining illness diagnosed in up to ~11% of patients with advanced HIV disease (Low et al. 2016; B-Lajoie et al. 2016).

Diagnosis of toxoplasmosis can be established by direct detection of the parasite or by serological techniques (Montoya & Liesenfeld 2004; O. Villard et al. 2016). Testing for serum *Toxoplasma* IgG and IgM antibodies, is essential in pregnancy and in immunocompromised patients at risk for the more severe disease forms, such as congenital, cerebral or ocular toxoplasmosis (O. Villard et al. 2016; Montoya & Liesenfeld 2004; Robert-Gangneux & Darde 2012). Detection of IgG is possible within 3-4 weeks after acute infection, peaks within 2 to 3 months, and usually remains detectable throughout life. The detection of IgG antibodies and absence of IgM antibodies indicates an old infection. However, if test results are positive for both IgG and IgM, interpretation is difficult, as positive results may signify either a recent infection or low levels of IgM antibodies from a previous infection (Liesenfeld et al. 1997; Guy 2014). IgG avidity testing may help dating infection in pregnant women to document whether infection was acquired during pregnancy. In immunocompromised patients, definite diagnosis relies on the detection of *Toxoplasma* DNA in cerebrospinal fluid, blood, bronchoalvolar lavage or other tissues, but sensitivity is <60% (Robert-Gangneux & Belaz 2016). In AIDS patients, a negative IgG/IgM result rules out acute toxoplasmosis or reactivation of past infection.

Many assays are available on the market, to detect anti-*Toxoplasma* IgG and IgM with variable performance. In HICs, serology routinely relies on

immunoassays, several of which have been evaluated by comparison to a gold-standard method (i.e. the dye-test). Overall, three IgG techniques (Platelia Toxo IgG® (Biorad), ECLIA® (Roche Diagnostics), Vidas Toxo IgG II (BioMérieux)) showed a high sensitivity (>95%) and a high NPV (>98%) for anti-Toxoplasma IgG detection (Villard et al. 2016; Maudry et al. 2009; Petersen et al. 2005; Hofgärtner et al. 1997) together with excellent performance for IgM detection in acute infection (Villard et al. 2016) Although no study has focused on the performance of immunoassays on a cohort of HIV-infected patients, their specificity has been evaluated with sera from patients with inflammatory diseases autoimmune diseases, or other infections (Villard et al. 2016). A western-blot assay (Western-blot Toxo IgG II, LD Bio) also showed a very good sensitivity and NPV (99%) (Franck et al. 2008) and is now routinely used in France as a reference method to confirm low IgG titers. However, all these techniques are expensive and/or require specific equipment, staff training and electricity supply, so are most appropriate for tertiary care labs.

More basic techniques are suitable for LIC, including agglutination assays and LFD. Several agglutination assays have been evaluated on the same sera panel, and displayed variable performances; they can detect either IgG, or IgG and IgM (Villard et al. 2012). A high sensitivity was observed with two assays (NPV 100%), but false positive results were obtained with sera from patients with autoimmune disease and/or syphilis, thus their use in an African population needs further evaluation. The Toxo ICT® LFD (LDBio) can detect both IgG and IgM and has been evaluated in several studies, with promising performances (Mahinc et al. 2017; Chapey et al. 2017). This point-of-care test is inexpensive and designed to be done on whole blood (15 µL), and could be very useful for bedside use, but field studies on HIV patients in LMICs are required.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis and screening	Immuno-assays are laboratory-based requiring equipment and electricity supply	Use of alternative diagnostics such as PCR	Difference in performance of the available commercial kits (LFD, western blots and ELISAs) poorly known in HIV patients
Rapid screening using ICT (15 min)	Need for quick result not possible if blood samples must be shipped to lab III or IV for immunoassay	Bedside use of LFD could help precising diagnostic performance for excluding cerebral and disseminated toxoplasmosis in AIDS	Quality assurance
High negative predictive value for CT in patients with focal neurological signs		Pregnancy screening in LMICs not studied	Use in primary care labs or community-based setting requires evaluation of LFD in LMICs
Avoids unnecessary toxic treatment by high dose cotrimoxazole if patient seronegative			
Cotrimoxazole prophylaxis necessary in Toxoplasma IgG seropositive with CD4 counts <200			
>5 top-performing commercially available assays suitable either for level II, III, IV labs			

Table 14.

Summarises the strengths, weaknesses, opportunities and challenges (SWOC) analysis of *Toxoplasma* IgG detection as an essential in vitro diagnostic in AIDS. Abbreviations - See P4.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

Characteristics	Immunoassays	Agglutination assays	LFD	Western Blot
Turnaround time	On demand [a]	On demand	On demand	On demand
Time to result	1 to 2 hours [b]	15 min to 2.5 hours	15 min	5 hours
Handiness	Needs trained technician staff	Easy	Very easy	Easy / technician staff
Storage of reagents	2 – 8°C	2 – 8°C	RT	2 – 8°C
Need for maintenance/ electricity supply	Yes	No	No	No
Ease of result interpretation	Easy	Reading may be ambiguous	Easy	Easy
Cost	High	Low	Low	High
Intended use	Lab-based (level III or IV) [c]	Lab-based (level II)	Primary care lab or community-based settings	Lab-based (level III or IV)

Table 15.
The strengths and weaknesses of different methods for detection anti-*Toxoplasma* antibodies suitable for LMICs. Abbreviations - See P4.
a Except with microplate ELISA. **b** Except with microplate ELISA which are performed by batch, once or more a week, depending on the number of sera.
c Except ELFA assay (Vidas, BioMérieux) which could be used in level II labs.

Performing toxoplasma serology in patients with advanced HIV disease would; 1) improve knowledge of the epidemiology of this disease, 2) allow targeting of high dose cotrimoxazole treatment (reduce unnecessary toxicity and resistance development) and avoid misdiagnosis with resistant TB or cryptococcosis, 3) prevent congenital toxoplasmosis in pregnant women with low CD4+ counts, by targeting cotrimoxazole prophylaxis in seropositive women, and 4) a positive test in AIDS would facilitate better management of paradoxical toxoplasmosis-immune reconstitution inflammatory syndrome, in the absence of CT scanning.

There are five good commercial assays, including one LFD and 3 ELISAs. Immunoassays and agglutination assays are laboratory based. The LFD (20 mins) could be bedside (detection of both IgG and IgM on whole blood). An area of research need is to understand the utility of toxoplasma serology in starting anti-*Toxoplasma* treatment in HIV patients presenting with neurological signs versus using the test to stop empirical therapy as is now common practice. There are separate strong arguments for toxoplasma serology in pregnancy (**Table 16**). Indeed, as toxoplasmosis is highly prevalent in sub-Saharan countries, the risk of acquisition of toxoplasmosis during pregnancy is high. Thus, serological screening early in pregnancy could allow: 1) educational intervention in seronegative women to prevent infection, and 2) to repeat serology in seronegative women to detect infection during pregnancy and thereby to start prenatal cotrimoxazole treatment, and screen babies at birth. These tests require universal integration into antenatal programs for maximum benefit.

IN CONCLUSION

The immunoassays and agglutination assays received a moderate recommendation given the lack of data from LICs in AIDS and relative infrequency in high HIV prevalence countries. In MICs with high *Toxoplasma* prevalence, as in Latin America, a strong recommendation was made. Should the *Toxoplasma* LFD show high performance for either starting or stopping therapy for *Toxoplasma* encephalitis, then a strong recommendation would be made for LMICs.

Strengths	Weaknesses	Opportunities	Challenges
For diagnosis and screening	Immuno-assays are laboratory-based requiring equipment and electricity supply	Bedside use/primary care setting of LFD could help women adherence to screening	Availability of echographic ultrasound to detect potential foetal abnormalities in case of infection acquired during pregnancy
Cost-effective screening if LFD used (detection of both IgG and IgM with high sensitivity)	Few data on the prevalence of congenital toxoplasmosis in LMICs	Seroconversion should be confirmed in tertiary care lab using an immuno-assay	Quality assurance
Identify seronegative women at risk of infection during pregnancy	Pregnancy screening in LMICs not studied	Determine the optimal frequency and timing of testing in pregnancy in LMICs	
Allows prevention education			
Repeated testing to diagnose seroconversion in pregnancy allows treatment to prevent congenital toxoplasmosis			
Cotrimoxazole prophylaxis in Toxoplasma-seropositive women with CD4 counts <200			
>5 top-performing commercially available assays suitable either for level II, III, IV labs			

Table 16.
Summarises the strengths, weaknesses, opportunities and challenges (SWOC) analysis of *Toxoplasma* IgG detection as an essential in vitro diagnostic in pregnancy. Abbreviations - See P4.

‘*Pneumocystis* PCR is a promising tool with sensitivity values ranging from ~86% to 100% compared with other methods of PCP diagnosis. However, at the present, this technigue is only appropriate for MICs and tertiary hospitals in LICs.’

Jahit Sacarlal, MD, MPH, PhD
Universidade Eduardo Mondlane, Maputo, Mozambique

Pneumocystis polymerase chain reaction

***Pneumocystis jirovecii* is a ubiquitous opportunistic human fungal pathogen that causes *Pneumocystis* pneumonia (PCP) in patients with T-cell mediated immune defects.**

PCP and Kaposi’s sarcoma were the key indicators of the global HIV/AIDS pandemic. Human to human transmission has led to dozens of healthcare associated outbreaks. Although the incidence has declined since the introduction of cotrimoxazole prophylaxis and combination antiretroviral therapy (ART), PCP remains the most important AIDS-defining opportunistic infection in the western world, but infection is poorly documented in most low-income countries because of the lack of diagnostic capability (Masur et al. 1981; Huang et al. 2011). Therapy of PCP with high dose cotrimoxazole is at least 70% effective, but carries considerable toxicity. Concurrent corticosteroids reduce mortality from PCP in AIDS, but may accelerate other opportunistic infections.

As for most invasive fungal infections, a delay in initiation of an appropriate antimicrobial therapy for PCP is associated with poor clinical outcome (Li et al. 2014). For many years, microscopy has been the cornerstone for diagnosis of PCP since *P. jirovecii* does not grow in culture. Detection of beta-D-glucan in blood is very sensitive, but not specific as glucan is a major cell wall constituent of many fungi (Damiani et al. 2013). Real-time polymerase chain reaction (PCR) and immunofluorescence (IF) microscopy are faster and more accurate alternative methods of PCP diagnosis. The sensitivity of PCR is about 15-20% better than classical staining (Giemsa, Diff-Quick, Gomori methenamine-silver or Toluidine O blue) (Alanio et al. 2016). All lower respiratory samples can be used for microscopy and PCR, but bronchoalveolar lavage has been preferred in developed countries with sensitivity of 90% to 99%. Induced and expectorated sputum are both adequate if the sample is of high quality’, the staining method is highly sensitive or PCR is used and the experience of the institution (Choe et al. 2014; Nowaseb et al. 2014). In young children, nasopharyngeal aspirates are the only realistic means of making the diagnosis currently, and only PCR is applicable to such specimens (Samuel et al. 2011).

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

IN CONCLUSION

There was a strong consensus recommendation of PCP PCR for use in MICs and specialist hospitals and reference laboratories in LICs. Simpler technical solutions will allow adoption in all LMICs.

Strengths	Weaknesses	Opportunities	Challenges
Only useful for diagnosis	Requires specialised equipment	Research to find new generation PCR with shorter turnaround time	No clear threshold to distinguish colonisation from disease, especially for non-HIV patients
Allows determination of fungal load	Require infrastructure with laboratory level 2/3 and with stable electricity power	Reagent/kit shipping at room temperature	
Turnaround time~4 hours	All current kits required shipping on dry ice	Simple means to distinguish colonisation from infection	
High sensitivity and specificity	Trained personal	Reduction in childhood mortality from pneumonia	
~8 commercially available assays	High costs associated with maintenance and acquisition of equipment	Diagnosis and isolation may reduce person to person transmission in hospital wards and outpatients	
No microscopy training required	Needs fridge and freezers for sample storage	Exclusion of diagnosis will reduce toxicity of high dose cotrimoxazole	
	Expensive		
	Time consuming compared to other rapid tests		

Table 17.
Strengths, weaknesses, opportunities and challenges (SWOC) analysis of PCP PCR detection as an essential in vitro diagnostic. Abbreviations - See P4.

Emmanuel Fajardo
Médecins Sans Frontières, Access Campaign,
Geneva, Switzerland

‘Patients benefit most from antifungal therapeutic drug monitoring (TDM) in the presence of marked inter-patient variability in drug levels, as well as significant drug interactions. TDM is recommended when drug levels correlate with drug efficacy and/or toxicity. Itraconazole, voriconazole and flucytosine fulfil these criteria.’

Professor Alessandro C. Pasqualotto
Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

Antifungal drug monitoring

The essential antifungal agents itraconazole, voriconazole, and flucytosine are recommended and are widely used for the prophylaxis and treatment of many superficial, invasive, allergic, and endemic mycoses and a number of AIDS-related opportunistic fungal infections including cryptococcal meningitis and disseminated histoplasmosis (Denning & Hope 2010; WHO 2015).

Antifungal treatment is usually prescribed for weeks or months, and sometimes for years. With long-term treatment and the fact that pharmacokinetics of antifungal agents can vary between patients for various reasons (unpredictable absorption, compliance, metabolism, elimination, different generic formulations or drug–drug interaction), leading to inconsistent serum concentrations; therapeutic drug monitoring (TDM) allows treatment optimisation by ensuring therapeutic serum levels, with additional benefits of avoiding toxicities from supra-therapeutic drug levels or development of resistance and treatment failure due to sub-therapeutic drug levels (Ashbee et al. 2014; Schelenz et al. 2015; Stott & Hope 2017).

Guidelines recommend TDM for flucytosine, itraconazole and voriconazole (Ashbee et al. 2014). TDM for flucytosine is recommended for neonates and those with abnormal renal function, but not for short term therapy of cryptococcal meningitis in AIDS as safety is not compromised in these patients. TDM is recommended for most patients on long term itraconazole for aspergillosis. Voriconazole monitoring is of particular importance in children, following dose changes, after a shift from intravenous to oral treatment or following a change in the patient’s clinical condition (Schelenz et al. 2015; Hope et al. 2008; Ullmann et al. 2018; Denning et al. 2016).

Improvement in efficacy and safety are the main reasons for the recommendation of TDM for systemic antifungal agents, especially in the immunocompromised patients and those with disseminated diseases (Ashbee et al. 2014). The best evidence for itraconazole TDM is related to antifungal prophylaxis and the occurrence of breakthrough infections and not toxicity. It’s unclear whether TDM improves survival or not, and this remains an important area for future research.

Available methods for antifungal TDM includes the bioassay, high performance liquid chromatography, liquid chromatography-mass spectrometry and tandem mass spectrometry, each with its own advantages and disadvantages (Ashbee et al. 2014). Bioassay is the preferred method to measure flucytosine and itraconazole levels.

Quality control assessment is a key requirement for triazole TDM (Bruggemann et al. 2009), coupled with the costs of equipment, costs associated with development and running of assays and equipment maintenance.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

IN CONCLUSION

There was a strong consensus recommendation for antifungal drug monitoring in middle-income countries under the following circumstances:-

- **Itraconazole for the long term treatment for chronic aspergillosis and disseminated histoplasmosis**
- **Voriconazole in children, following dose changes, after a shift from intravenous to oral treatment or following a change in the patient's clinical condition**
- **Flucytosine for neonates and those with abnormal renal function**

Data from Stott & Hope (Stott & Hope 2017), Ashbee et al (Ashbee et al. 2014), Schelenz et al (Schelenz et al. 2015), Pasqualotto et al (Pasqualotto et al. 2007) and Pascual et al (Pascual et al. 2008). Abbreviation: HPLC: High performance Liquid Chromatography.

Antifungal agent	Strengths	Weaknesses	Opportunities	Challenges
Itraconazole **Methods** Bioassay HPLC	Improved safety Improved efficacy Reduction in risk of resistance (low levels) Reduction in cost (high levels) Objective assessment for compliance Results within 24-48 hours Relatively cheap Minimum equipment requirement	Difficult to distinguish levels of two or more azoles in the same sample Itraconazole metabolite is bioactive, so target therapeutic levels vary by method of assay	Studies on survival benefits Blood spot assays for remote monitoring	Pharmacokinetic variability of all itraconazole formulations Requires a well-established laboratory and flow of samples to be cost effective Non-linear pharmacokinetics The accumulation of itraconazole occurs slowly and target trough concentrations are only reached after 7–15 days of dosing Quality assurance
Voriconazole **Methods** Bioassay HPLC Tandem mass spectrometry	Improved safety, especially with slow metabolisers and older people Improved efficacy, especially in children Reduction in cost (high levels) Fast. Results in less than 10 hours Highly specific	Routine equipment maintenance required Requires trained personnel to perform the test and read Expensive to acquire equipment Few clinical data on voriconazole TDM value	Alternative cheaper methods for drug monitoring	Inter-individual pharmacokinetic variability Ethnic differences in pharmacokinetics Non-linear pharmacokinetics in adults Optimal dose adjustment for out of range levels Quality assurance
Flucytosine **Method** Bioassay	Improves safety in neonates and renal dysfunction Results within 24-48 hours Relatively cheap Minimum equipment requirement Good clinical data supporting routine use in clinical care	Requires stable power during incubation Impact on clinical outcome is not known	Quality controls for techniques and control organisms Assessment of clinical (safety & mortality) benefits of flucytosine monitoring	Progessive renal impairment with amphotericin B therapy Quality assurance

Table 18. Strengths, weaknesses, opportunities and challenges (SWOC) analysis of antifungal drug monitoring as an essential in vitro diagnostic. Abbreviations - See P4.

‘Rapid diagnosis of infection is a lifesaving measure in the management of advanced HIV cases by adding blood culture, as well as routine bacterial and fungal cultures to the essential diagnostic list.’

Prof. Afia Zafar

The Aga Khan University, Karachi, Pakistan

Direct microscopy and Blood, Fungal and Bacterial cultures

Infection is responsible for over 8 million deaths annually.

Pneumonia and bloodstream infections (BSI) caused by bacteria, fungi and mycobacteria are important causes of illnesses in community and hospital settings (Jacob et al. 2013; Larru et al. 2016; McCarty & Pappas 2016). Common pathogens include *Streptococcus pneumoniae*, *Staphylococcus aureus*, gram negative bacteria, *Candida* spp, *Aspergillus* spp, *Mycobacterium tuberculosis* and non-tuberculous mycobacteria (Huson et al. 2014; Jacob et al. 2013). The major opportunistic invasive mycoses in AIDS are *Pneumocystis* pneumonia, cryptococcosis, disseminated histoplasmosis, coccidioidomycosis and disseminated talaromycosis (Chastain et al. 2017b; Limper et al. 2017). Accurate rapid diagnosis is critical for survival.

Considered a diagnostic ‘gold standard’ for ages, direct microscopy on an appropriate specimen allows presumptive identification of a pathogen to genus level which in turn guides empiric antimicrobial therapy (Guarner & Brandt 2011). Examples of characteristic phenotypic characteristics that may provide a presumptive identification include e.g. demonstration of yeast cells with capsular ‘halo’ in *Cryptococcus* spp, spherules with multiple endospores in *Coccidioides* spp, gram positive cocci as seen in staphylococcal and streptococcal infections and ‘beaded’ cells acid-fast bacilli suggestive of *M. tuberculosis*, to mention but a few (Murray et al. 2013).

Blood culture has for decades been considered the ‘gold standard’ test for the detection of pathogens in blood specimens. Modern automated systems have a higher yield for most pathogens. Common bacteria causing sepsis in AIDS (such as Salmomellae) are best diagnosed with blood culture; disseminated histoplasmosis, disseminated cryptococcosis, talaromycosis and tuberculosis in AIDS will usually yield a positive blood culture, although *H. capsulatum* and *M. tuberculosis* grow so slowly that patients die before they turn positive (Schelenz et al. 2015; Jacob et al. 2013). Early administration of an effective antimicrobial improves prognosis in bloodstream infections (Kumar et al. 2006). Identification and susceptibility testing of pathogens in blood specimens guides clinical selection of optimal antimicrobial therapy (Schelenz et al. 2015). *Candida* bloodstream infection is more common in most middle income countries (MICs) than high income countries (HICs), contributing to neonatal sepsis and deaths in critical care and following major surgery; blood culture is the only realistic means of establishing the diagnosis of invasive candidiasis (Pappas et al. 2018).

Culture of respiratory samples is almost always positive for most “typical” bacteria causing respiratory tract infections (Murray et al. 2013). For mycobacteria, acid-fast (Ziehl-Neelsen) staining and culture remain at the core of any diagnostic algorithm with the sensitivity of 20-70% and specificity of 95-98% for AFB microscopy

Test	Purpose	Reason for inclusion	Caveats
Direct Microscopy	Rapid identification of bacteria, fungi, parasites, mycobacteria on many clinical samples and tissue sections (stained and unstained) Detection of organisms is based on morphological characteristics Possibility of initiation of appropriate therapy	Allows rapid identification to genus level (sometimes to species or species complex level) Cost effective Fast (2-4 hours) Minimal equipment requirement Light microscopes do not require electricity supply Staining and use of optical brighteners increases sensitivity 2 or more pathogens can be identified and differentiated by microscopy Result often influences treatment with potential to improve outcome	Usually not definitive identification apart from multi-cellular parasites and some fungi Dependant on personnel with specific microscopic skills Sensitivity varies with agent, source and quality of specimen and observer experience Quality assurance Viability: cannot differentiate between alive and dead cells Difficult to quantify and replicate Not an option where no lab facility

Table 19 (Part A) Purposes, reasons for inclusion and caveats of direct microscopy, fungal culture, bacterial culture and mycobacterial culture as essential diagnostic tests. Abbreviations - See P4.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

and the sensitivity of 95% and the specificity of 98% for culture based diagnosis (Azadi et al. 2018). In chronic pulmonary histoplasmosis, *Histoplasma* is isolated in culture of sputum or bronchoalveolar lavage in about 75% of patients (Hage et al. 2011). Fungal yield from respiratory samples is much lower in disseminated histoplasmosis. Culture is positive in only about 50% of patients with chronic pulmonary aspergillosis (Hope et al. 2005); high volume sputum culture has been shown to improve respiratory yields of *Aspergillus* spp. (Fraczek et al. 2014).

In disseminated histoplasmosis, culture of blood, respiratory specimens or tissues such as bone marrow are considered the standard diagnostic method. The highest culture yield is from bone marrow aspirate/ biopsy - positive in about 75% of cases (Hage et al. 2011). *Coccidioides* spp are rarely recovered in blood cultures, culture of respiratory secretions provides a modest sensitivity leaving histology as the diagnostic standard of care for the diagnosis of disseminated coccidioidomycosis (Guarner & Brandt 2011). Meanwhile, blood cultures plus skin biopsy for histology, culture or direct microscopy is the standard of diagnosis for disseminated talaromycosis (Guarner 2017; Chastain et al. 2017a). **(Table 19 A-C)** considers the purposes, reasons for inclusion and caveats of direct microscopy, fungal culture, bacterial culture and mycobacterial culture as essential diagnostic tests.

IN CONCLUSION

Direct microscopy was strongly endorsed as an essential diagnostic investigation in LMICS. Bacterial, fungal and mycobacterial culture are also essential microbiological investigations, including blood culture. Point of care tests for the diagnosis of bacterial and other infections are needed.

Test	Purpose	Reason for inclusion	Caveats
Fungal Culture	Identification and speciation of fungal pathogens Allows for antifungal susceptibility testing	Allows for targeted therapy Antifungal stewardship Resistance detection and guides antifungal selection Genus and often species identification has profound treatment and epidemiological significance Speed and accuracy of fungal culture reports impacts outcome Measure response to treatment Store strains for future use including genotyping and vaccine development Characterize the agent – for epidemiological purposes	Less sensitive than non-culture diagnostics for most fungal pathogens Blood and tissue cultures are slow (2-6 weeks) Biosafety level 3 laboratory requirement for dimorphic fungi Quality assurance Uninterrupted supply of electricity and at least 2 incubators at different temperatures

Table 19 (Part B) Purposes, reasons for inclusion and caveats of direct microscopy, fungal culture, bacterial culture and mycobacterial culture as essential diagnostic tests.
Abbreviations - See P4.

Test	Purpose	Reason for inclusion	Caveats
Bacterial Culture	Identification and speciation of bacteria Allows for drug susceptibility testing of bacterial pathogens Very few non-culture-based tests for common bacterial infections	Obtain definitive identification and characterization Usually positive for pathogen Determine most likely pathogen Allows detection of antibacterial resistance Characterize the agent - for epidemiological purposes	24-72 hour turnaround time Labor intensive Technically demanding - experienced staff Quality assurance Some bacteria do not grow such as Legionella, Mycoplasma, Leptospira Uninterrupted supply of electricity Automated blood culture systems are expensive
Test	**Purpose**	**Reason for inclusion**	**Caveats**
Mycobacterial Culture	Identification and speciation of tuberculous and nontuberculous mycobacteria Allows for drug susceptibility testing	Automatic culture systems positive in 7-10 days Guides anti-TB agent selection Allows for resistance monitoring Probably can be omitted on samples that are positive and fully susceptible by direct molecular testing	Low sensitivity for tissue and CSF samples Turnaround time; 2-6 weeks High cost Uninterrupted supply of electricity Requires sophisticated biosafety level 3 laboratory which is expensive to establish and maintain

Table 19 (Part C) Purposes, reasons for inclusion and caveats of direct microscopy, fungal culture, bacterial culture and mycobacterial culture as essential diagnostic tests.
Abbreviations - See P4.

Dr Tom M. Chiller
Chief, Mycotic Diseases Branch, CDC, USA

‘ Internal and external quality assessment are critical aspects of laboratory quality management in order to ensure consistently high diagnostic reporting for patient care partly through continuing technical education’

Prof. Jean-Pierre Gangneux
Rennes 1 university, Rennes, France

External Quality Assurance

Quality assurance is a set of quality management systems including diagnostic test documentation, use of standard operating procedures and quality control samples, and the utilization of external quality assessment schemes (EQAS) to ensure laboratories consistently provide correct and reliable test results.

EQAS aims at evaluating the accuracy of the entire testing process from receipt and analysis of sample to reporting of results (World Health Organization 2016). EQAS allows objective assessment of quality of testing of patients’ specimens as well as quality of services delivered, which are required by accreditation and regulatory bodies such as ISO 15189 regulation for medical laboratories and ISO 22870 for point-of-care tests (Sciacovelli et al. 2010; Kettelhut et al. 2003).

In most developed countries, EQA programmes are well established and have contributed greatly to improving the quality of care at all levels of the health care system (James et al. 2014). Additionally, international organisations like the United Kingdom National External Quality Assessment Scheme (UK NEQAS) have many centres across Europe, Asia, Africa, South American and the Middle East. Participation in an EQA program provides valuable data and information which allow comparison of performance and results among different test sites.

More recently, the Asia Fungal Working Group (AFWG) initiative survey of laboratory practices for diagnosis of fungal infection in seven Asian countries reported that only 56% participated in an EQAS, and only 43% conducted regular formal staff training (Chindamporn et al. 2018). There is clearly a need to facilitate improved participation, as this will lead to improved laboratory performance in the mid term.

EQAS is an integral part of continuous quality improvement and represents an educational stimulus to laboratory staff. It should not be punitive. For tests such as microscopy and culture, this is the only option for quality assessment as regulatory approval does not apply. For non-microscopy and culture tests certification by a regulatory authority, including prequalification by the WHO, is a necessary first step to ensuring quality standards, but does not address individual laboratory performance. EQAS therefore is a critical aspect of laboratory quality management to ensure consistently high diagnostic reporting for patient care through continuing technical education.

References available online at: https://www.gaffi.org/global-fungal-infection-forum-3-in-kampala/

Benefits of EQAS are numerous and include;

1. To enhance patient care and safety through improved laboratory practice
2. To characterise test bias and imprecision across multiple methods
3. To correlate specific method variables with bias and imprecision
4. To identify interfering substances and quantify their effects across multiple methods
5. To provide clinical laboratories with reliable information for replacing unsatisfactory methodologies
6. To identify clinical laboratories that are at risk for poor performance
7. To satisfy accreditation and regulatory requirements
8. To identify training needs
9. To act as an early warning for systematic problems associated with equipment, kits or personnel (post-market surveillance)
10. To provide an insight into and to improve national performance levels

Adapted from (**Table 3)** in Plebani et al (Plebani et al. 2008) with additional points added.

Examples of EQA schemes	India	UK	France	Finland	Netherlands
Mycology (culture, antifungal susceptibility)	●	●	●		
Aspergillus serology	●	●	●		
Cryptococcus serology		●	●		
Toxoplasma serology		●	●	●	
Antifungal drug monitoring					●

Table 20.
Available EQA programmes in Mycology, *Toxoplasma* serology and Antifungal drug monitoring.
Abbreviations - See P4.

WHO Model List of Essential In Vitro Diagnostics

The World Health Organization (WHO) published the first edition of the Model List of Essential In Vitro Diagnostics (EDL) in May 2018.

The EDL is intended to complement the WHO Model List of Essential Medicines (EML) and enhance its impact. WHO recognises that In Vitro Diagnostics (IVDs) are an essential component to advance universal health coverage, address health emergencies, and promote healthier populations.

The EDL outlines a group of IVDs that are recommended by WHO for use at various levels of a tiered national health care system. The EDL is not intended to be prescriptive with respect to the IVDs listed or the levels at which such IVDs can/should be used; rather country programmes should make the ultimate decisions about which IVDs are selected and where they are implemented, based on national or regional burden of disease, unmet needs and priorities.

The 1st EDL is presented by health care facility level in two tiers:

- **Tier I:** IVDs for Primary health care where no laboratories are available, only minimal facilities such as microscopy and possibility of self-testing or point of care testing
- **Tier II:** IVDs for Health care facilities with clinical laboratories, including district hospital/laboratory, regional/provincial/specialised laboratories, and national reference laboratory.

Based on the selection criteria described below, the EDL consists of:

- A group of general laboratory tests that can be used for routine patient care as well as for the detection and diagnosis of a wide array of disease conditions – communicable and NCDs.e.g. clinical chemistry, serology, haematology, microbiology and mycology..
- IVDs designed for the detection, diagnosis and monitoring of each of the following WHO key disease areas: HIV, TB, malaria, HBV/HCV, and HPV and syphilis.

The EDL does not list specific test brands, but rather consists of IVDs described according to their biological targets. Where specific products in categories of tests contained in the EDL have been prequalified by WHO or are recommended by a WHO disease programme, a link is provided to that information, which is updated regularly.

The EDL will be expanded and updated annually with the intention to ultimately cover a broad, comprehensive spectrum of disease. WHO will issue a call for applications to add IVD test categories to the next edition of the EDL in mid-2018. The call will request applicants to provide information on clinical accuracy or impact of the proposed IVDs. The first EDL will be expanded significantly over the next few years, incorporating tests for other important areas such as antimicrobial resistance, additional NCDs, emerging pathogens, emergencies and outbreaks, and neglected tropical diseases. It is foreseen that the EDL will be an important tool to increase access to appropriate, affordable, and quality-assured IVDs, particularly where they are most needed to address health priorities.

http://www.who.int/medical_devices/diagnostics/WHO_EDL_2018.pdf

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- Universal access to diagnostics for serious fungal disease
- Universal access to antifungal agents
- Accurate data on the number and severity of fungal infections
- Health professional education related to better recognition and care for patients with serious fungal disease

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