**Diagnostics Survey** Africa 2022

# DIAGNOSTICS FOR FUNGAL DISEASE IN AFRICA

## A GAFFI SURVEY 2022

African Union

AFRICA CDC
Centres for Disease Control and Prevention
Safeguarding Africa's Health

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# Introduction from the Africa Centres for Disease Control and Prevention (Africa CDC)

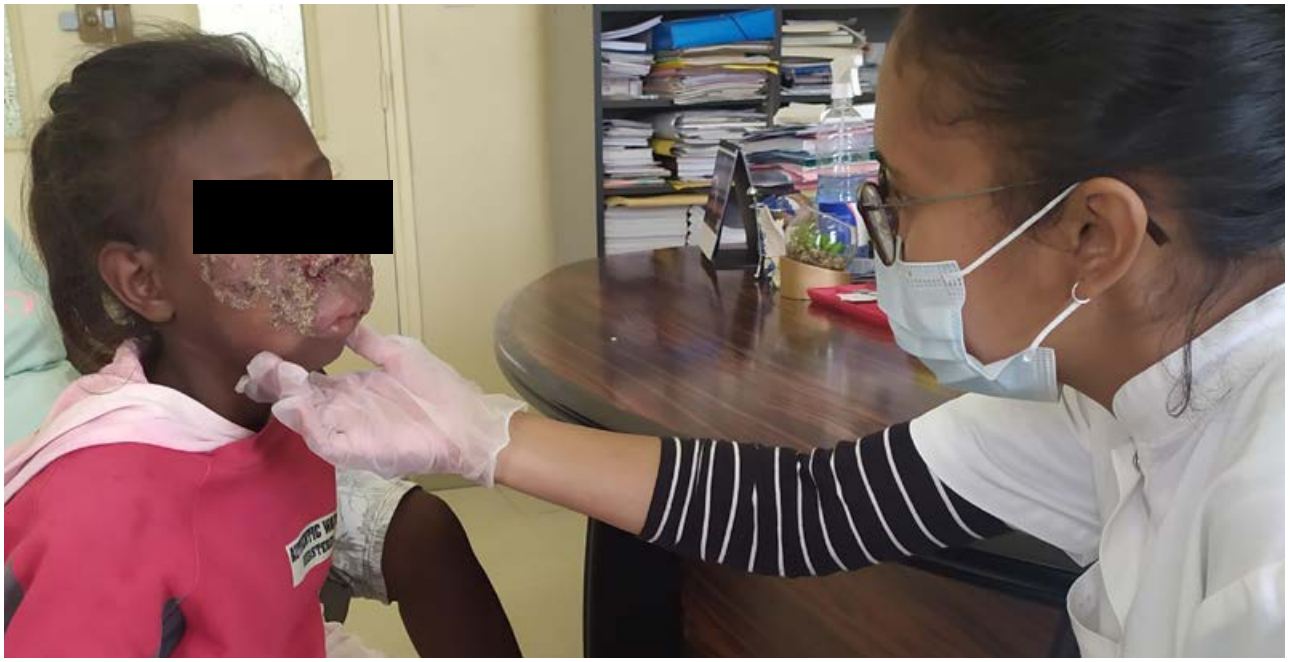
In Africa, despite the high morbidity and mortality in at-risk populations, fungal diseases continue to be neglected. Limited availability of affordable diagnostic and healthcare workers, vertical health systems, and poor access to antifungal drugs on the continent contribute to premature deaths from this disease.

GAFFI in collaboration with experts in 48 African Union Member States, conducted the first-ever in-depth situational survey to comprehensively evaluate access to diagnostics and treatment for fungal diseases and identify existing gaps. The findings of this survey provide a solid foundation for identifying targeted, cost-effective interventions for better access to diagnosis and treatment in the most at-risk populations in Africa.

Following this survey, high-level decision-makers, experts, and representatives from civil society organizations gathered at virtual consultation meetings organized by the Africa CDC and GAFFI to review the findings of this survey, agree on shared priorities for sustainable improvement and catalyze the momentum for urgent deployment of interventions to improve access to diagnostics and treatment of fungal diseases in Africa.

The priorities of support identified are in line with the New Public Health Order, Africa CDC's vision for the continent. The continent needs strong African public health institutions, expanded manufacturing of diagnostics and therapeutics, investment in the public health workforce, increased domestic investment in health and respectful, action-oriented partnerships. Fungal diseases are tractable as a public health threat in the continent, starting with significant expansion in diagnostics.

Addressing fungal diseases as a priority on a continent with a substantial burden of diseases such as HIV/AIDS and TB is not only a public health urgency but also a moral imperative.



# Contents

# Executive summary

The African continent is home to over a billion people, and has a high burden of HIV infection and fungal skin diseases, an increasing number of people with cancer and chronic lung disease, with many more treated with therapies that affect the immune system.

Accurate diagnosis is crucial for optimal care and reducing unnecessary antimicrobial prescribing. Serious fungal disease is often relatively silent and needs to be actively sought to maximise the chance of survival and minimise disability. Many new fungal disease diagnostic tests using lateral flow technology and molecular techniques have been launched in the last 10 years. In addition to microscopy, histopathology and fungal culture, some have been deemed 'Essential' by the World Health Organization (list of essential in vitro diagnostics, EDL).

Systemic market failure to incorporate into care packages the latest generations of rapid tests is commonplace on the African continent. This detailed survey, with multiple validation steps in country, finds some countries with almost no tests and others with an almost complete portfolio, although no African country is fully equipped to meet the challenges of fungal diseases, even in the private sector. The survey reports on data from 48 of 55 African Union Member States.

**Current diagnostic status for some diagnostics include:**

- 23 countries are using cryptococcal antigen (CrAg) testing often or occasionally for fungal meningitis in AIDS but 22 do not use it (EDL, 2018).
- Very few countries test for *Histoplasma* antigen in urine (EDL, 2019).
- 7 countries offer molecular testing for *Pneumocystis* pneumonia in AIDS and cancer, but 37 do not (EDL, 2021).
- 4 countries provide *Aspergillus* antibody testing often or occasionally to diagnose chronic pulmonary aspergillosis (often mistaken for TB) (EDL, 2021).
- For asthma diagnosis, 18 countries use spirometry regularly or occasionally. Severe fungal asthma is responsive to oral antifungal therapy.
- 27 countries provide diagnostics for fungal keratitis, a debilitating condition which results in at least 10% of the affected eyes perforating and 100% chance of blindness without therapy (EDL, 2018).
- 4 countries provide *Aspergillus* antigen testing often or occasionally for fungal pneumonia in COVID-19, influenza and leukaemia (EDL, 2021).

The report also details each country's capabilities in diagnostics, with single page summaries (Appendix 4).

Some of the diagnostic tests such as CT and MR imaging, blood culture and histopathology are required for a broad range of conditions, including fungal disease. The financial burden of diagnostics usually falls directly on patients and families, and few countries fully support diagnostics for fungal disease.

GAFFI highlights the many opportunities to integrate diagnostics into different levels of the health system. It also summarises who should be tested and the ideal turnaround time for results. A final section addresses surveillance and research needs across the continent.

This reports highlights the still long road to travel to offer even WHO approved Essential Diagnostics to many of the continent's citizens.

# Acknowledgements

This survey was led by Emma Orefuwa, supported by Richard Penney and David Denning. It was only possible because of the remarkable contributions from a large body of committed professionals from across the African continent, as well as many who assisted in networking (page 6).

This report was internally funded by GAFFI, without any direct external report. A substantial proportion of GAFFI's efforts are enabled through voluntary gifts of time. GAFFI receives donations from a wide variety of sources, including other foundations, industry, societies and private individuals.

GAFFI's partners are listed here: weblink

and in each annual report: weblink

and in each Global Fungal Infection Forum report weblink (resources tab).

### Images

Skin consultation (page 2) and skin biopsy (Lala Soavina Ramarazatovo, Antananarivo), *Aspergillus* hyphae direct microscopy (Shamithra Sigera, Colombo), positive histoplasmin skin reaction (Rita Oladele, Lagos), running PCR assay (Sabelle Jallow, Johannesburg), lumbar puncture (David Meya, Kampala), spirometry (Pauline Ndagire, Kampala), bronchoscopic image (Jeremiah Chakaya, Nairobi) slit lamp examination and corneal sampling handling (Simon Arunga, Mbarara). All other images were from David Denning's longstanding educational library, and previously unpublished.

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# Introduction

Accurate diagnosis is integral to good medical practice. Most serious fungal diseases are clinically quiet, until they are advanced, rather like cancer, and early diagnosis is critical to a good outcome. The last few years have seen a positive transformation in diagnostics for fungal disease - more sensitive tests, simplicity, a better understanding of which at-risk patients to apply them to, speed of result and reduced cost. Several of the best performing tests are now listed as Essential by the World Health Organization, and expanded access to everyone across the world is now required.

GAFFI's recent estimations of the burden of fungal diseases and associated premature mortality, indicates that globally expanded access to these diagnostics, linked with access to generic antifungals, could reduce global deaths by 1.3 million per year. This survey of the current status of diagnostic access in Africa for fungal disease is intended as a benchmark for the future, for each country.

Investment by countries and hospitals in fungal diagnostics directly supports many of the Sustainable Development Goals (see panel right).

GAFFI presents here data for 48 African Union Member States with populations over 1 million, the current situation with respect to clinical diagnostic procedures necessary for making most fungal disease diagnoses, radiology capacity, access and radiologist interpretation and mycology diagnostics, for laboratories but also for community and local hospital use in some cases.

### How access to diagnostics impacts positively on the Sustainable Development Goals (SGD)

- **SDG3** Ensuring healthy lives and promoting well-being for all at all ages
- **SDG1** Promoting the health needs of the poor - especially HIV/AIDS, TB and NTDs.
- **SDG4** Supporting high-quality education for all to improve health and health-equity - healthcare worker education in particular.
- **SDG8** Promoting health employment as a driver of inclusive economic growth - directly with more laboratory personnel and expanded roles in community care, radiology and information technology, and indirectly in supply chains for diagnostics and antifungal agents.
- **SDG9** Promoting national R&D capacity and manufacturing of affordable essential medical products - notably epidemiology studies, surveillance and laboratory consumables and computers.
- **SDG12** Promoting responsible consumption of medicines to combat antimicrobial resistance - notably widespread adoption of rapid and sensitive diagnostics, antifungal surveillance and antifungal stewardship programs.
- **SDG16** Empowering strong local institutions to develop, implement, monitor and account for ambitious national SDG responses - notably development of critical mass in fungal disease diagnosis and management with surveillance networks.
- **SDG17** Mobilising partners to monitor and attain the health-related SDGs - development of public health mycology, on a strong epidemiology and surveillance background.

SUSTAINABLE DEVELOPMENT GOALS

# Key diagnostic tests for important fungal conditions

| Fungal condition | Key tests | Clinical setting |
|---|---|---|
| Cryptococcal meningitis | Cryptococcal antigen (serum and CSF), lumbar puncture, fungal culture of CSF | New HIV patients, HIV admission to hospital & HIV clinics |
| *Pneumocystis* pneumonia | CXR, CT scan chest, *Pneumocystis* PCR (respiratory samples or nasopharyngeal aspiration), serum Beta D glucan, bronchoscopy | New HIV patients, HIV admission to hospital (adults and children), cancer patients with pneumonia |
| Candidaemia and invasive candidiasis | Blood culture, peritoneal or abdominal drain microscopy & culture, serum Beta D glucan | ICU, renal failure, premature babies, abdominal surgery patients, chronic ambulatory peritoneal dialysis |
| Invasive aspergillosis | *Aspergillus* antigen, microscopy and culture (respiratory samples), CT scan, bronchoscopy | Lung cancer, leukaemia and lymphoma patients, ICU (including severe influenza and COVID-19), advanced HIV |
| Disseminated histoplasmosis | *Histoplasma* antigen (urine and serum) | Advanced HIV and other immunocompromised people |
| Fungal keratitis | Corneal scraping, microscopy and fungal culture | Ophthalmology |
| Chronic pulmonary aspergillosis | CXR, CT scan, *Aspergillus* antibody, fungal culture (sputum) | Lung disease, especially TB patients |
| Fungal asthma | Spirometry, CXR, *Aspergillus* IgE, total IgE, sputum fungal culture | Lung disease |
| Skin fungal NTDs (mycetoma, chromoblastomycosis, sporotrichosis) | Skin biopsy, microscopy, fungal culture, histopathology | Community services and dermatology |
| Ringworm, tinea capitis, onychomycosis etc | Microscopy & fungal culture (skin, hair or nail samples) | Community services and dermatology |
| Recurrent vaginal candidiasis | Microscopy and fungal culture | Community services, STD clinics and gynaecology |

**Table 1. Key diagnostic tests for important fungal conditions, stratified by urgency and clinical setting**

CAPD = chronic ambulatory peritoneal dialysis; STD = sexually transmitted diseases; CXR = chest X-ray; ICU = intensive care unit; CSF = cerebrospinal fluid; PCR = polymerase chain reaction.

- Immediately life-threatening
- Immediately sight-threatening
- Major morbidity and life-threatening
- Major morbidity
- Significant morbidity

# Survey methodology

**GAFFI conducted the survey in seven phases:**

1. Questionnaire development with later iterative improvements.
2. Each questionnaire was then completed by one or more respondents in each country using a snowball sample starting with GAFFI Ambassadors and existing networks of contacts. Respondents were encouraged to reach out to colleagues in areas where they did not have first-hand knowledge. In larger countries, in order to ensure thorough coverage, additional responses were sought from different parts of the country.
3. Each response was inputted for analysis by the GAFFI team and checked against other data sources (such as company sales, and other NTD programs). Data was compiled and visualised using QGIS software and Natural Earth vectors to design maps showing each diagnostic's coverage across the continent.
4. A video conference call with each respondent and team members to review the data and check full understanding of the question (with interpreters on the call if required),
5. An additional external validation check was then carried out from public (i.e. literature) or private sources.
6. The next validation step was held via video conference call with country stakeholders in relevant topics (i.e., HIV/AIDS, laboratory coordination) and/or Ministry of Health representatives, and the national laboratory service, as well as the initial questionnaire respondent(s). Collected data and country profiles were distributed to relevant local stakeholders and experts, to verify data and/or correct inaccuracies.
7. The final validation step consisted of a series of five regional webinars presenting the survey methodology and results to each of Africa CDC's regions in October 2022. Each country profile was shared before and after the webinars and feedback requested, or confirmation that the profile was substantially correct. Country profiles were translated into French and Portuguese for francophone and lusophone countries respectively.

One-page profiles were also created for each country, summarising the data collected alongside basic information about the country, demographic data, key health indicators relevant to fungal disease and its health system (Appendix 4). This information included country-specific data on: HIV (provided by UNAIDS); TB (WHO); asthma world health survey 2003 and other data (To et al, 2012); cancer (Globocan); GDP (IMF); total health expenditure (World Bank); domestic health expenditure (WHO); area, population and age structure (CIA World Factbook).

**Table 2. Diagnostic tests and procedures surveyed**

| Clinical procedures | Lab tests ( *Listed on the WHO's Essential Diagnostic List ) | Radiology |
|---|---|---|
| Bronchoscopy | Direct microscopy* | Chest X-ray |
| Spirometry | Blood culture* | CT scan |
| Corneal scraping | Histopathology* | MRI scan |
| Lumbar puncture | Culture* | Radiologist reporting |
| Skin biopsy | Cryptococcal antigen test (CrAg)* | |
| | *Histoplasma* antigen test* | |
| | *Aspergillus* antigen test* | |
| | *Aspergillus* antibody test* | |
| | *Pneumocystis* PCR test* | |
| | CD4 count (criteria and assay)* | |

# RAPID ANTIGEN AND ANTIBODY TESTS

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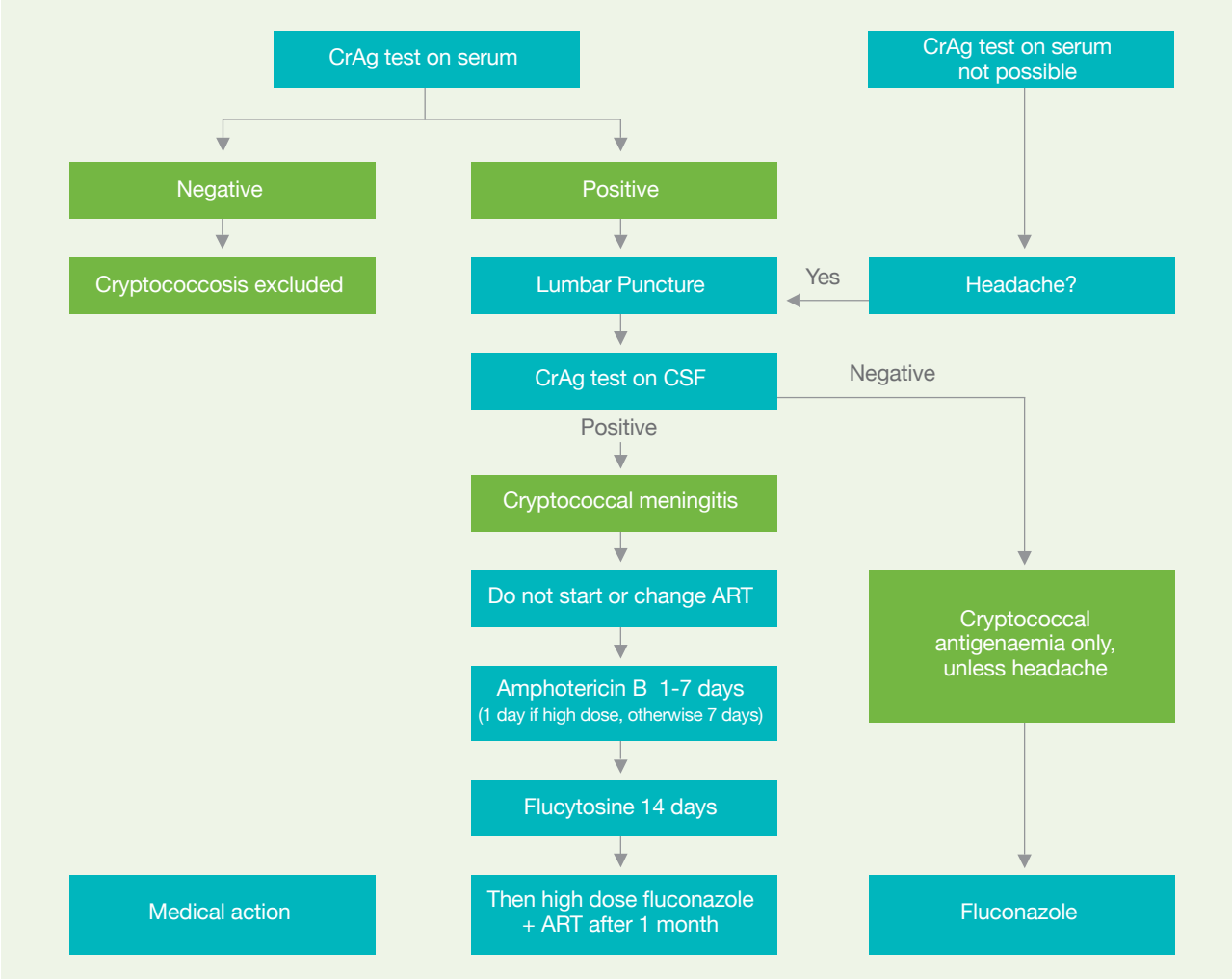
# Cryptococcal antigen (CrAg)

*Cryptococcus* spp. cause meningitis. These fungi shed a complex sugar (antigen) during human infection which is slow to clear from the bloodstream and CSF. Detection of this antigen is the basis of the cryptococcal antigen test, initially as a latex agglutination assay, then an EIA and now a lateral flow assay.

The simplicity and excellent performance of this test (sensitivity and specificity of >99% (Jarvis, 2011)), combined with its low cost, place it as one of the very best tests in all of microbiology; and it does rapidly diagnose a treatable life-threatening infection.



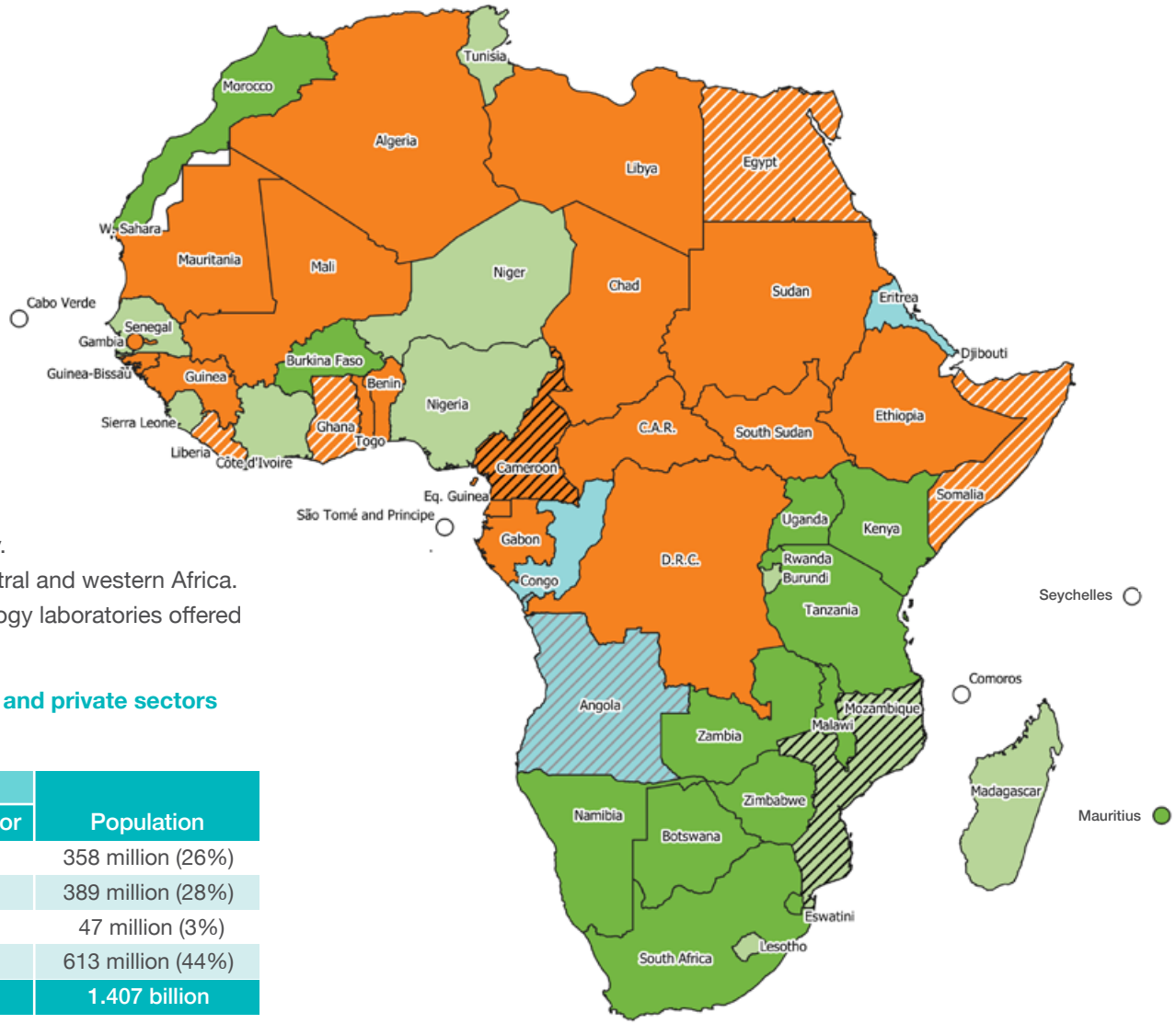

**Right**
**At risk HIV patients (new, admitted to hospital, <200 CD4 count)**

**CrAg** was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended for screening and diagnosing cryptococcal meningitis in people with advanced HIV disease as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b)

World Health Organization

# Availability of CrAg testing by country

- No Data
- Not publicly available / used rarely
- Publicly available / used rarely
- Privately available / used rarely
- Publicly available / used occasionally
- Privately available / used occasionally
- Publicly available / used often
- Privately available / used often
- Population < 1 million




**Key points**

1. Most testing is now done by qualitative lateral flow assay.
2. Access is much better in eastern and southern, than central and western Africa.
3. Within Nigeria, only 45% of 22 tertiary hospital microbiology laboratories offered any CrAg testing (Osaigbovo, 2021).

**Availability of cryptococcal antigen testing in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 14 (29%) | 9 (19% | 358 million (26%) |
| Occasional | 9 (19%) | 5 (10%) | 389 million (28%) |
| Rarely | 3 (6%) | 6 (13%) | 47 million (3%) |
| Never | 22 (46%) | 28 (58%) | 613 million (44%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# CrAg testing. Additional Information

### Cost and ease of use

1. Cost per assay is approximately $4 including shipping and plasticware.
2. It takes very little technical time or specialised skill.

### CrAg screening

1. It is a highly cost-effective intervention.
2. It is associated with an incremental cost-effectiveness ratio of $6.14 per disability-adjusted life year (DALY) averted compared to no screening (Ramachandran, 2017).
3. Implementation of CrAg screening in Uganda (as an example) was projected to cost only $1.52 more per person, with a corresponding 40% relative reduction in cryptococcal-associated mortality.

### Guidelines

CrAg is recommended by the WHO and numerous country guidelines for patients with advanced HIV disease.

'Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy' weblink

'WHO guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children, 2018' weblink

Updates listed here: weblink

### Suppliers

There are several suppliers of cryptococcal antigen tests, listed here: weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Adults with Advanced HIV Disease, including newly hospitalised patients, are the major group to be routinely tested. In areas with a high HIV prevalence, patients presenting with neurological features (confusion, headache, stroke-like syndrome, neck stiffness or photophobia) should be dually tested for HIV and CrAg, the CrAg test less important if they are HIV negative. Other immunocompromised patients with these features should also be tested for CrAg, although the sensitivity of serum CrAg is lower.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | Yes |
| Skin rash, or lumps | Yes if HIV+ |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | Sometimes |
| Critically ill and ICU | |
| Fever and sepsis | Yes if HIV+ |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Possibly | Possibly |
| Local HIV clinics | Yes | Preferably |
| Local hospitals | Yes | Preferably |
| Referral hospitals | Yes | Yes |
| Teaching/specialist hospitals | Yes | Yes |

# *Aspergillus* IgG antibody

The symptoms and radiology of chronic pulmonary aspergillosis are similar to those of TB (Baluku, 2021). *Aspergillus* IgG antibody levels play a critical role in the diagnosis and exclusion of chronic pulmonary aspergillosis (CPA) as well as in the monitoring of treatment (Page, 2015). Recovery of *Aspergillus* in culture is difficult but when it grows is generally discounted as a contaminant unless experienced staff are involved, so the diagnosis can be elusive.

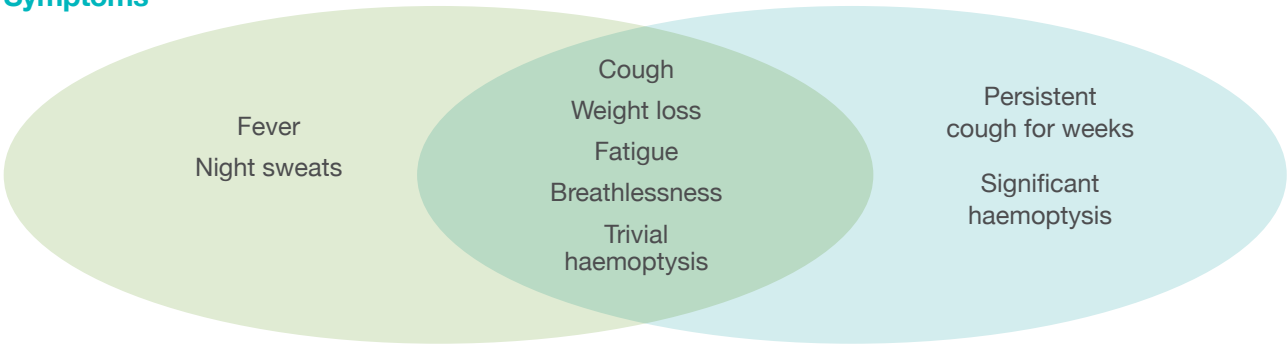
*Aspergillus* IgG antibody is detectable in 80-92% of patients with CPA, being falsely negative in a few patients with subtle immunodeficiency. *Aspergillus* IgG antibody is just as useful in HIV positive people as those who are negative.

### Laboratory methodologies

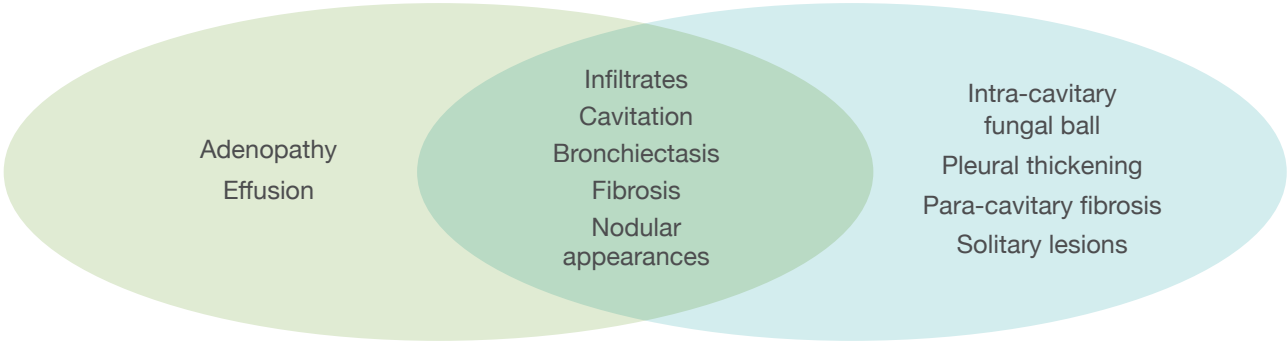
There are 4 laboratory methodologies used: Enzyme-linked immunosorbent assay (ELISA), automated immune detection, lateral flow and the less sensitive and slow precipitins test. The lateral flow assay has an excellent performance but is not quantitative, unlike ELISA and the automated platforms. Quantification is useful in monitoring treatment response and relapse.

## Similarities and differences between TB and CPA

### Symptoms



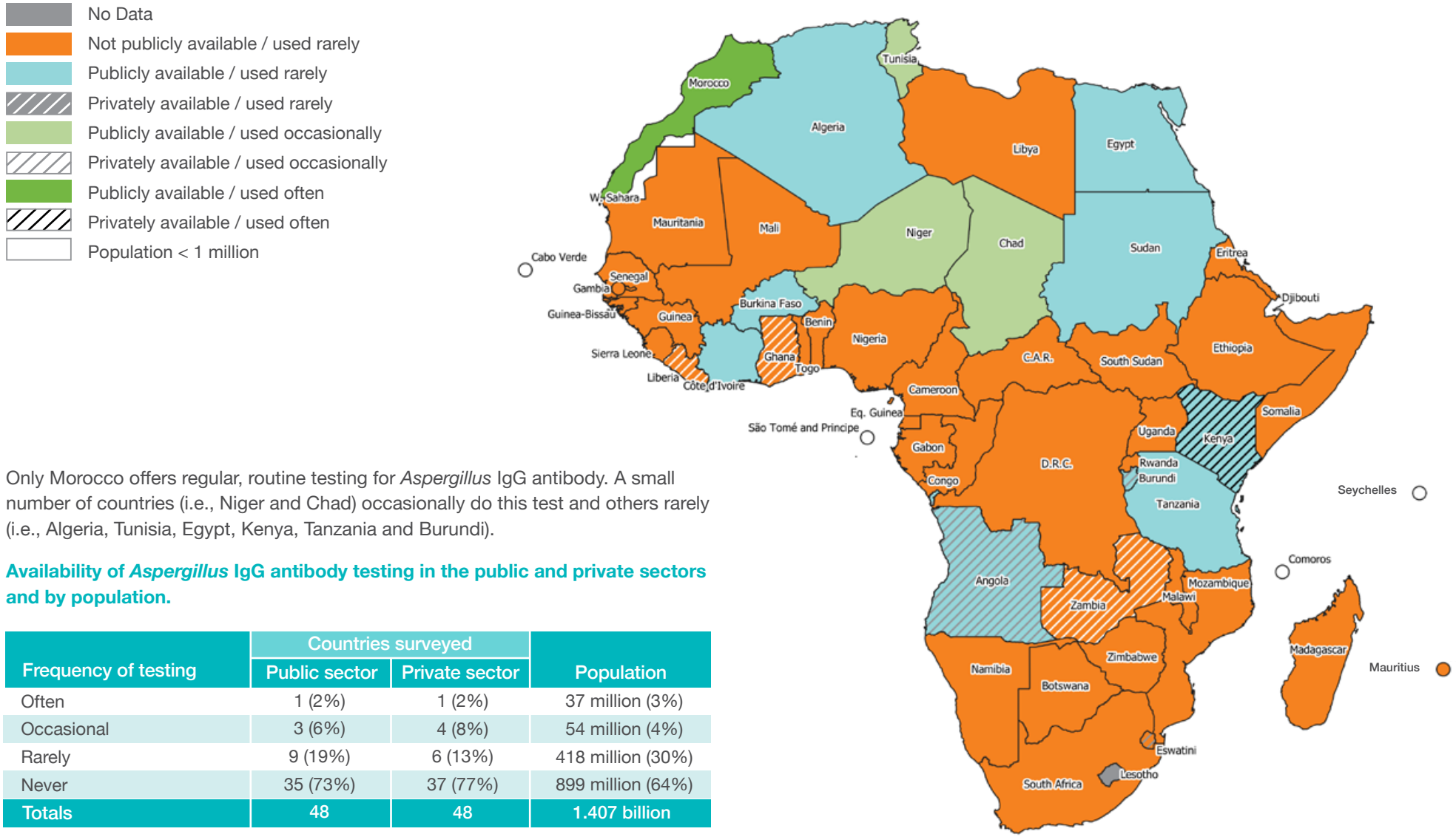

### Radiological features




***Aspergillus*** IgG antibody was added to the Essential Diagnostics List in 2021 (3rd EDL, 2021).
It is recommended as an aid to the diagnosis of chronic pulmonary aspergillosis as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b)

World Health Organization

# Availability of *Aspergillus* IgG testing by country




Only Morocco offers regular, routine testing for *Aspergillus* IgG antibody. A small number of countries (i.e., Niger and Chad) occasionally do this test and others rarely (i.e., Algeria, Tunisia, Egypt, Kenya, Tanzania and Burundi).

**Availability of *Aspergillus* IgG antibody testing in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 1 (2%) | 1 (2%) | 37 million (3%) |
| Occasional | 3 (6%) | 4 (8%) | 54 million (4%) |
| Rarely | 9 (19%) | 6 (13%) | 418 million (30%) |
| Never | 35 (73%) | 37 (77%) | 899 million (64%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# *Aspergillus* IgG testing. Additional information

### Cost and ease of use

Most *Aspergillus* antibody assays are $4 to $8 each.

### Guidelines

*Aspergillus* IgG antibody testing is an intrinsic component of the diagnosis of both chronic and allergic pulmonary aspergillosis as documented in guidelines (Denning, 2016; Hurrass, 2017).

Updates listed here: weblink

### Suppliers

There are several suppliers of *Aspergillus* antibody tests, listed here: weblink

Evidence submitted to the WHO to support inclusion of *Aspergillus* IgG antibody in the 3rd Model List of Essential in Vitro Diagnostics can be found here:

weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Adults with unconfirmed pulmonary TB, poorly responsive TB during therapy and patients with recurrent clinical or radiological features after completing therapy for pulmonary TB should be tested for *Aspergillus* IgG antibody. Patients with cavitation, pleural thickening and infiltrates around cavities are likely to have chronic pulmonary aspergillosis.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | No | No |
| Local TB clinics | Yes | Preferably |
| Local hospitals | Yes | Preferably |
| Referral hospitals | Yes | Yes |
| Teaching/specialist hospitals | Yes | Yes |

# *Aspergillus* antigen

The *Aspergillus* antigen test is primarily of value to diagnose invasive (and chronic) aspergillosis.

Invasive aspergillosis is almost always fatal unless diagnosed and treated promptly. It is silent in its initial clinical manifestations and cultures are very insensitive so unless clinical suspicion is very high, and appropriate non-culture-based tests are done, the majority of patients do not survive. In Italy only 11% of invasive aspergillosis cases in AIDS patients were diagnosed in life (Antinori, 2009).

The dominant assay globally has been a sandwich ELISA (BioRad), although other companies now offer similar assays. Recently 2 lateral flow assays have been introduced. One detects galactomannan — a sugar produced by *Aspergillus* - and the other detects a protein antigen.

Over 50 studies of *Aspergillus* antigen detection in serum have been done, mostly in leukaemia patients. A meta-analysis found the sensitivity to be ~78% and the specificity 85% (Leeflang 2015). In bronchoalveolar lavage fluid, a meta-analysis of 17 studies in cancer and transplant patients found a sensitivity of 88% and specificity of 81% (Heer 2019). Levels fall slowly with successful therapy and rise or remain unchanged in patients who fail antifungal therapy. Sputum is not an adequate clinical sample to test *Aspergillus* antigen but tracheal samples from intensive care can be used.

In addition to HIV and leukaemia, invasive aspergillosis is associated with COVID and influenza.

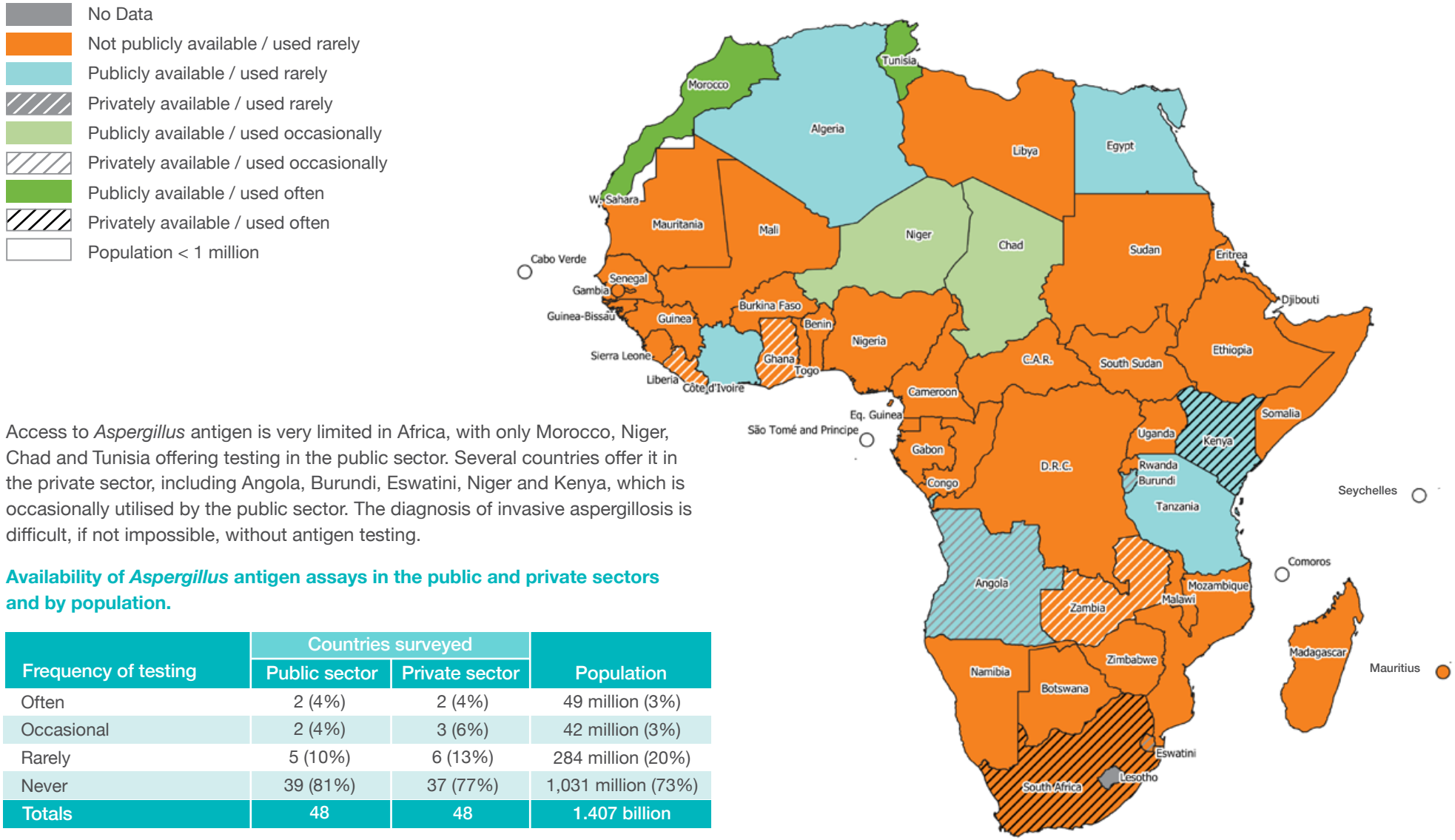
Some patients with other fungal infections have a cross reaction with *Aspergillus* antigen, notably those with talaromycosis and histoplasmosis, but the assay is less sensitive for these infections.

**Below**
**An example of the BioRad ELISA Aspergillus antigen test.**


***Aspergillus*** antigen was added to the Essential Diagnostics List in 2021 (3rd EDL, 2021). It is recommended to aid in the diagnosis of invasive aspergillosis in immunocompromised patients as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b).

World Health Organization

# Availability of *Aspergillus* antigen testing by country




Access to *Aspergillus* antigen is very limited in Africa, with only Morocco, Niger, Chad and Tunisia offering testing in the public sector. Several countries offer it in the private sector, including Angola, Burundi, Eswatini, Niger and Kenya, which is occasionally utilised by the public sector. The diagnosis of invasive aspergillosis is difficult, if not impossible, without antigen testing.

**Availability of *Aspergillus* antigen assays in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 2 (4%) | 2 (4%) | 49 million (3%) |
| Occasional | 2 (4%) | 3 (6%) | 42 million (3%) |
| Rarely | 5 (10%) | 6 (13%) | 284 million (20%) |
| Never | 39 (81%) | 37 (77%) | 1,031 million (73%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# *Aspergillus* antigen testing. Additional information

### Cost and ease of use

Depending on the system, the cost per assay of *Aspergillus* antigen is in the $10-$15 range.

### Guidelines

*Aspergillus* antigen testing is recommended for the diagnosis of invasive aspergillosis by all guidelines that detail diagnostic testing, including the Middle East (Al Abdely, 2014), UK (Schelenz, 2015), Europe (Ullman, 2018) and guidelines specifically for influenza (Netherlands) (SWAB, 2017), solid organ transplants (USA) (Hussein, 2019) and COVID-19 (Hashim, 2021). The recommendations are strong for diagnosis using bronchoalveolar lavage (including intensive care samples), but weaker for serum, except in neutropenia patients not on mould-active antifungal prophylaxis. It is also recommended for testing bronchoalveolar lavage fluid from suspected chronic pulmonary aspergillosis patients (Denning, 2016).

Updates listed here: weblink

### Suppliers

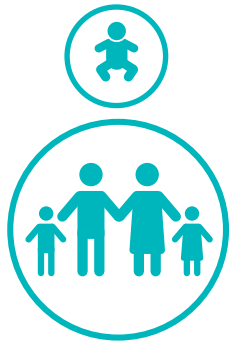
There are several suppliers of *Aspergillus* antigen tests, listed here: weblink

Evidence submitted to the WHO to support inclusion of *Aspergillus* antigen in the 3rd Model List of Essential in Vitro Diagnostics can be found here:

weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. The best sample is a bronchoalveolar lavage and this should be tested for *Aspergillus* antigen in all immunocompromised patients. Serum is a good sample for testing in neutropenic patients and may be positive in other immunocompromised people. Other respiratory samples (such as tracheal aspirate taken in intensive care) may also have high levels of *Aspergillus* antigen.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes if cancer or HIV |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Yes |
| Fever and sepsis | |



**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | No | No |
| Local TB clinics | No | No |
| Local hospitals | Preferably | No |
| Referral hospitals | Preferably | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

# LABORATORY FUNGAL DIAGNOSTIC TESTS

gaffi.org

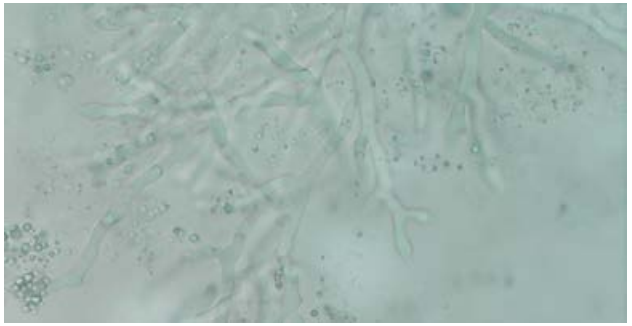
# Direct microscopy

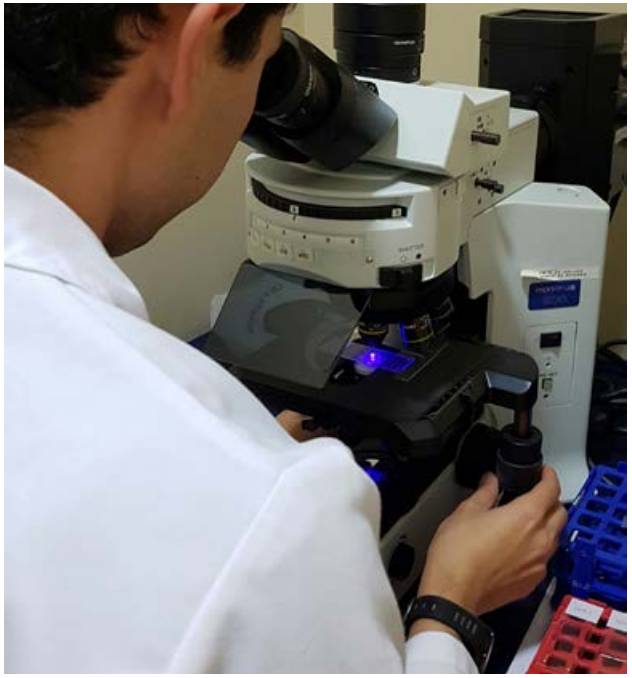
Direct microscopy for fungal hyphae or yeast cells is quick and highly specific if positive, but often negative. It takes considerable skill and training to do well, and is time consuming; too few trained staff are available. The commonest samples submitted to labs are nails, skin scrapings and vaginal samples. Less common samples are hair, sputum, bronchoscopy, urine and needle aspirations. As skin, nail and hair fungal infections affect ~1 billion people and 70% of women develop vaginal candidiasis, the number of samples is large, varying by local practice and disease prevalence.

Most samples are simply inspected directly without any pre-processing. For diluted samples, such as bronchoalveolar lavage or urine, centrifugation is used, and the sediment examined – sometimes using a cytocentrifuge. The most common stains used for microscopic evaluation are Gram stain, calcofluor white (CFW) (requiring fluorescence microscopy) and potassium hydroxide (KOH). More specialised stains (Giemsa, periodic acid Schiff, Gomori methenamine silver or Papanicolaou) can also be used, especially in cytology.

### Availability of India ink direct microscopy for CSF

India ink microscopy is less sensitive than cryptococcal antigen (CrAg) but is faster than culture. If routine and fast CrAg testing is available for CSF, then India ink microscopy is unnecessary.





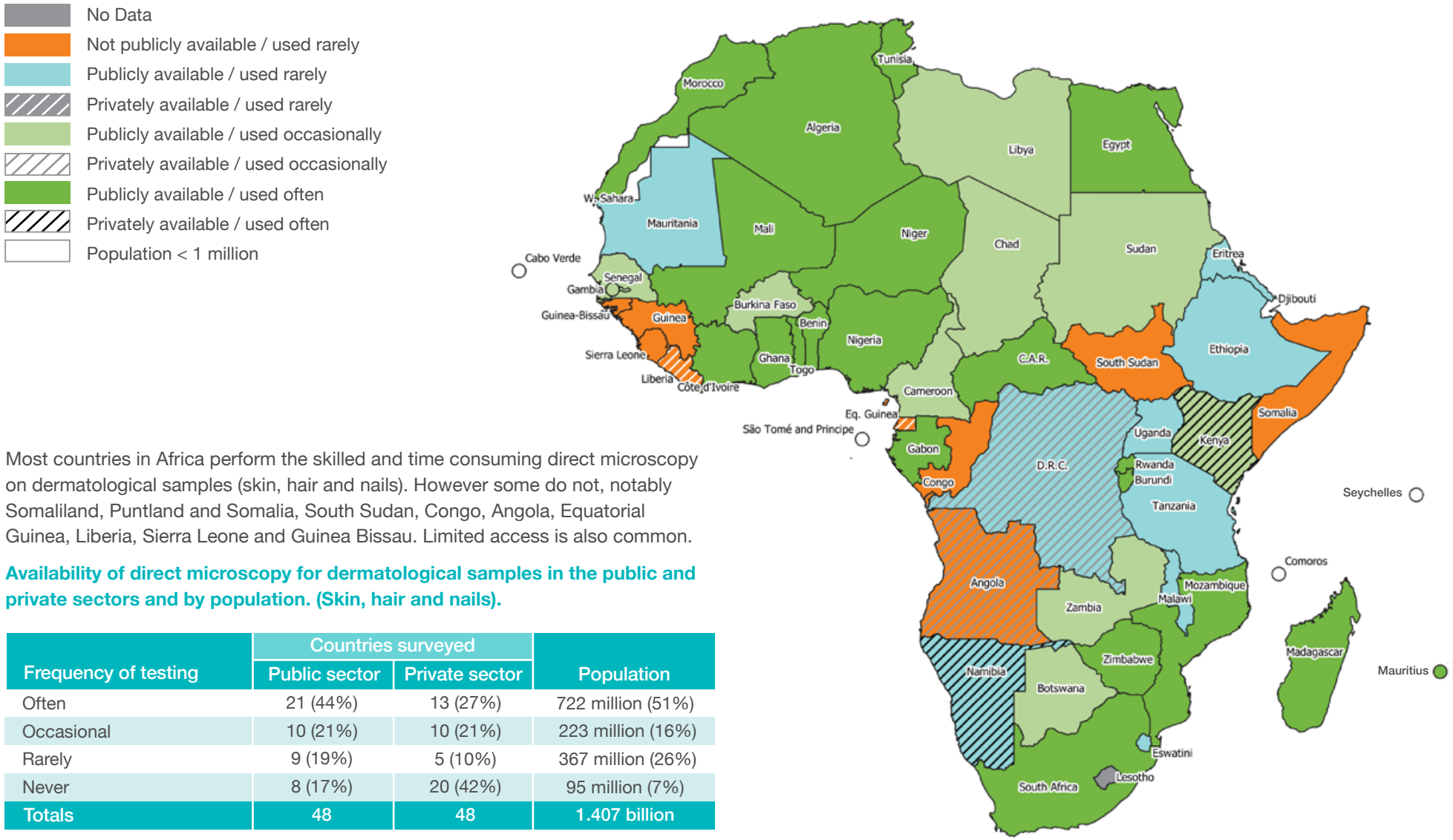
**India ink direct microscopy of CSF in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 23 (48%) | 12 (25%) | 471 million (34%) |
| Occasional | 13 (27%) | 13 (27%) | 665 million (47%) |
| Rarely | 6 (13%) | 5 (13%) | 213 million (15%) |
| Never | 6 (13%) | 18 (38%) | 58 million (4%) |
| **Totals** | **48** | **48** | **1.407 billion** |

**Microscopy** of multiple specimen types was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended for assessment of cells for infection, neoplasia, inflammatory and degenerative disorders under cytology as a general IVD for use in clinical laboratories (EDL 3, Section II.a).

World Health Organization

# Availability of direct microscopy for dermatological samples

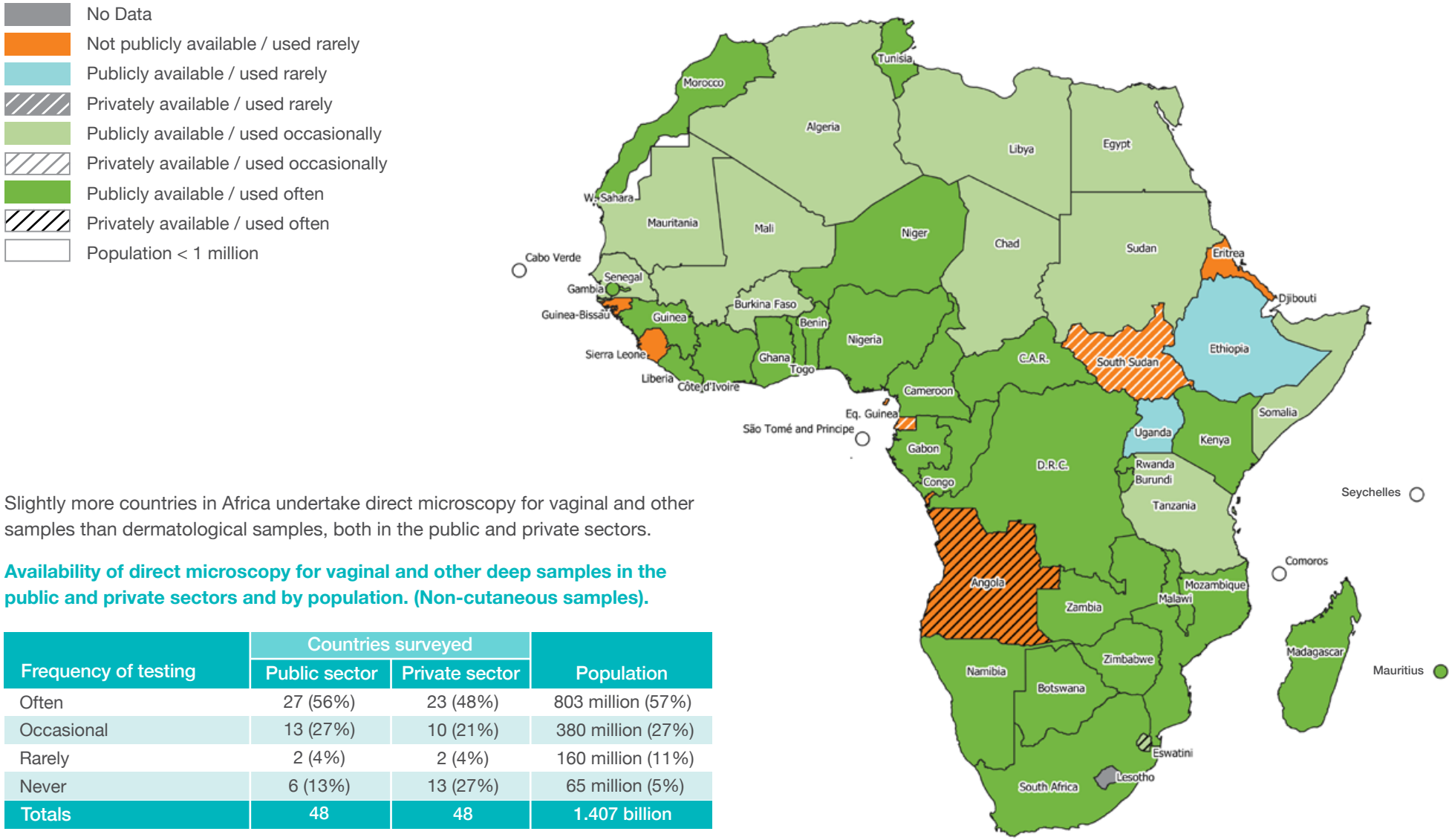



Most countries in Africa perform the skilled and time consuming direct microscopy on dermatological samples (skin, hair and nails). However some do not, notably Somaliland, Puntland and Somalia, South Sudan, Congo, Angola, Equatorial Guinea, Liberia, Sierra Leone and Guinea Bissau. Limited access is also common.

**Availability of direct microscopy for dermatological samples in the public and private sectors and by population. (Skin, hair and nails).**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 21 (44%) | 13 (27%) | 722 million (51%) |
| Occasional | 10 (21%) | 10 (21%) | 223 million (16%) |
| Rarely | 9 (19%) | 5 (10%) | 367 million (26%) |
| Never | 8 (17%) | 20 (42%) | 95 million (7%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Availability of direct microscopy for vaginal and other deep samples




Slightly more countries in Africa undertake direct microscopy for vaginal and other samples than dermatological samples, both in the public and private sectors.

**Availability of direct microscopy for vaginal and other deep samples in the public and private sectors and by population. (Non-cutaneous samples).**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 27 (56%) | 23 (48%) | 803 million (57%) |
| Occasional | 13 (27%) | 10 (21%) | 380 million (27%) |
| Rarely | 2 (4%) | 2 (4%) | 160 million (11%) |
| Never | 6 (13%) | 13 (27%) | 65 million (5%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Direct microscopy. Additional information

### Costs and ease of use

The consumable and chargeable costs of direct microscopy are about $3, although each sample can take 10 minutes of experienced technical time to read.

### Guidelines

1. Direct microscopy of hair is recommended in all guidelines addressing tinea capitis (Mayser, 2020).
2. Microscopy is strongly recommended for onychomycosis (Seebacher, 2007, Ameen, 2014).
3. For HIV-infected patients, direct microscopy of skin samples is recommend by the WHO for diagnosis (WHO, 2014).
4. An international consensus of experts recommended microscopy for the diagnosis of skin NTDs (mycetoma, chromoblastomycosis and sporotrichosis) (Hay, 2019).
5. Diagnosis of vulvovaginal candidosis should involve the combination of clinical features and the microscopic detection of yeasts and (pseudo-) hyphae (German guidelines) (Farr, 2021).
6. Direct microscopy with fluorescent brighteners from clinical specimens is strongly recommended for the diagnosis of invasive aspergillosis and mucormycosis by the European Confederation of Medical Mycology and others (Ullmann, 2018; Cornely, 2019).
7. The WHO 2018 cryptococcosis guidelines recommend the use of India ink microscopy on CSF only if CrAg is not available.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. All skin, hair, nail and vaginal samples should be examined microscopically unless an alternative quick test is done (i.e. PCR or *Candida* antigen). All ocular specimens and also bronchoscopy and tracheal samples from intensive care and immunocompromised patients should be examined microscopically. Biopsy or aspiration samples from collections or abnormal tissue (i.e. nasal biopsy) should also be examined.
If *Pneumocystis* PCR is not available, then microscopy for *Pneumocystis* on sputum or bronchoscopic specimens in immunocompromised patients is important.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | Yes |
| Eye symptoms with corneal abnormality | Yes |
| Persistent lung symptoms | Some |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Yes |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Yes | No |
| Local TB clinics | Yes | No |
| Local hospitals | Yes | Possibly |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

# Fungal culture

Most fungi can be recovered from clinical samples by culture, although many grow poorly or very slowly. Approximately 600 species of fungi (of an estimated 3.5 million globally) cause human disease but ~30 species cause >99% of human disease. Fungal media are superior to bacterial media for growing fungi. Some fungi, such as *Histoplasma* spp. and dermatophytes, are slow growing, so plates should be kept for up to 3 weeks before discard.

Species level identification of fungi isolated in culture is mandatory for many reasons. However, identification requires considerable skill and experience. Culture is needed to detect resistance to antifungals but in some cases, species level identification gives important information such as:

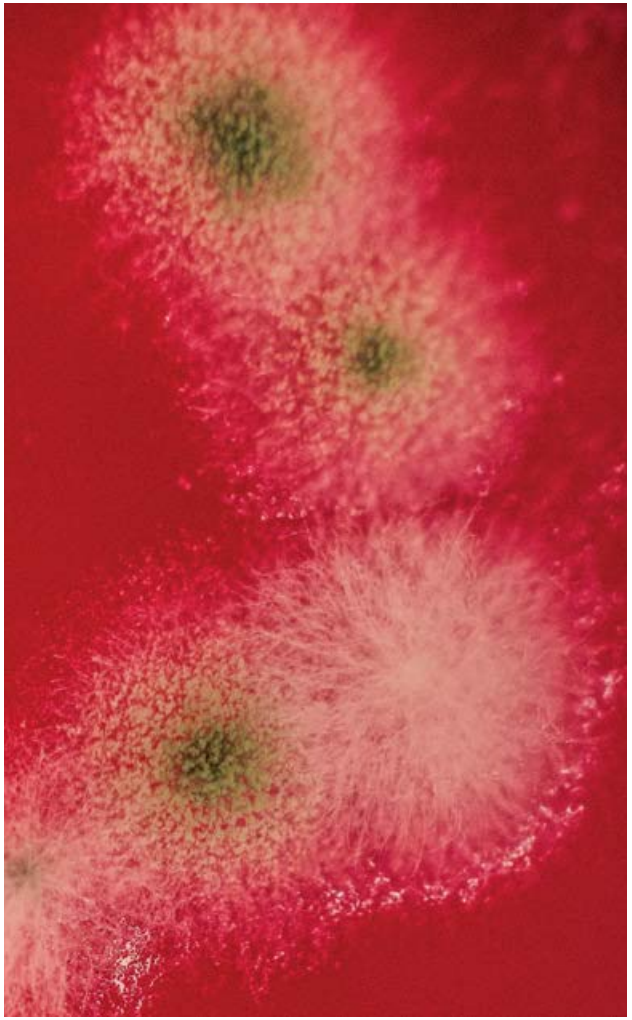
- *Candida krusei* is fluconazole resistant
- All *Mucorales* are voriconazole resistant
- *Aspergillus terreus* is amphotericin B resistant

Susceptibility testing can be done on most fungi to detect acquired resistance.

### Class 3 pathogenic fungi

Category 3 pathogens can cause severe human disease and may be a serious hazard to employees. They may spread in the community, but there is usually effective prophylaxis or treatment available. Most human pathogenic fungi are not a risk to laboratory workers, but a few are, notably the endemic fungi *H. capsulatum, Blastomyces* spp., *Coccidioides* spp. and *Talaromyces marneffei*. Only the first two are present in Africa; the last two could be imported. *Cladophialophora bantiana* is a rare cause of brain abscess in otherwise healthy people. A category level 3 laboratory is recommended for handling these cultures.

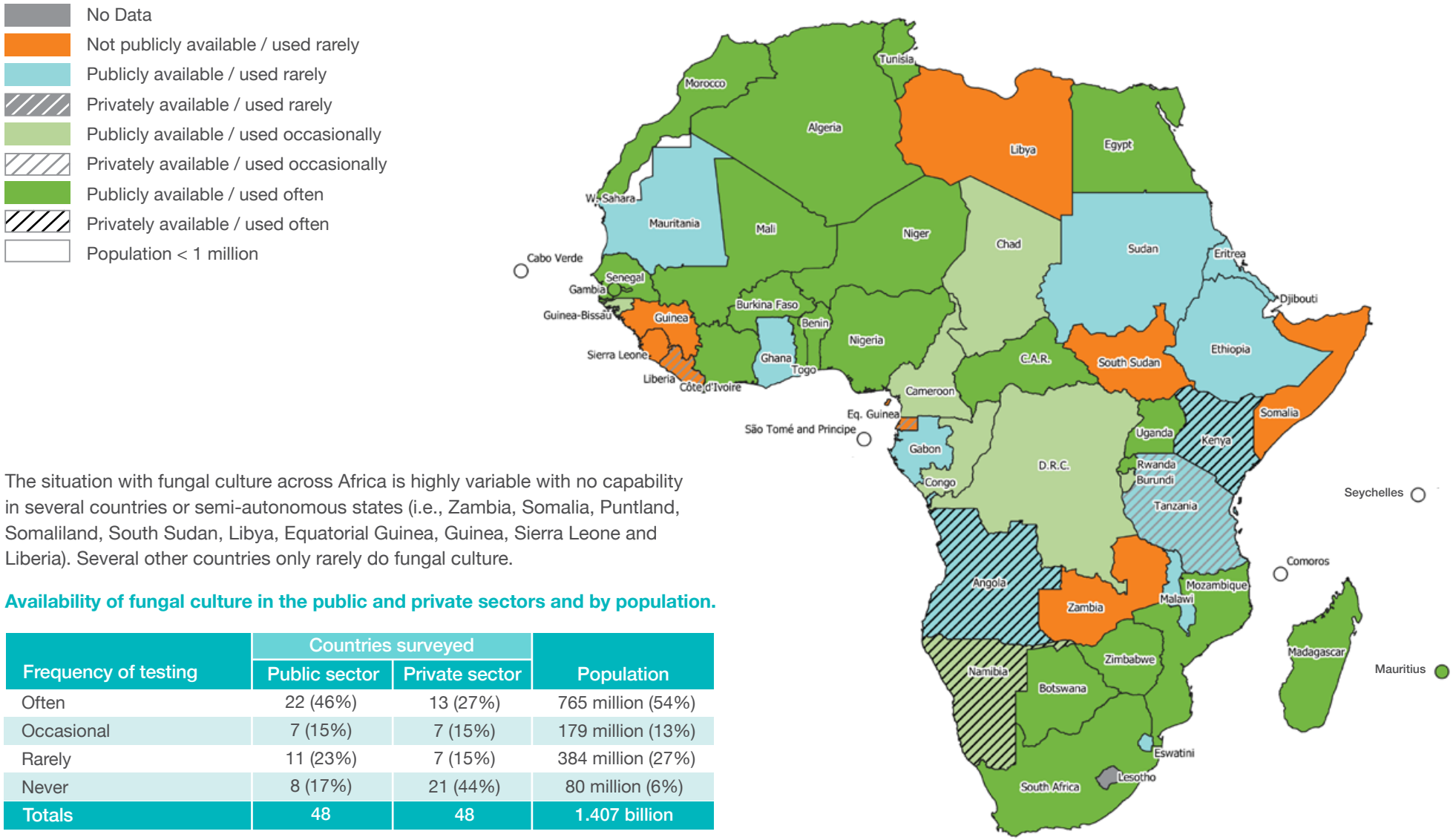
Laboratories able to handle Class 3 pathogens have been mapped across Africa (see Appendix 2). Some of these laboratories do not yet have protocols for handling fungi.



**Culture** of multiple specimen types was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended as an initial step in detection and identification of bacterial and fungal species for selection of appropriate antimicrobial regimens as a general IVD for use in clinical laboratories (EDL 3, Section II.a).

World Health Organization

# Availability of fungal culture by country




The situation with fungal culture across Africa is highly variable with no capability in several countries or semi-autonomous states (i.e., Zambia, Somalia, Puntland, Somaliland, South Sudan, Libya, Equatorial Guinea, Guinea, Sierra Leone and Liberia). Several other countries only rarely do fungal culture.

**Availability of fungal culture in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 22 (46%) | 13 (27%) | 765 million (54%) |
| Occasional | 7 (15%) | 7 (15%) | 179 million (13%) |
| Rarely | 11 (23%) | 7 (15%) | 384 million (27%) |
| Never | 8 (17%) | 21 (44%) | 80 million (6%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Fungal culture. Additional information

### Costs and ease of use

The cost of an agar plate containing media suitable for culturing fungi is under $3. However it can take considerable technical time and expertise, and additional costs (such as extra media, reagents, identification kits, molecular identification) to finally identify positive cultures. If susceptibility testing is done (as recommended for many isolates), this is an additional cost, of at least $25, and often more.

### Guidelines

1. Fungal culture is recommended for almost all samples, if a fungal infection is considered possible, in almost all guidelines as a component of diagnosis (Schelenz, 2015). The only common exception is vaginal samples, where culture is only recommended for recurrent or recalcitrant cases (BASH, 2019).
2. All isolates cultured should be identified to genus and preferably complex level, if species level is not possible.
3. *Candida* spp. from deep sites (including central venous catheter tips) should be susceptibility tested, if possible (Cuenca-Estrella, 2013).
4. As azole resistance rates rise, so susceptibility testing of *Aspergillus* spp. becomes more necessary, but only for isolates from patients who will be treated or are on therapy and where local environmental surveys show the presence of resistant *A.fumigatus* strains (Ullmann, 2018).

Updates listed here: weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. All skin, hair, nail, vaginal, sputum and ocular samples should be plated for fungal culture. Bronchoscopy, tracheal or abnormal tissue (i.e. nasal or brain biopsy) samples from intensive care and immunocompromised patients should be cultured for fungi. Urine in hospitalised and catheterised patients should be cultured for *Candida* spp.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | CSF culture |
| Skin rash, or lumps | Yes |
| Eye symptoms with corneal abnormality | Yes |
| Persistent lung symptoms | Yes |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Blood culture |
| Fever and sepsis | Blood culture |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Yes | No |
| Local TB clinics | Yes | No |
| Local HIV clinics | Yes | No |
| Local hospitals | Yes | Possibly |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

There are several suppliers of fungal media, agar plates and identification systems or kits. Additional information on fungal culture is provided here: weblink

# Blood culture

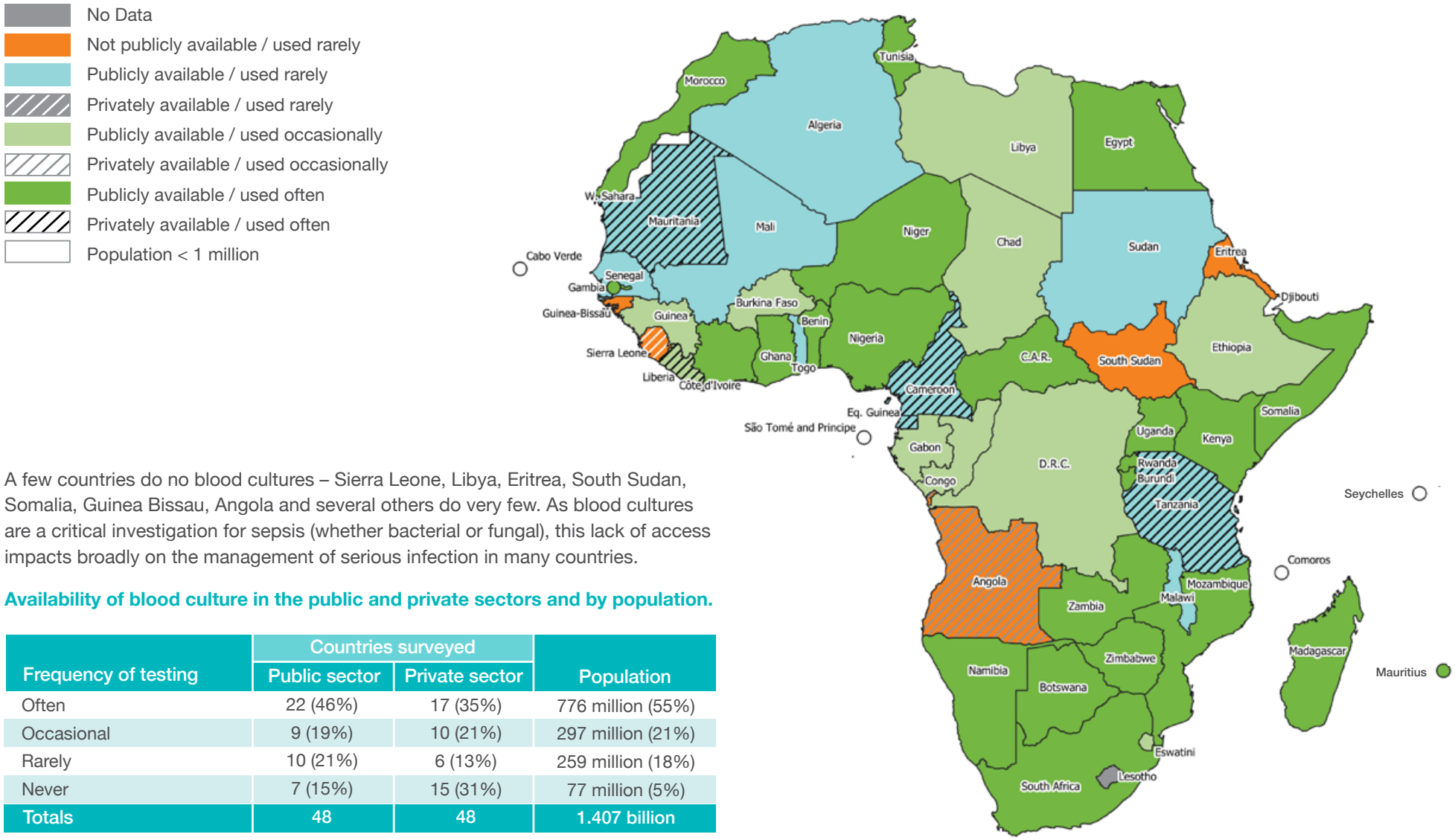
Blood culture is the most important test to define causes of sepsis in adults and children and diagnose endocarditis (heart valve infection) (Weiss, 2020; Gould, 2011). The most common fungi found in blood are *Candida* species, and the precise species and its susceptibility pattern are important for optimal care. Once *Candida* has been grown, antifungal therapy can be started and usually antibacterial therapy stopped, contributing to antimicrobial resistance control (Denning, 2017).

There is an important time dependency for both bacterial and fungal sepsis, in terms of taking the sample, speed of result and starting therapy. Delay in taking a blood culture by 50 minutes contributes 1 additional death in 35, whereas a 2 hour delay increases the number of deaths by 1 in 14 (Pruinelli, 2018). This is especially true of *Candida* bloodstream infection, as without culture, the diagnosis will almost always be missed, and remain untreated.

**Culture** of venous blood was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended for the detection of bacterial and fungal bloodstream infections (sepsis) as a general IVD for use in clinical laboratories (EDL 3, Section II.a).

World Health Organization

# Availability of blood culture by country




A few countries do no blood cultures – Sierra Leone, Libya, Eritrea, South Sudan, Somalia, Guinea Bissau, Angola and several others do very few. As blood cultures are a critical investigation for sepsis (whether bacterial or fungal), this lack of access impacts broadly on the management of serious infection in many countries.

**Availability of blood culture in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 22 (46%) | 17 (35%) | 776 million (55%) |
| Occasional | 9 (19%) | 10 (21%) | 297 million (21%) |
| Rarely | 10 (21%) | 6 (13%) | 259 million (18%) |
| Never | 7 (15%) | 15 (31%) | 77 million (5%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Blood culture. Additional Information

### Costs and ease of use

There are several different commercial blood culture systems, almost all with an alert when a bottle turns positive. The typical cost of blood culture is $15 to $20. although each bottle may cost as little as $3. If positive, additional costs will be incurred in identification of the organism and susceptibility testing, if done.

### Guidelines

1. The Surviving Sepsis Campaign recommends blood cultures are taken before antibiotics are given in all cases of clinical sepsis (Weiss, 2020).
2. Two blood cultures are optimal if endocarditis is suspected, or three sets in patients with a chronic or subacute presentation (Gould, 2012).

Updates listed here: weblink

### Suppliers

There are numerous suppliers of blood culture bottles and systems.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Hospitalised and immunocompromised patients (including Advanced HIV Disease) should have blood cultures done, and include media capable of growing fungi. If a yeast is seen on gram stain, it is usual to do a germ tube test to distinguish *Candida albicans* from other species that are more likely to be fluconazole resistant.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | Yes if HIV+ |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Yes |
| Fever and sepsis | Yes if HIV+ |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | No | No |
| Local HIV clinics | No | No |
| Local hospitals | Preferably | Possibly |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

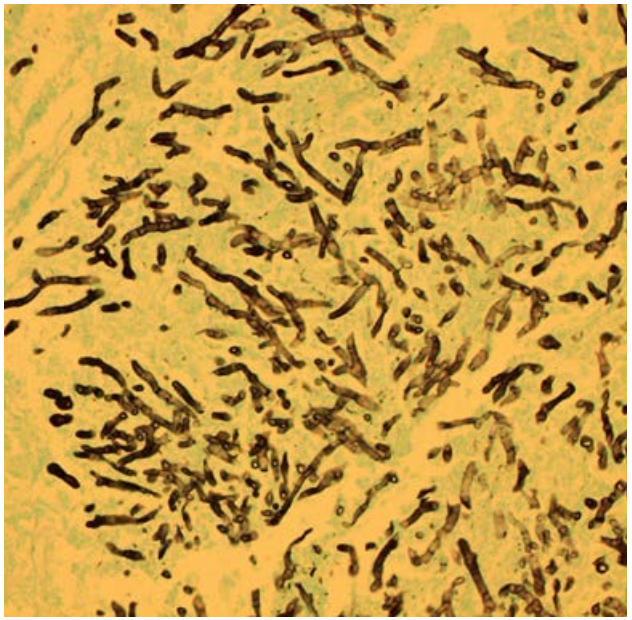
# Histopathology

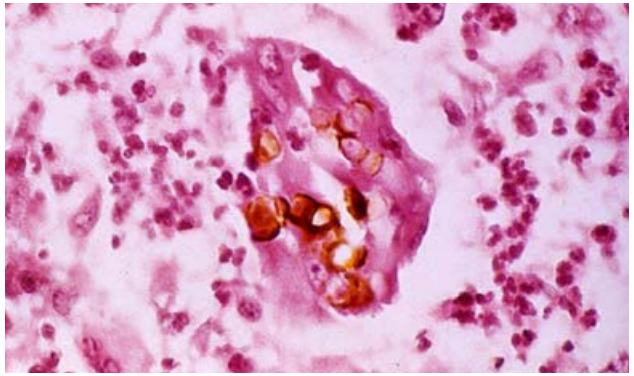
Detection of fungi in tissue from surgical or fibreoptic scope biopsy specimens, needle aspiration specimens, dab imprints, or autopsy tissues can provide definitive proof of invasive fungal disease. Fungal disease is an occasional mimic of cancer and histopathology provides the correct diagnosis (Guarner, 2011).

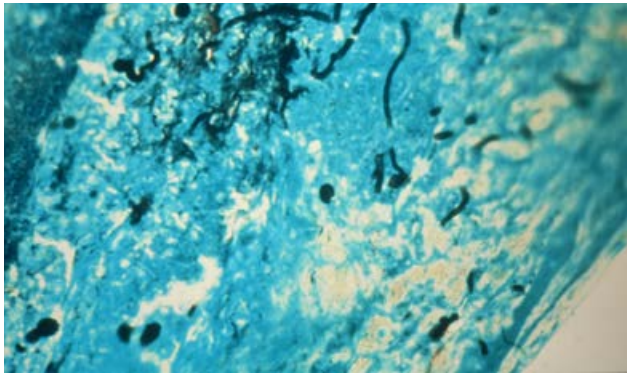
All tissues are stained with haematoxylin and eosin (H&E), but hyphae and yeasts are often invisible. Specialised stains for fungi should be done in parallel with standard stains, in immunocompromised patients (Schelenz, 2015). The additional stains for fungi are Grocott (methenamine) silver (GMS) stain or periodic acid–Schiff (PAS) (Fernandez-Flores, 2014). Usually Ziehl–Neelsen stain for acid-fast organisms and Gram stain is also done for bacteria and fungi. To see fungi in tissue, the GMS stain is more sensitive than PAS, but as it also stains tissue reticulin and the inflammatory cell lysosomes so the background can be a challenge to interpret. The morphology of any tissue reaction to fungal infection adjacent to fungal structures is better visualised with PAS than with GMS, relevant to describing granulomatous reactions or eosinophil reactions or a lack of reaction as in *Pneumocystis* infection, for example.

Reporting standards have been published, as determining the precise fungus involved can be a challenge but can be inferred with some highly characteristic features (Schelenz, 2015). Cultures from tissue are often negative. Paraffin-embedded blocks can be used for DNA extraction and sequencing to identify the responsible fungus but is not always successful.

1. Most African countries have a histopathology service, but some only for specific diseases such as leishmaniasis.
2. In Libya, histopathology is done, but fungal stains are not.
3. In other countries including Somalia, Puntland, Somaliland, South Sudan, Eritrea, Angola, Sierra Leone and Guinea Bissau there is essentially no histopathology service.
4. In many other countries, there is a very limited service, including Mauritania, Senegal, Algeria, Mali, Cameroon, Tanzania, Equatorial Guinea, Togo, Malawi and Angola.



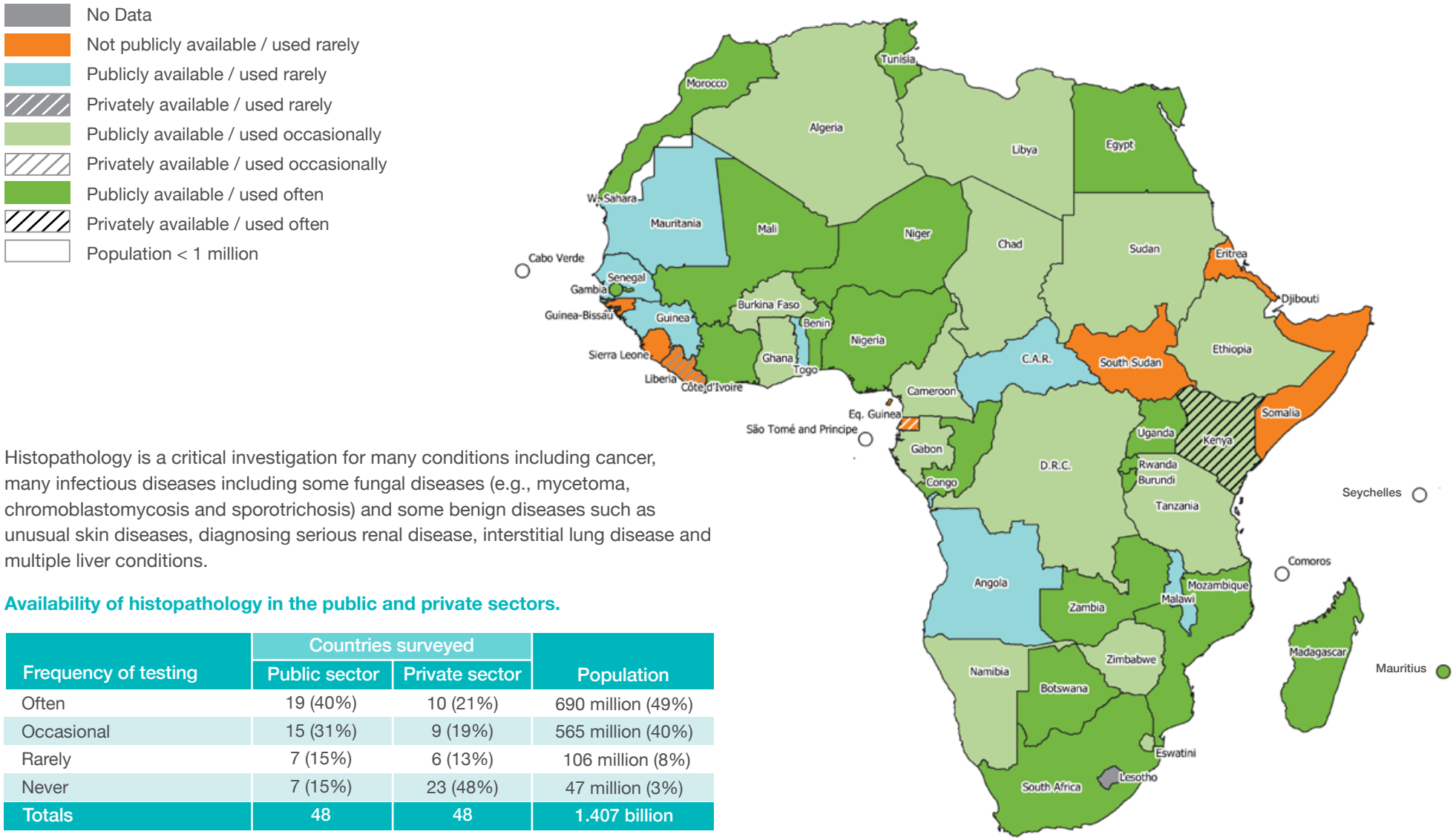




**Histopathology** was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended for assessment of tissue for infection, neoplasia, inflammatory and degenerative disorders as a general IVD for use in clinical laboratories (EDL 3, Section II.a).

World Health Organization

# Availability of histopathology by country




Histopathology is a critical investigation for many conditions including cancer, many infectious diseases including some fungal diseases (e.g., mycetoma, chromoblastomycosis and sporotrichosis) and some benign diseases such as unusual skin diseases, diagnosing serious renal disease, interstitial lung disease and multiple liver conditions.

### Availability of histopathology in the public and private sectors.

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 19 (40%) | 10 (21%) | 690 million (49%) |
| Occasional | 15 (31%) | 9 (19%) | 565 million (40%) |
| Rarely | 7 (15%) | 6 (13%) | 106 million (8%) |
| Never | 7 (15%) | 23 (48%) | 47 million (3%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Histopathology. Additional Information

### Costs and ease of use

The majority of histopathology charges were in the range of $20 to $50, occasionally more.

### Guidelines

Summary recommendations of the importance and reporting standards for histopathology of fungal disease are published (Schelenz, 2015).

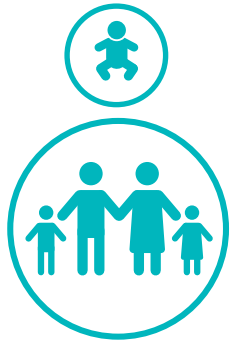
Updates listed here: weblink

### Suppliers

There are many suppliers of standard reagents for histopathology which are inexpensive.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. All tissues, including biopsies, from immunocompromised and diabetic patients should be stained for fungi (usually a GMS or PAS stain). If possible, such stains should be done concurrently with standard H&E staining and reported rapidly. Patients with chronic skin lesions with a possible mycetoma, chromoblastomycosis or sporotrichosis diagnosis should also be examined for fungi.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | Yes |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Selected patients |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | |



**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

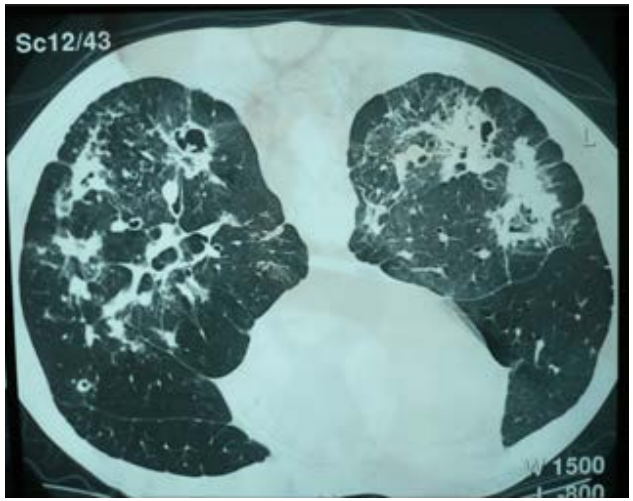
| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | No | No |
| Local HIV clinics | No | No |
| Local hospitals | Yes | No |
| Referral hospitals | Yes | Yes |
| Teaching/specialist hospitals | Yes | Yes |

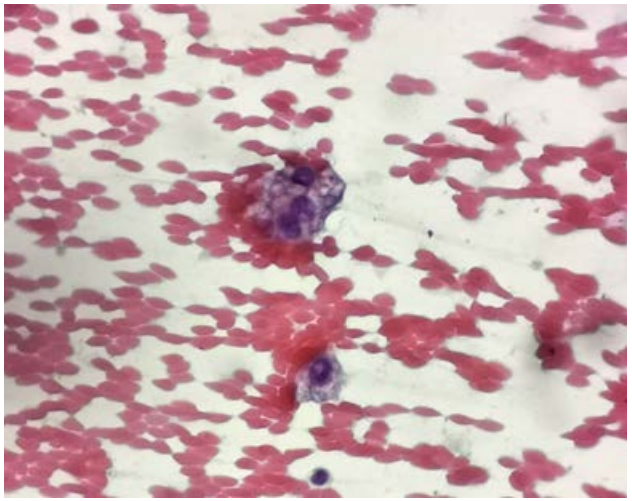
# *Histoplasma* antigen

*Histoplasma* is a geographically localised fungus, linked to bats and bird guano. It is more common in rural areas. Exposure can be mapped with skin testing for past exposure. Some areas of the world have more than 25% population with past exposure and are considered hyperendemic. Undiagnosed and treated, disseminated histoplasmosis is always fatal in AIDS and is often mistaken for TB.

Few studies have been performed to ascertain the incidence of histoplasmosis in HIV-infected people in Africa but where they have been done, histoplasmosis was present (Mapengo, 2022: Oladele, 2022)

At least 90% of patients with AIDS-related histoplasmosis can be diagnosed by detecting *Histoplasma* antigen in urine and serum (Caceres, 2019). Alternative means of making the diagnosis are invasive, painful and with less sensitivity including skin or bone marrow biopsy and culture. PCR could be an alternative to antigen detection but there are no commercialised PCR assays for *Histoplasma.*

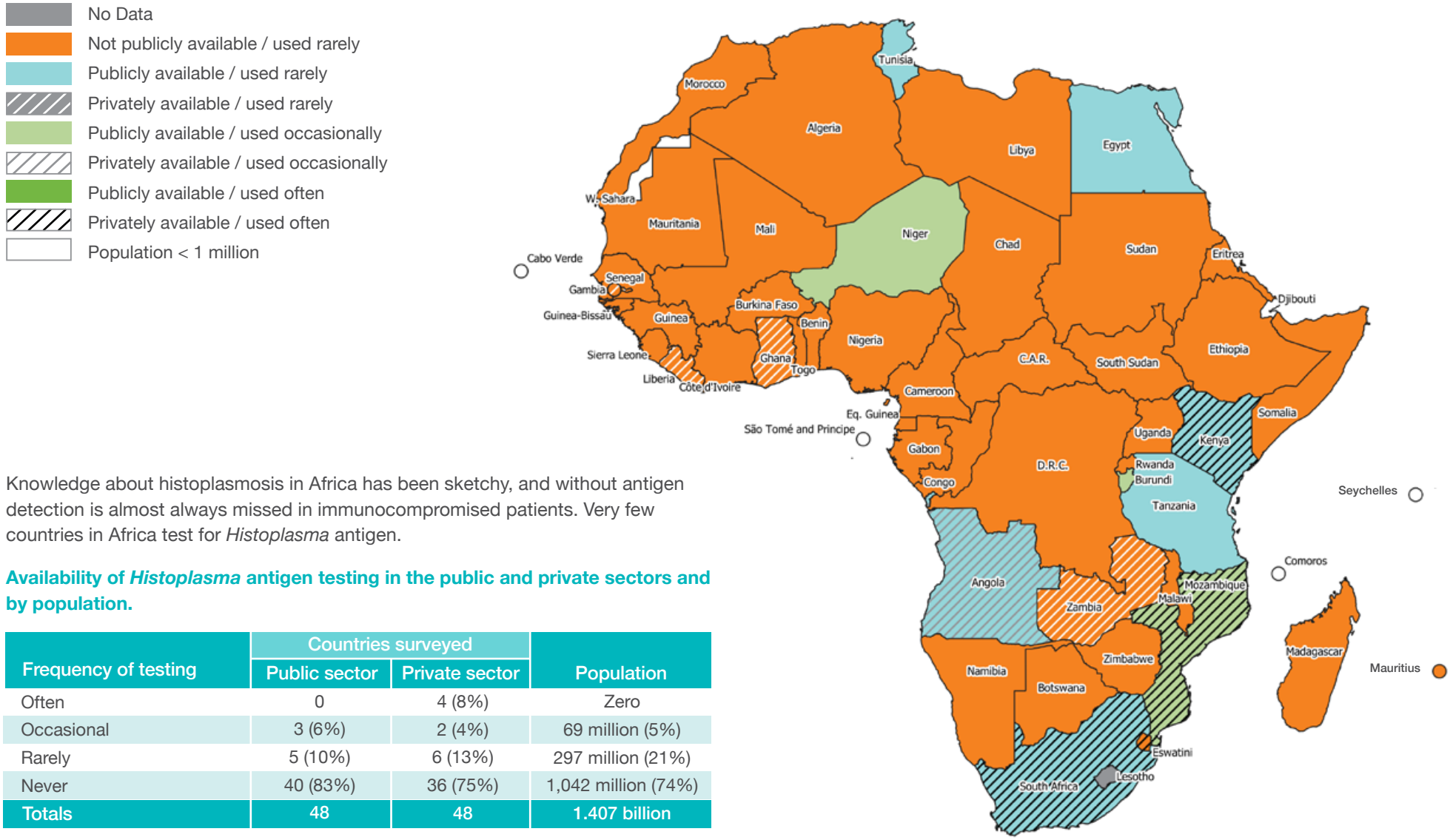



***Histoplasma*** antigen detection in urine was conditionally approved as an Essential Diagnostic List in 2019 by WHO and fully incorporated in 2021 (3rd EDL, 2021). It is recommended as an aid to the diagnosis of disseminated histoplasmosis as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b).

World Health Organization

# Availability of *Histoplasma* testing by country




Knowledge about histoplasmosis in Africa has been sketchy, and without antigen detection is almost always missed in immunocompromised patients. Very few countries in Africa test for *Histoplasma* antigen.

**Availability of *Histoplasma* antigen testing in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 0 | 4 (8%) | Zero |
| Occasional | 3 (6%) | 2 (4%) | 69 million (5%) |
| Rarely | 5 (10%) | 6 (13%) | 297 million (21%) |
| Never | 40 (83%) | 36 (75%) | 1,042 million (74%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# *Histoplasma* testing. Additional information

### Cost and ease of use

1. The per assay cost of antigen detection using either an ELISA format or LFA is under $15 per sample.
2. Lateral flow assays are slightly simpler to do, but about the same cost, or lower if a single sample.
3. The training of technical staff to perform ELISA tests on a routine basis takes 1 day.

### Guidelines

In 2020, PAHO and WHO issued guidelines on the diagnosis and management of disseminated histoplasmosis in AIDS (PAHO, 2020). These recommend antigen-based diagnosis in all patients in endemic areas for histoplasmosis.

In 2007, the Infectious Diseases Society of America updated its guidelines (Wheat, 2007), and since then, several others have been published including those for critical care (Limper, 2011), transplant recipients (Miller, 2019) and most recently an internationally authored global guideline (Thompson, 2021).

Updates listed here: weblink

### Suppliers

There are several suppliers of *Histoplasma* antigen tests, listed here: weblink

Evidence submitted to the WHO to support inclusion of Aspergillus antigen in the 3rd Model List of Essential in Vitro Diagnostics can be found here: weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Adults with Advanced HIV Disease, including newly hospitalised patients, are the major group to be tested. It is not clear if children need testing. In areas with a very low incidence of histoplasmosis, the test may be done when other diagnoses have been excluded. Other immunocompromised patients with pancytopenia, sepsis, hepatosplenomegaly and gastrointestinal features should be tested. Every effort should be made to get urine from patients as this is the best sample.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | Yes if HIV+ |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes if HIV |
| Leukaemia and neutropenia / corticosteroids | Some patients |
| Critically ill and ICU | Some patients |
| Fever and sepsis | Yes if HIV+ |

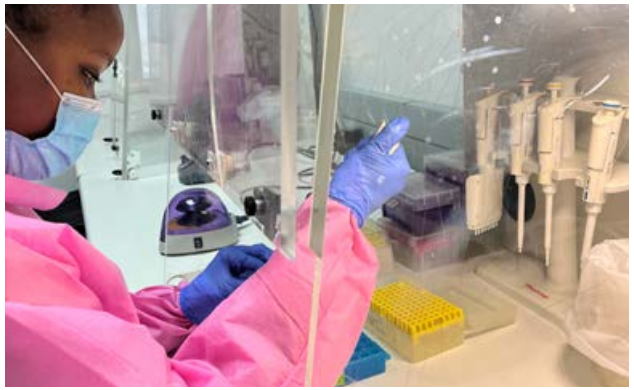
**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Possibly | No |
| Local HIV clinics | Preferably | Possibly |
| Local hospitals | Preferably | Possibly |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

# *Pneumocystis* PCR

Each species of *Pneumocystis* has co-evolved with its mammalian host and the human species is *Pneumocystis jirovecii*. This fungus is so well adapted to the human lung it cannot grow on agar, and laboratory diagnosis relies on microscopy or PCR. The usual sample type is expectorated or induced sputum or bronchoalveolar lavage, with equivalent sensitivity.

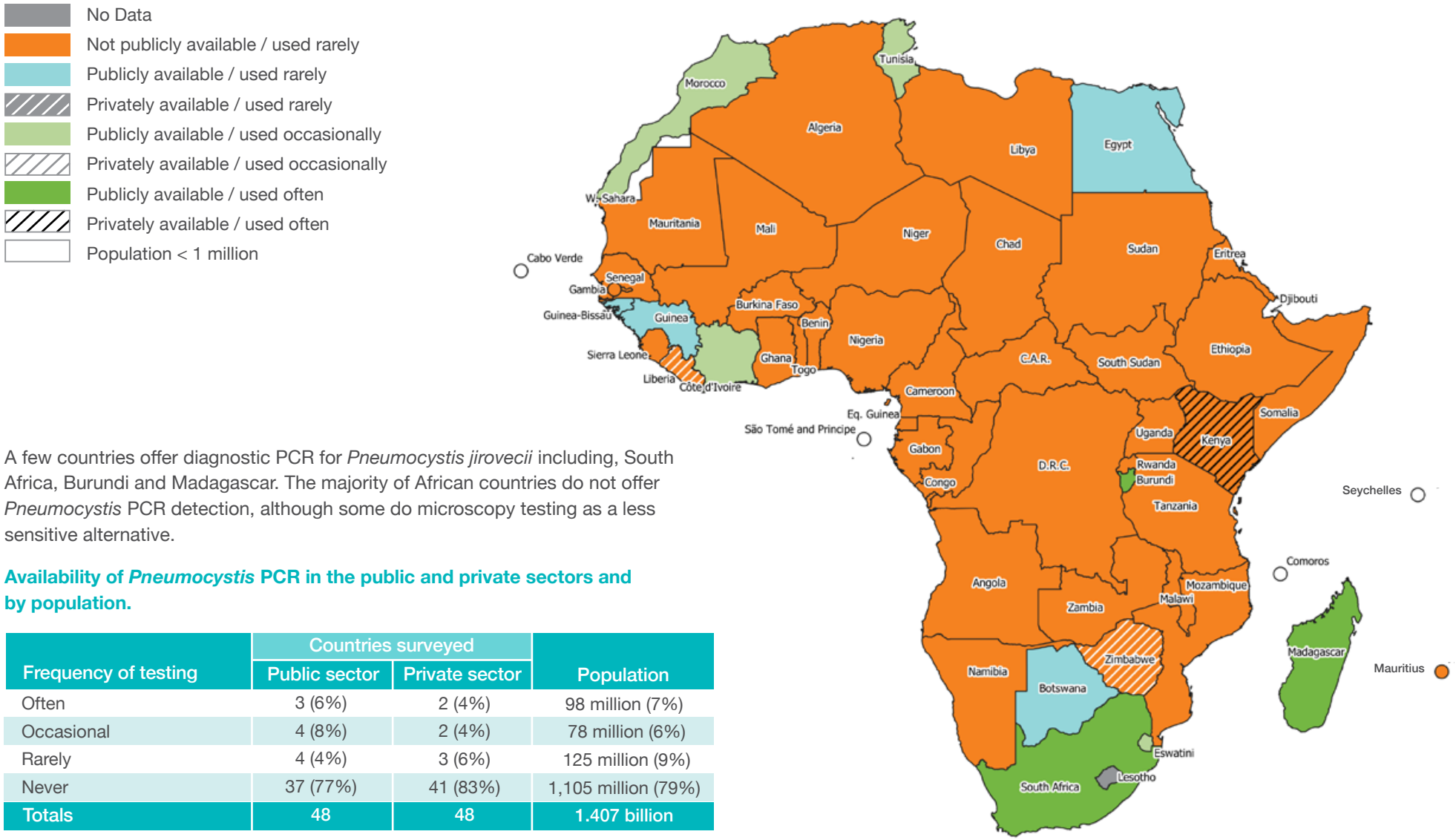
Microscopy is less sensitive (70-90%), than PCR (97%) (Wills, 2021). *Pneumocystis* PCR has an excellent negative predictive value (NPV) of ≥99% sufficient to rule out the diagnosis when PCR is negative (Summah, 2013; Fan, 2013). PCR can also be used on nasopharyngeal aspirates in babies and young children. Direct detection of co-trimoxazole resistance mutation is also possible with PCR, but not with microscopy.




***Pneumocystis*** PCR was added to the Essential Diagnostic List in 2021 (3rd EDL, 2021). It is recommended as an aid to the diagnosis of PCP as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b).

World Health Organization

# Availability of *Pneumocystis* PCR by country




A few countries offer diagnostic PCR for *Pneumocystis jirovecii* including, South Africa, Burundi and Madagascar. The majority of African countries do not offer *Pneumocystis* PCR detection, although some do microscopy testing as a less sensitive alternative.

**Availability of *Pneumocystis* PCR in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 3 (6%) | 2 (4%) | 98 million (7%) |
| Occasional | 4 (8%) | 2 (4%) | 78 million (6%) |
| Rarely | 4 (4%) | 3 (6%) | 125 million (9%) |
| Never | 37 (77%) | 41 (83%) | 1,105 million (79%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# *Pneumocystis* PCR. Additional Information

### Costs and ease of use

Cost per assay is variable depending on the supplier and volumes. DNA extraction costs are $5 to $10 per sample. Assay cost is approximately $20 to $25. It is necessary to have a system for processing respiratory samples (i.e., as for TB or pneumonia), and at least a PCR thermocycler, as used for many COVID-19 assays. This is the most technically challenging of the fungal diagnostic assays, but the widespread use of PCR for COVID-19 and other pathogens has probably ensured skills are available in many healthcare facilities.

### Guidelines

1. The diagnosis of *Pneumocystis* pneumonia by PCR is recommended by the WHO (2017) in patients with HIV who present with respiratory symptoms.
2. There are also guidelines on diagnosis of PCP from the Fifth European Conference on Infections in Leukaemia (Alanio, 2016) and the American Society of Transplantation Infectious Diseases Community of Practice for transplant patients (Fishman et al, 2019).

   Updates listed here: weblink

### Suppliers

There are several suppliers of *Pneumocystis* tests, listed here: weblink

The commercially available tests in 2020 presented to the WHO supporting the application for *Pneumocystis* PCR to be an Essential Diagnostic are listed here: weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. *Pneumocystis* PCR on sputum or bronchoscopic specimens is helpful in immunocompromised patients. In babies and young children nasopharyngeal specimens can be analysed by PCR (but not microscopy). Blood and tissue can also be analysed with PCR.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes if HIV+ or cancer |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Some |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | No | No |
| Local TB clinics | Yes | No |
| Local HIV clinics | Preferably | No |
| Local hospitals | Yes | No |
| Referral hospitals | Yes | Possibly |
| Teaching/specialist hospitals | Yes | Yes |

# CLINICAL DIAGNOSTIC PROCEDURES

gaffi.org

# Lumbar puncture

Lumbar puncture is used primarily to diagnose or exclude meningitis or encephalitis, and also occasionally for non-infectious conditions such as possible Guillain-Barre syndrome or subarachnoid haemorrhage. It is a skilled procedure done for all ages.

### The procedure

Skin antisepsis followed by local anaesthetic is used to ensure sterility and reduce the discomfort in the skin and deep tissues while the long needle is inserted into the lumbar canal. In meningitis, especially in cryptococcal and tuberculous meningitis, the cerebrospinal fluid (CSF) pressure may be elevated, and the lumbar puncture has therapeutic benefit as well as being critical to the diagnosis. For this reason, a manometer is used to measure CSF pressure, if available.

It usually takes an experienced physician about 20 minutes to do a lumbar puncture and one assistant is usually required, partly to calm and reassure the patient, partly to assist with drawing up local anaesthetic and partly for sample collection. Ideally a blood sample for blood glucose is collected at the same time to allow comparison of CSF and blood glucose levels.

### Laboratory sample processing

Samples of CSF are submitted urgently to the laboratory to test for a cell count (red and white cells, and whether neutrophils or lymphocytes), total protein, glucose (CSF and blood), gram stain and bacterial culture. In the context of HIV, CrAg is mandatory (regardless of the other results) as is a fungal culture and usually a stain for acid fast bacilli and molecular assay for TB. Multiple other assays can be done, depending on the initial results and whether a viral infection is considered. The lab should store the CSF for a few days refrigerated after these initial assays to allow further testing if clinically appropriate.

**Figure 1.**

The standard lumbar puncture kit for adults includes:

1. The sterile LP bevelled needle (of different gauges), which is about 12 cm (5 inches) long and contains a stylet.
2. A sterile 3-way stopcap to insert into the needle once in the lumbar space.
3. A sterile manometer to measure CSF pressure. The manometer fits snuggly onto the top port of the 3-way stopcock.

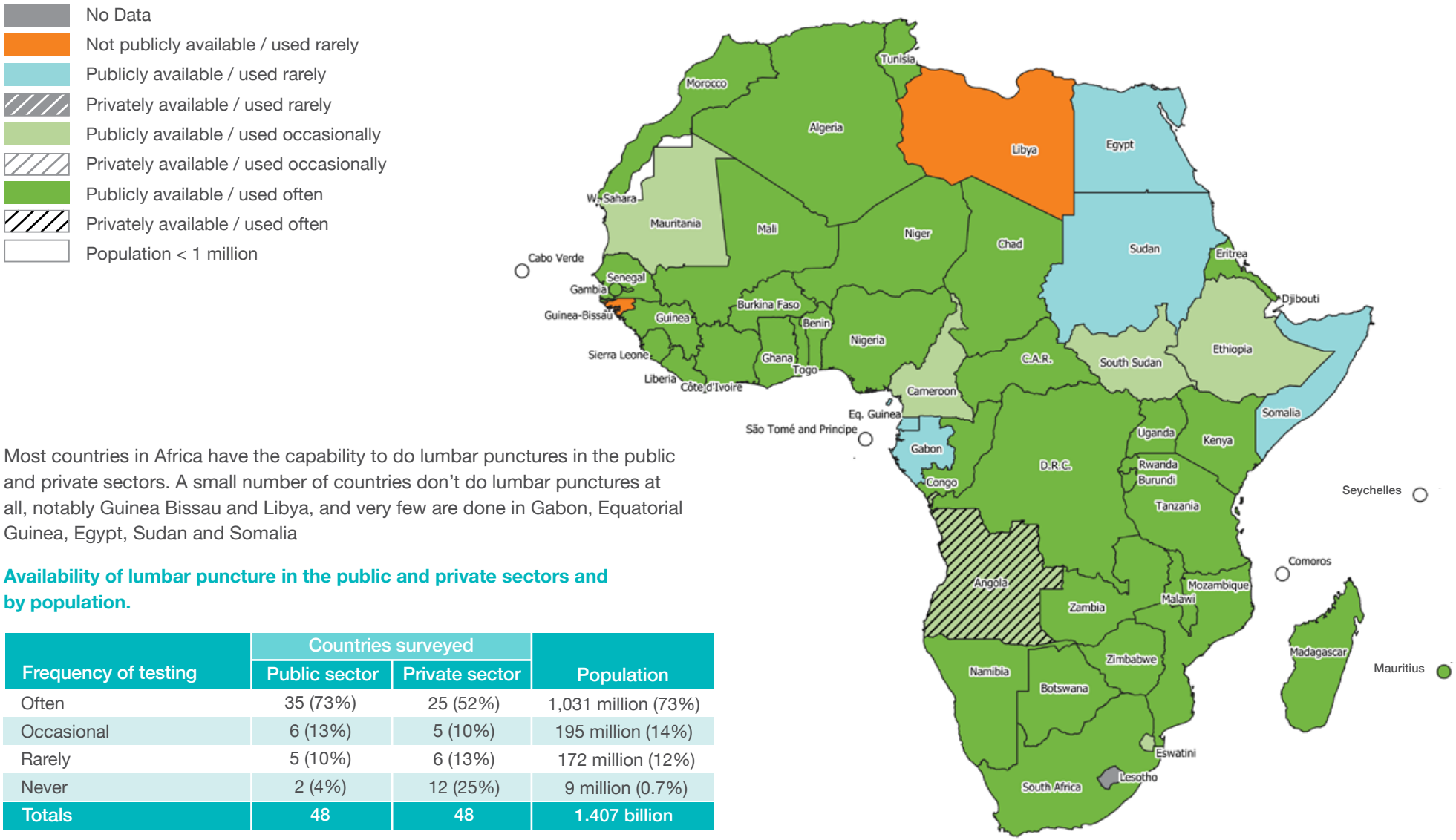
Separately are 3 sterile sample pots and a blood glucose tube into which some CSF is placed to measure CSF glucose. Not shown are the swabs for sterilising the skin and local anaesthetic. In many hospitals in Africa, there is no regular supply of lumbar puncture kits and doctors expend considerable time searching for all the components necessary for the procedure.

It is also rare to have plastic manometers, and sometime IV tubing is used as a substitute.

**Figure 2.**

Lumbar puncture in Uganda (measuring CSF pressure with a manometer). The patient is lying on his side with his back vertical and his knees drawn up as much as possible (to open up the lumbar space). The LP needle has been inserted horizontally and the stopcap and manometer are being used to measure CSF pressure and then the clear cerebrospinal fluid is collected into a sample tube. Usually 4 samples are collected, 1 of which is a glucose tube.

# Availability of lumbar puncture by country




Most countries in Africa have the capability to do lumbar punctures in the public and private sectors. A small number of countries don't do lumbar punctures at all, notably Guinea Bissau and Libya, and very few are done in Gabon, Equatorial Guinea, Egypt, Sudan and Somalia

**Availability of lumbar puncture in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 35 (73%) | 25 (52%) | 1,031 million (73%) |
| Occasional | 6 (13%) | 5 (10%) | 195 million (14%) |
| Rarely | 5 (10%) | 6 (13%) | 172 million (12%) |
| Never | 2 (4%) | 12 (25%) | 9 million (0.7%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Lumbar puncture. Additional information

### Costs and ease of use

The cost of a lumbar puncture needle (~$0.22) and sample tubes ($0.40 each), local anaesthetic and skin antisepsis are small. The cost of a manometer and 3-way stopcock is higher ($10). The laboratory costs of routine tests of CSF are typically <$20 in Africa, but are higher the more tests are done. The procedure is not usually charged to the patient in public hospitals.

### Guidelines

Lumbar puncture is recommended in ill patients with HIV and if meningitis is considered in:

'Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy' weblink

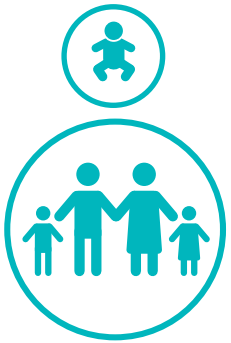
'WHO guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children, 2018' weblink

Most national guidelines recommend lumbar puncture for suspected meningitis. The WHO has a plan to minimize deaths from meningitis, which includes diagnosis with lumbar puncture and provision of rapid tests: weblink

The WHO issued a comprehensive Roadmap in 2020 with one of its 5 pillars being: 'Diagnosis and treatment, focused on speedy confirmation of meningitis and optimal management'. weblink

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Patients presenting with neurological features (confusion, headache, stroke-like syndrome, neck stiffness or photophobia), and without focal features should have a lumbar puncture to diagnose meningitis or encephalitis, at all age groups. Raised intracranial pressure and coagulation defects are the major contraindications.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | Usually |
| Skin rash, or lumps | Possibly |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | Some |



**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Possibly | No |
| Local HIV clinics | Preferably | No |
| Local hospitals | Yes | Yes |
| Referral hospitals | Yes | Yes |
| Teaching/specialist hospitals | Yes | Yes |

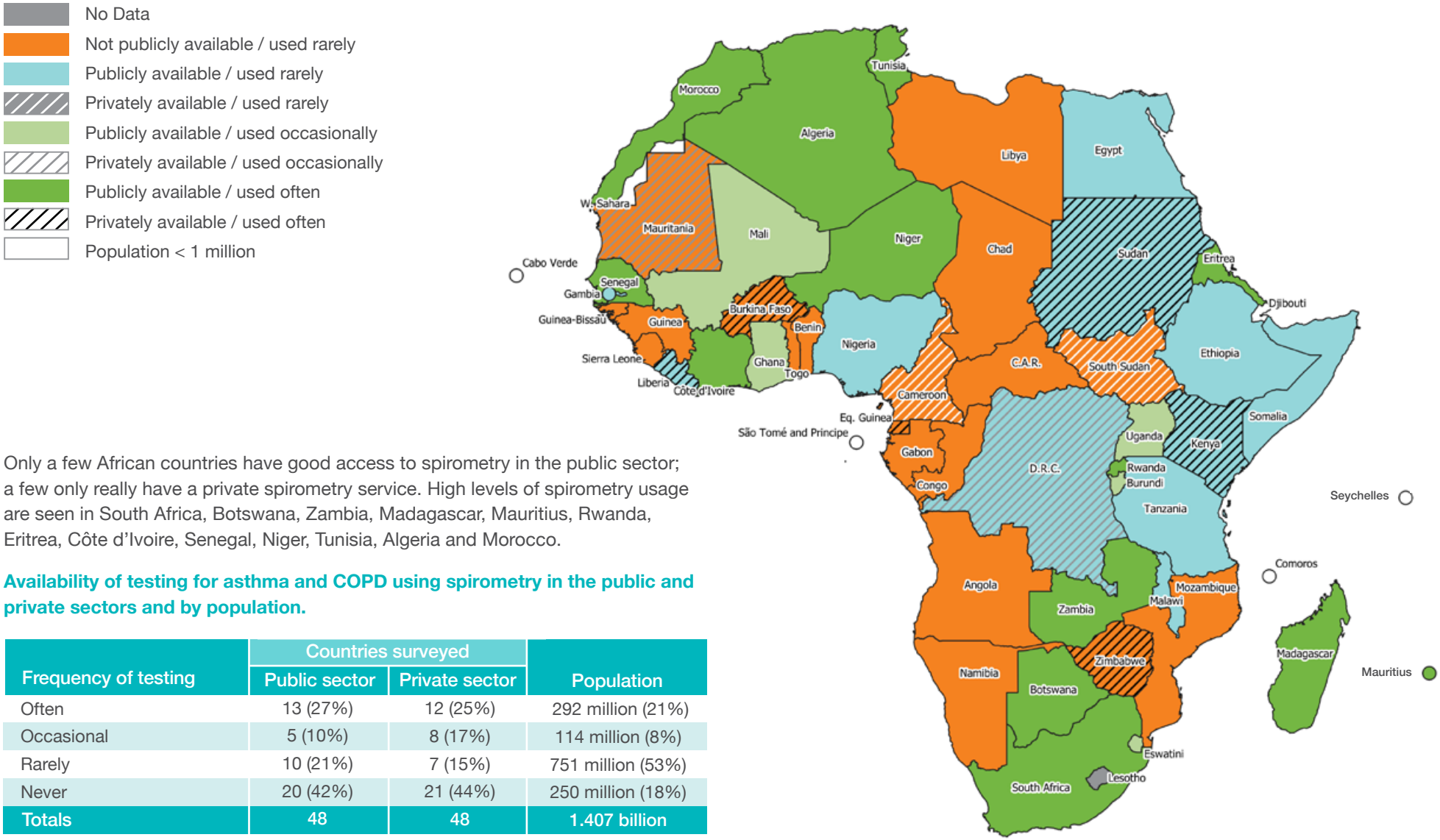
# Spirometry

Measurement of lung function is fundamental to good respiratory care. Spirometry delivers two main data points – the FVC (total air volume in the lungs that can be expelled, and therefore lung size and capacity) and FEV1 (air flow rate on expiration). Different people have different size lungs, which is related in part to height, sex and ethnicity.

Low lung volumes are indicative of lung disease. If FEV1 is reduced, it indicates airflow obstruction, which is diagnostic for asthma or COPD (or both). In asthma, there is usually variation through the day and week (unless late stage) whereas there is usually very little variation in chronic obstructive pulmonary disease (COPD). People with COPD usually have inflated lungs with a high FVC and airflow obstruction (low FEV1). Spirometry before and after medication (salbutamol or terbutaline inhaler) shows whether airflow obstruction is or is not reversible. If there is at least 10% improvement in airflow obstruction with an inhaler, asthma is the likely diagnosis.

There are different spirometers on the market, with more or less accuracy. However careful instruction and supervision of the patient doing spirometry are important to achieve reproducible results. Results should be compared with normal values for each ethnic group. Most values for Africa have been obtained from African Americans, with recent data published for some groups in Africa (van Rooyen, 2015; Ivanova, 2020).

# Availability of spirometry by country




Only a few African countries have good access to spirometry in the public sector; a few only really have a private spirometry service. High levels of spirometry usage are seen in South Africa, Botswana, Zambia, Madagascar, Mauritius, Rwanda, Eritrea, Côte d'Ivoire, Senegal, Niger, Tunisia, Algeria and Morocco.

**Availability of testing for asthma and COPD using spirometry in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 13 (27%) | 12 (25%) | 292 million (21%) |
| Occasional | 5 (10%) | 8 (17%) | 114 million (8%) |
| Rarely | 10 (21%) | 7 (15%) | 751 million (53%) |
| Never | 20 (42%) | 21 (44%) | 250 million (18%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Spirometry. Additional information

### Costs and ease of use

The lowest costs for doing spirometry were $5 to $27, but in several countries the cost to the patient was $100 to $200 per test.

### Guidelines

1. The Global Initiative for Asthma (GINA) recommends spirometry for all suspected asthma, and as a way of assessing severity and treatment response (GINA, 2020).
2. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) continues to recommend spirometry as it is 'required to make the diagnosis of COPD; the presence of a post-bronchodilator FEV1/FVC <0.70 confirms the presence of persistent airflow limitation.'

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Patients with persistent wheezing or breathlessness, with a negative TB test, should have spirometry done, primarily to diagnose asthma or COPD, but also other lung disorders.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where tests may be best performed |
|---|---|
| Community services | Preferably |
| Local TB clinics | Yes |
| Local HIV clinics | No |
| Local hospitals | Yes |
| Referral hospitals | Yes |
| Teaching/specialist hospitals | Yes |

# Bronchoscopy

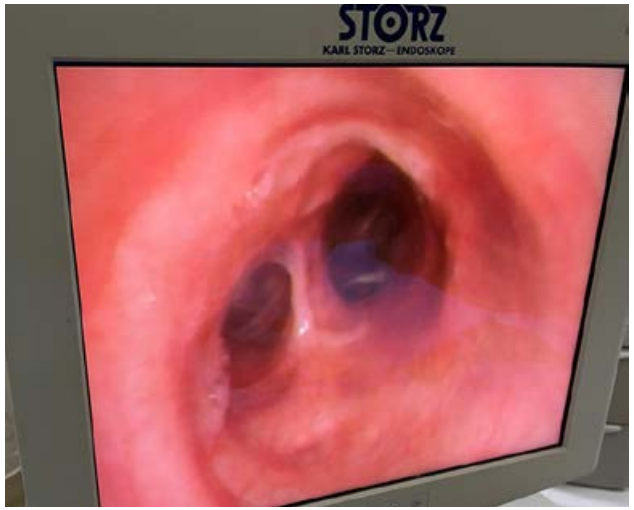
Bronchoscopy is an essential procedure in pulmonary medicine primarily for diagnosis but also for several therapeutic indications. It can be performed in patients who are awake and cooperative, or in mechanically ventilated patients.

Rigid bronchoscopy, usually during anaesthetic and thoracic surgery, was routine from the early 1900s, and flexible, fiberoptic bronchoscopes were introduced in the late 1960's. Numerous technical advances in bronchoscopy followed including transbronchial biopsy, bronchoalveolar lavage, needle aspiration and then biopsy (all for diagnosis), and then therapeutic interventions including laser therapy, endobronchial stent insertion, endobronchial ultrasound, bronchial thermoplasty for severe asthma and insertion of endobronchial valves for lung volume reduction (Panchabhai, 2015).

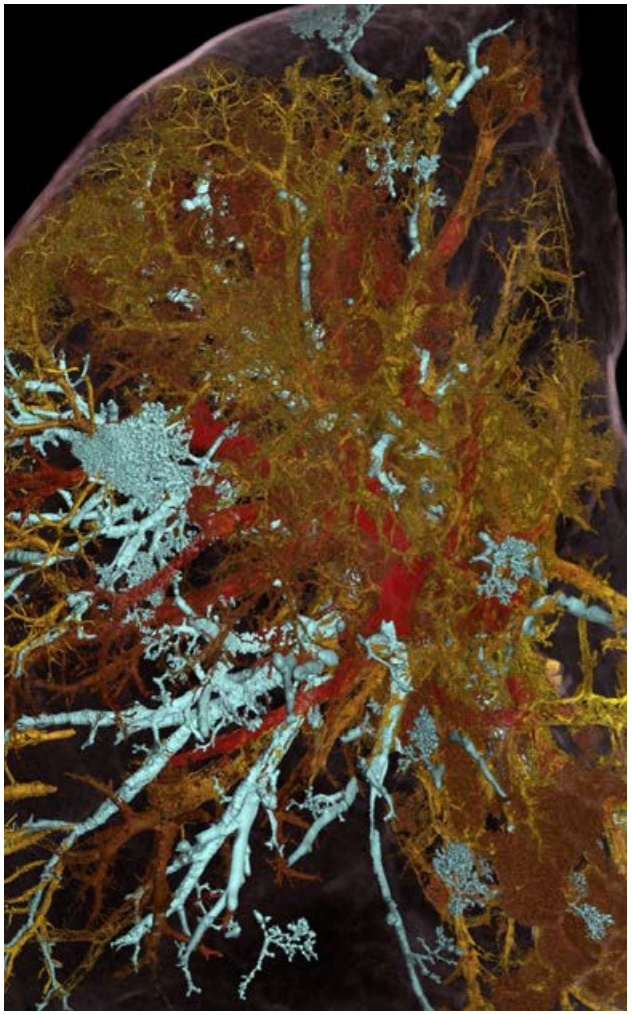
The most common indications for flexible bronchoscopy are suspected infections, malignancy, haemoptysis evaluation and interstitial lung disease. The common indications for therapeutic bronchoscopy are for removal of retained secretions and foreign bodies.

From the perspective of fungal diseases, inspection of the airways for tracheobronchitis and sample collection for mycological analysis are both critical. In addition, occasional patients with allergic aspergillosis in asthma or *Aspergillus* bronchitis get airway plugging, and removal of this thick mucus via bronchoscopy brings immediate relief.

The usual samples collected at bronchoscopy include bronchial biopsies, transbronchial biopsies and bronchoalveolar lavage or BAL. To collect BAL, 20-50 mL of warmed saline is rapidly instilled into one lobe (which can be directed by imaging findings) and then suctioned out. Usually, this procedure is repeated twice. This BAL sample is then submitted to the laboratory for cytology (for malignant cells and cell count), direct microscopy and culture for bacteria, fungi and mycobacteria, and mycobacterial PCR. If the patient is immunocompromised then virology (notably for cytomegalovirus), *Pneumocystis* PCR and/or *Aspergillus* antigen and PCR are usually requested.

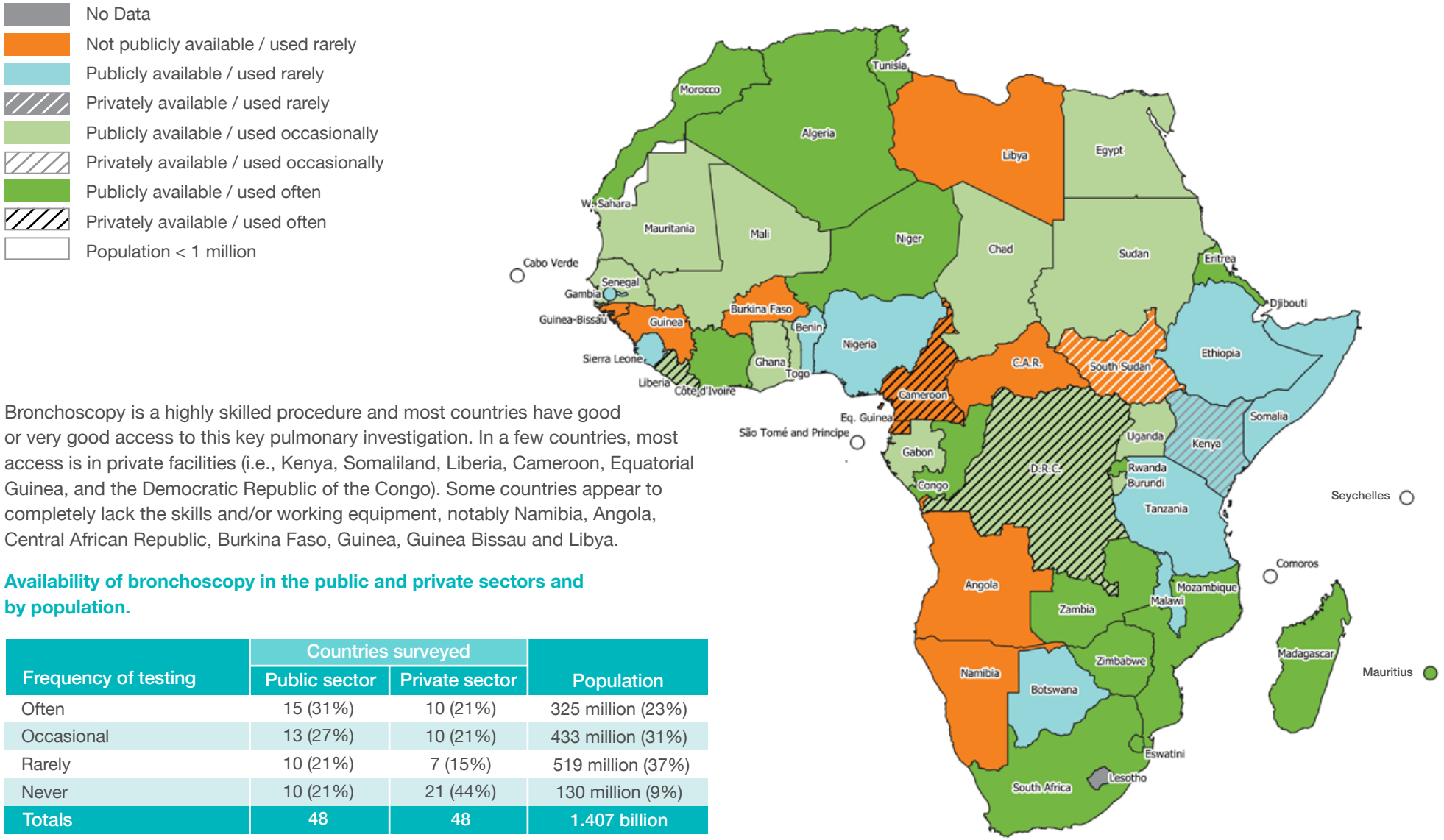



Bronchi dividing visualized in bronchoscopy done in Kenya.



Human lungs severely affected by COVID-19 (high risk for aspergillosis) using Hierarchical Phase-Contrast Tomography (HiP-CT) © ESRF weblink

# Availability of bronchoscopy by country




Bronchoscopy is a highly skilled procedure and most countries have good or very good access to this key pulmonary investigation. In a few countries, most access is in private facilities (i.e., Kenya, Somaliland, Liberia, Cameroon, Equatorial Guinea, and the Democratic Republic of the Congo). Some countries appear to completely lack the skills and/or working equipment, notably Namibia, Angola, Central African Republic, Burkina Faso, Guinea, Guinea Bissau and Libya.

**Availability of bronchoscopy in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 15 (31%) | 10 (21%) | 325 million (23%) |
| Occasional | 13 (27%) | 10 (21%) | 433 million (31%) |
| Rarely | 10 (21%) | 7 (15%) | 519 million (37%) |
| Never | 10 (21%) | 21 (44%) | 130 million (9%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Bronchoscopy. Additional information

### Costs and ease of use

Most bronchoscopies are charged at $30 to $80, but in some countries $100 to $300 is charged.

### Guidelines

Comprehensive guidelines on all aspects of bronchoscopy were published by multiple members from 3 societies in India in 2019 (Mohan, 2019). More detailed guidelines on specific diseases and how to handle samples, notably interstitial lung disease (Meyer, 2012), hypersensitivity pneumonitis (Raghu, 2020) and Covid-19 patients (Eber, 2021) have been separately published. Recently a meta-analysis of best practice for patient safety during bronchoscopy was also published (Strohleit, 2021).

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. The main reasons for bronchoscopy include evaluation of haemoptysis, to take samples for an unusual infection such as invasive aspergillosis or *Pneumocystis* pneumonia, to diagnose or exclude lung cancer, tracheobronchitis or tracheomalacia and to take samples to diagnose inflammatory lung disorders. There are also several therapeutic procedures done by bronchoscopy.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Some |
| Leukaemia and neutropenia / corticosteroids | Some |
| Critically ill and ICU | Usually |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where tests may be best performed |
|---|---|
| Community services | No |
| Local TB clinics | No |
| Local HIV clinics | No |
| Local hospitals | No |
| Referral hospitals | Possibly |
| Teaching/specialist hospitals | Yes |

# Skin biopsy and skin sample collection

There are several methods of collecting samples from skin lesions, depending on whether ulcerated or not, body location, clinical experience and equipment available. Small and potentially malignant lesions are usually subjected to excision biopsy. Larger and deep lesions are usually sampled with a punch or incision biopsy. In probable mycetoma lesions, grains can often be extracted from sinuses and submitted to the laboratory. Skin scraping from ulcerated lesions is often diagnostic with direct microscopy and/or culture but cannot be processed for histopathology. Burn wounds may require biopsy to diagnose mould infections. Very few comparative data are published on comparative diagnostic yield of these different approaches.

An international survey of 26 experts asked about the optimal diagnostic sampling method for skin fungal NTDs yielded considerable variation in sampling preferences (Hay, 2019). An ability to do a punch or incision biopsy was rated highest in terms of diagnostic yield.

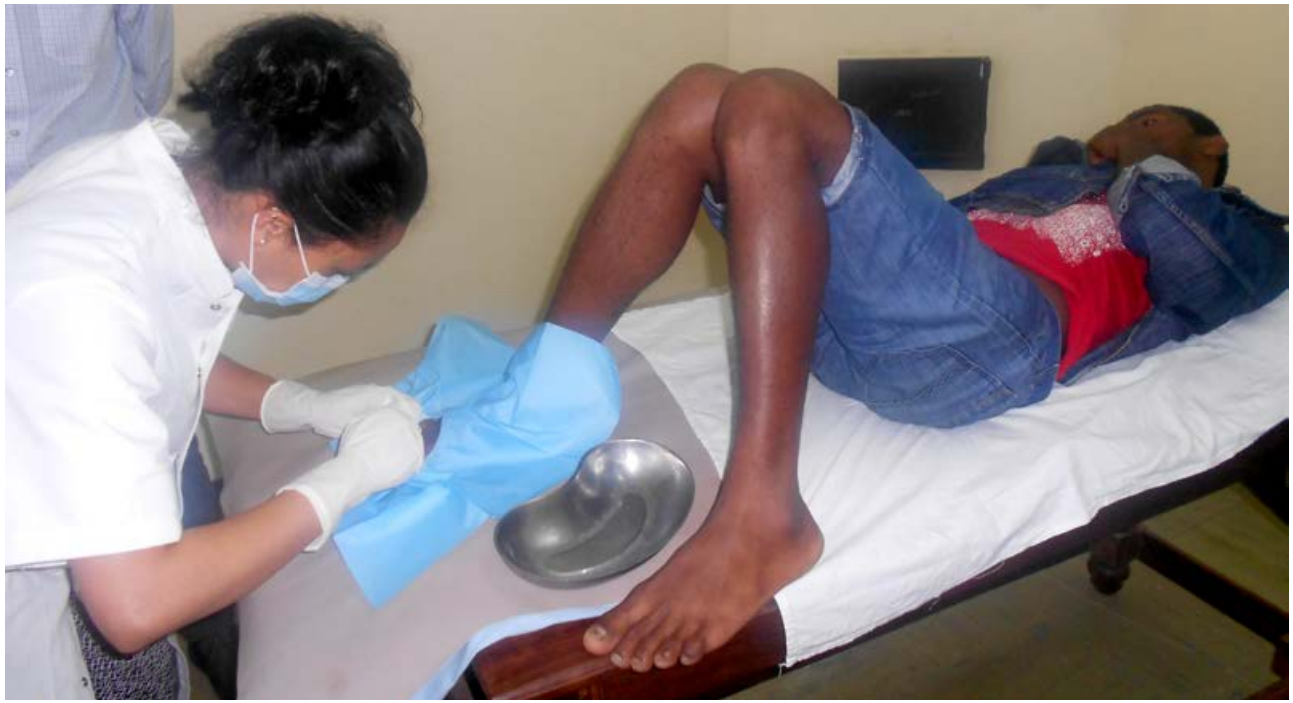
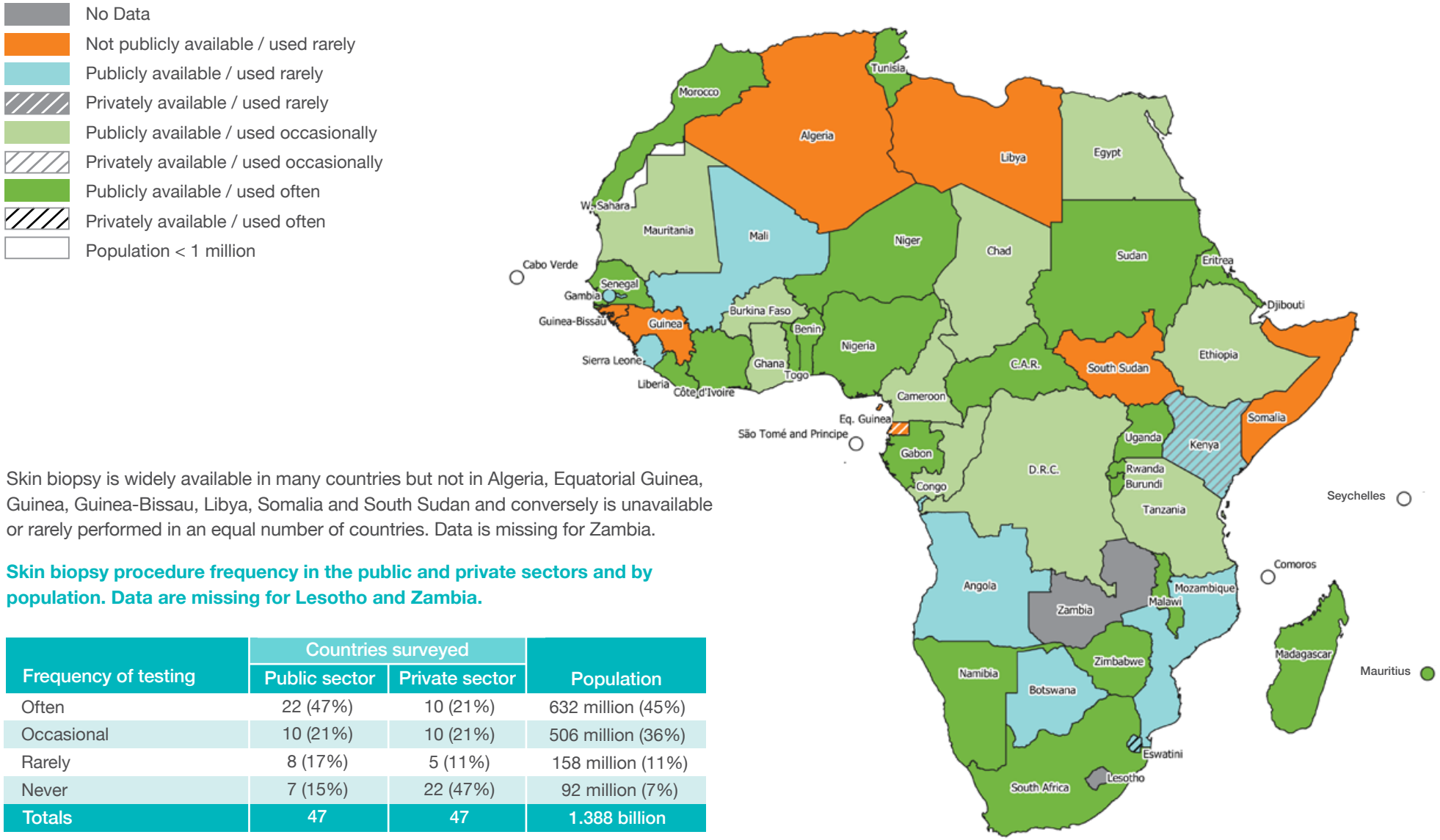


**Figure Below** Method of collecting material for laboratory investigation (positive responses) (Hay, 2019).

| Responders' choice | Swab from broken skin or sinuses | Impression smear | Skin scraping from broken skin or sinuses | Punch or incision biopsy or curettage | Excision biopsy |
|---|---|---|---|---|---|
| Mycetoma | 38% | 42% | 54% | 74% | 61% |
| Chromoblastomycosis | 8% | 29% | 67% | 79% | 42% |
| Sporotrichosis | 21% | 17% | 50% | 75% | 58% |

# Availability of skin biopsy and skin sample collection by country




Skin biopsy is widely available in many countries but not in Algeria, Equatorial Guinea, Guinea, Guinea-Bissau, Libya, Somalia and South Sudan and conversely is unavailable or rarely performed in an equal number of countries. Data is missing for Zambia.

**Skin biopsy procedure frequency in the public and private sectors and by population. Data are missing for Lesotho and Zambia.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 22 (47%) | 10 (21%) | 632 million (45%) |
| Occasional | 10 (21%) | 10 (21%) | 506 million (36%) |
| Rarely | 8 (17%) | 5 (11%) | 158 million (11%) |
| Never | 7 (15%) | 22 (47%) | 92 million (7%) |
| **Totals** | **47** | **47** | **1.388 billion** |

# Skin biopsy and skin sample collection

### Costs and ease of use

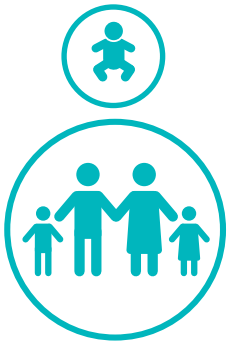
The procedure cost for a skin biopsy varies from $3 to $150, depending on the country.

### Guidelines

There are several different methods of taking a skin biopsy and many are summarised by Nischel et al (2008), with good photographs, useful practical details and technical considerations. Detailed guidance on site selection, likely diagnosis, biopsy technique and sample handling are published (Elston, 2016), but do not include examples of likely deep fungal skin infection. The indications for and complementary testing of skin lesions of possible fungal origin, including disseminated fungal infections are outlined by Miller et al (2018) in table 46. Skin/tissue biopsy is also required to diagnose filamentous fungal infection of burn wounds (Miller, 2018).

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Patients with chronic skin lesions with a possible mycetoma, chromoblastomycosis or sporotrichosis diagnosis should have a biopsy if alternative diagnostic samples (such as mycetoma grain) cannot be obtained. Burned patients with rapid onset wound discolouration should have an urgent biopsy for *Aspergillus* or Mucorales infection. New skin lesions in Advanced HIV Disease should be biopsied to exclude disseminated histoplasmosis, tuberculosis or cryptococcosis.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | Yes |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | Some |
| Critically ill and ICU | |
| Fever and sepsis | Some |



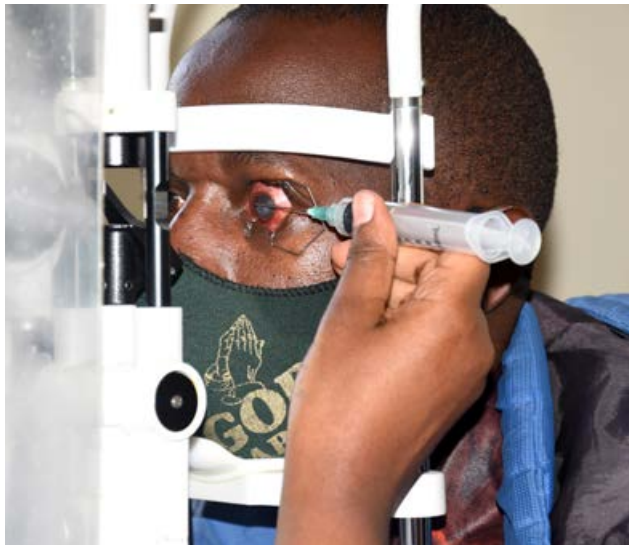
**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where tests may be best performed |
|---|---|
| Community services | No |
| Local HIV clinics | No |
| Local dermatology clinics | Preferably |
| Local hospitals | Possibly |
| Referral hospitals | Yes |
| Teaching/specialist hospitals | Yes |

# Corneal scraping

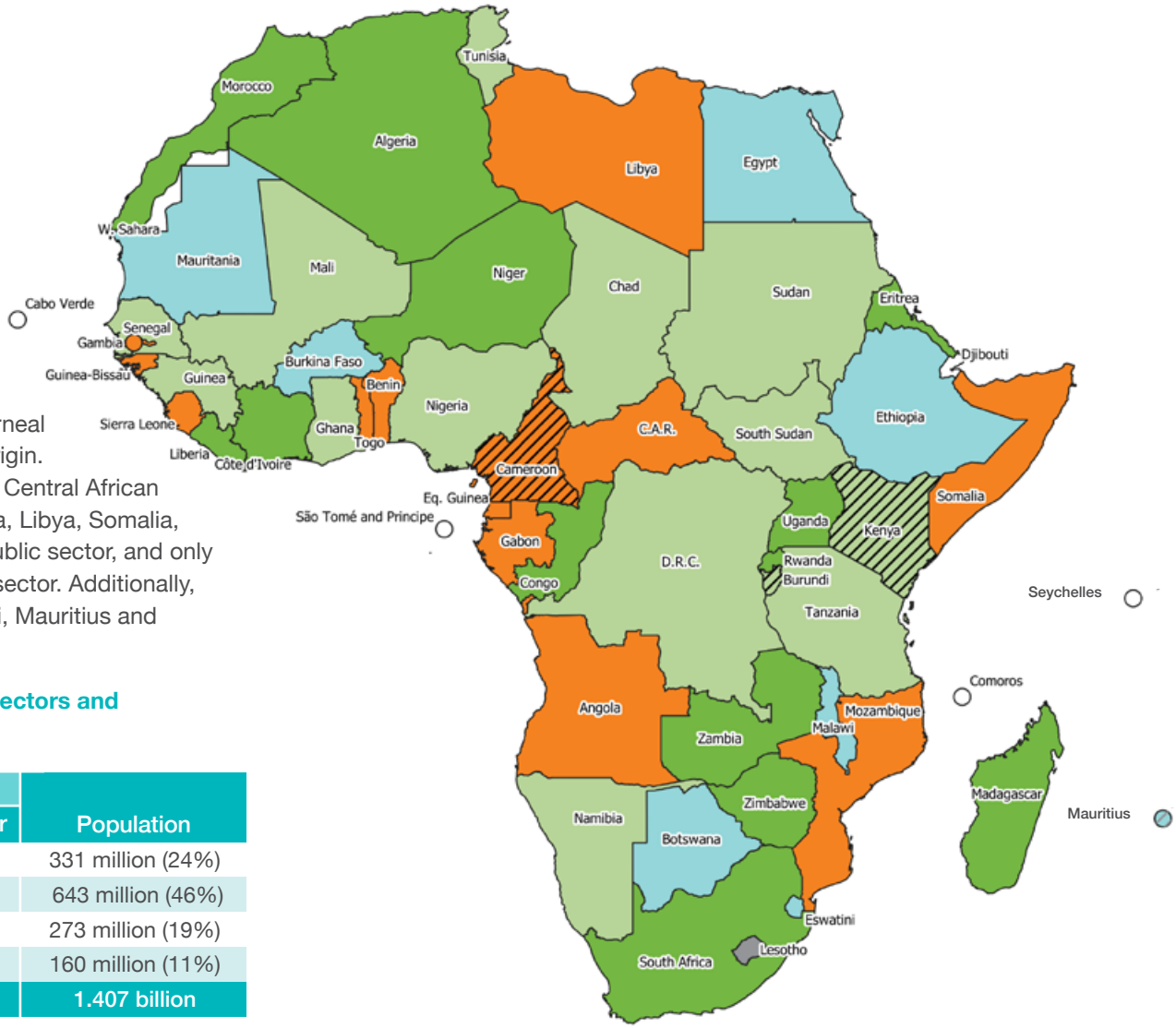
Corneal scrapings for direct microscopy and culture are required for definitive diagnosis of fungal keratitis. A corneal scrape is done with preservative-free anaesthetic and before fluorescein is used (to assess for foreign bodies). A sterile Kimura spatula, surgical blade, or hypodermic needle (21 or 23 gauge) is used to collect material from the base and edges of the corneal ulcer. The samples for microbiology are directly inoculated onto solid and liquid media in the clinic as well as multiple slides prepared for microscopy, for different stains. This optimal microbiological practice is unique to ocular microbiology, reflecting the many different possible organisms responsible and tiny samples obtained. Swabs are usually inadequate to confirm a diagnosis as fungi have generally penetrated deep into the cornea. Excessive scraping for more material risks scarring and therefore reduced vision on recovery.

Further general information on fungal keratitis and its diagnosis are provided by 2 recent reviews (Brown, 2020; Hoffman, 2021).



# Availability of corneal scraping by country

- No Data
- Not publicly available / used rarely
- Publicly available / used rarely
- Privately available / used rarely
- Publicly available / used occasionally
- Privately available / used occasionally
- Publicly available / used often
- Privately available / used often
- Population < 1 million




The aetiological diagnosis of microbial keratitis requires a corneal scrape and in Africa, over 50% of such cases are fungal in origin. Mozambique, Angola, Gabon, Equatorial Guinea, Cameroon, Central African Republic, Ghana, Togo, Sierra Leone, Guinea Bissau, Gambia, Libya, Somalia, Puntland and Somaliland do not have this capability in the public sector, and only in Cameroon (of these countries) is it available in the private sector. Additionally, in Botswana, Malawi, Ethiopia, Egypt, Burkina Faso, Eswatini, Mauritius and Mauritania, it is a rarely performed procedure.

**Availability of corneal scraping in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 13 (27%) | 8 (17%) | 331 million (24%) |
| Occasional | 14 (29%) | 8 (17%) | 643 million (46%) |
| Rarely | 8 (17%) | 6 (13%) | 273 million (19%) |
| Never | 13 (27%) | 26 (54%) | 160 million (11%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Corneal scraping. Additional information

### Costs and ease of use

The usual cost of doing a corneal scraping is about $100, but a little lower in some countries and is provided free in Nigeria. The direct costs including the local anaesthetic, scalpel, glass slides for microscopy and agar plates are under $7.

### Guidelines

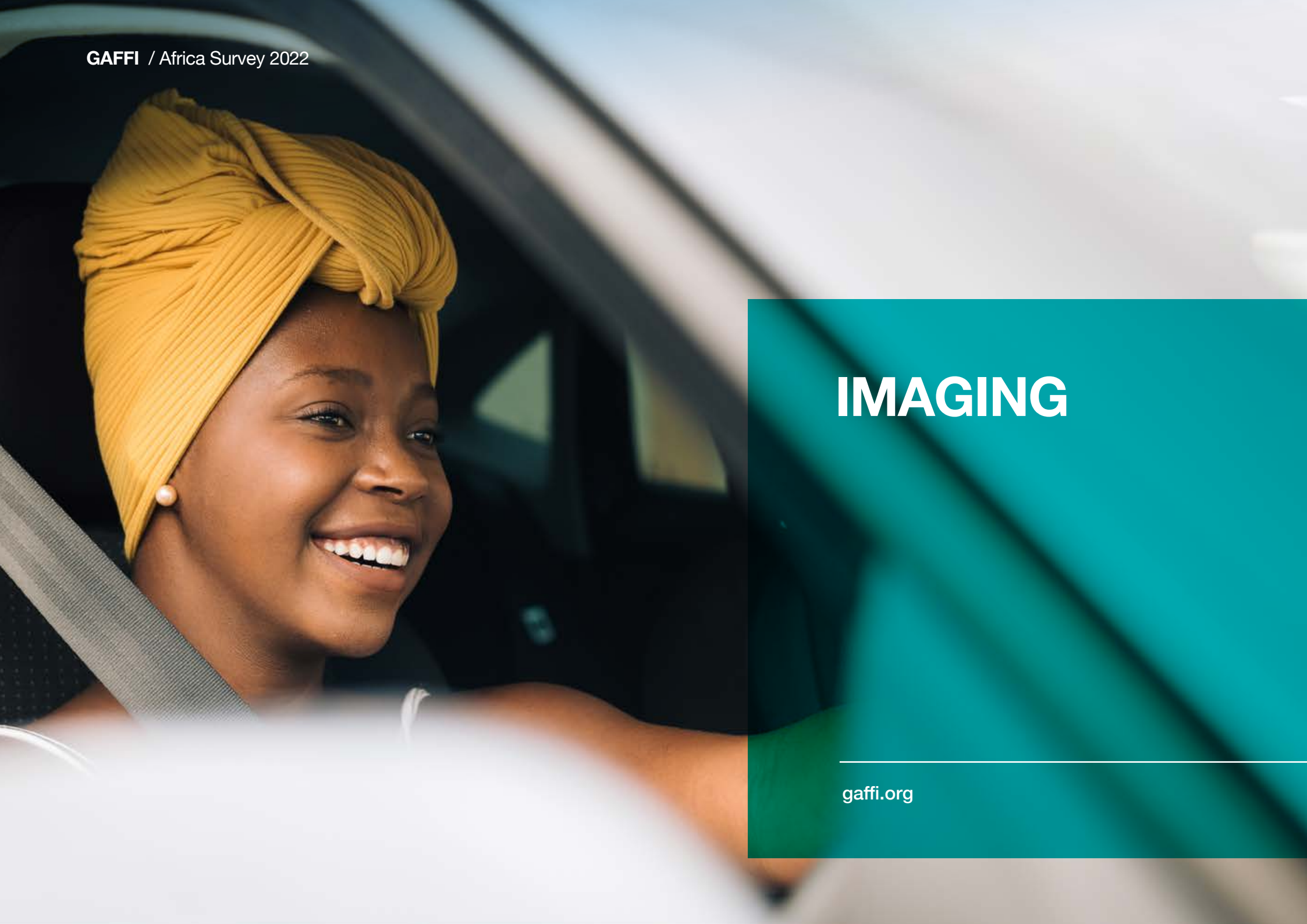
1. The 2004 guidelines for the management of corneal ulcer from the WHO Regional office for SE Asia mandated taking a corneal sample in all cases for microscopy, bacterial and fungal culture, with subsequent identification of any positive cultures (SEAR, 2004).
2. A specific guideline for Africa for fungal keratitis also mandates taking samples from ulcers for diagnosis (AAO, 2014).

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. Any corneal ulcer should be scraped to provide microscopy and culture specimens.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | Yes |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken |
|---|---|
| Community services | No |
| Local HIV clinics | No |
| Local eye health clinic | Preferably |
| Local hospitals | No |
| Referral hospitals | Preferably |
| Teaching/specialist hospitals | Yes |



# IMAGING

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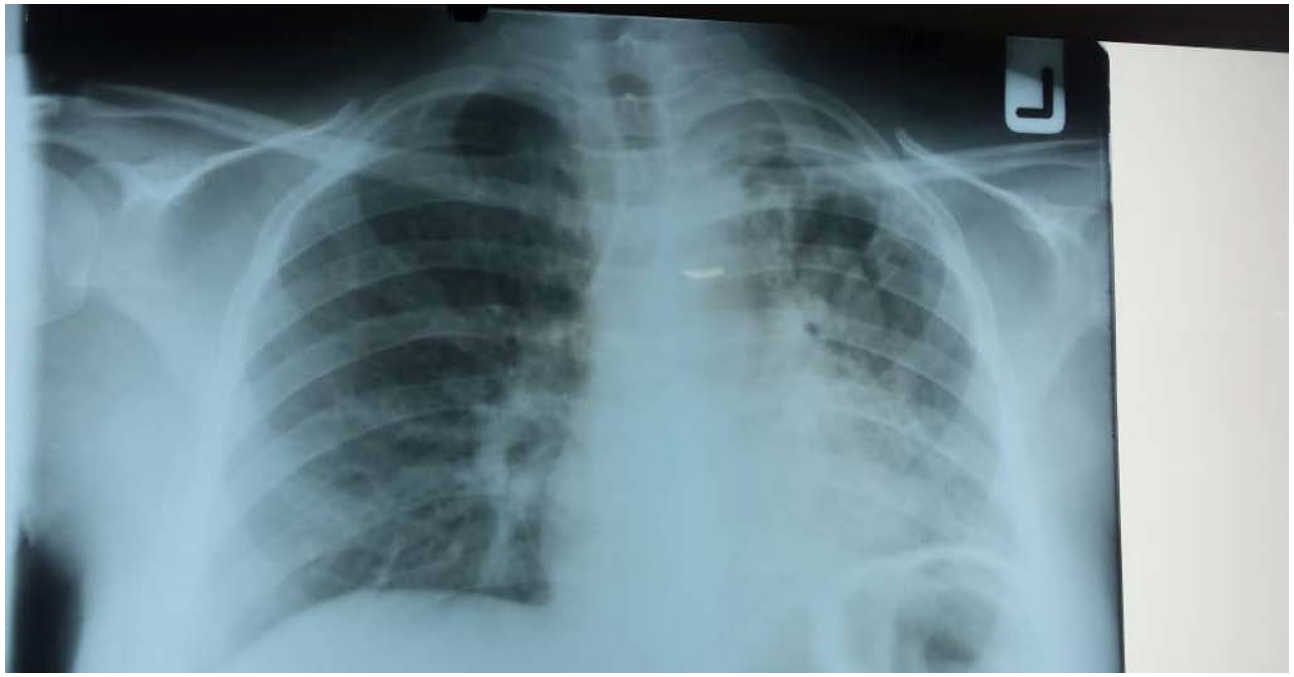
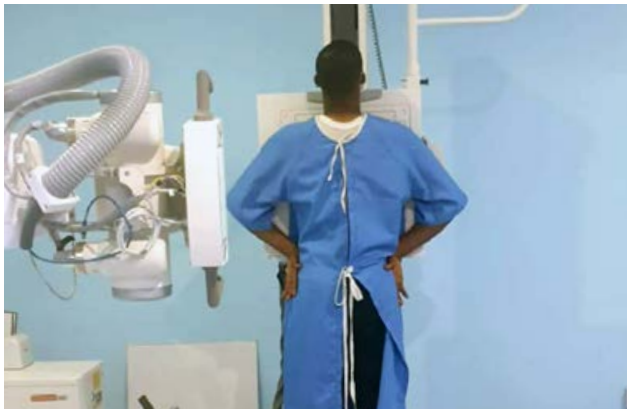
# Chest X-ray

The chest X-ray (or radiograph) is probably the most frequently performed radiological investigation globally. The lungs, heart, mediastinum and bones and soft tissues of the thorax are all visualised. The chest X-ray can carry substantial diagnostic information but interpretation is often complex, when abnormal. Some diagnoses are straightforward, such as enlargement of the heart, pneumothorax and lobar pneumonia, others much more subtle, such as bronchiectasis, *Pneumocystis* pneumonia and miliary tuberculosis.

In recent years, radiology imaging has been moving to digital platforms. This has several advantages. It is faster as chemical processing of X-ray film is not required and it allows transfer of images to other locations and computer enhancement and magnification, which aids interpretation. Less radiation is used to produce images of similar contrast.

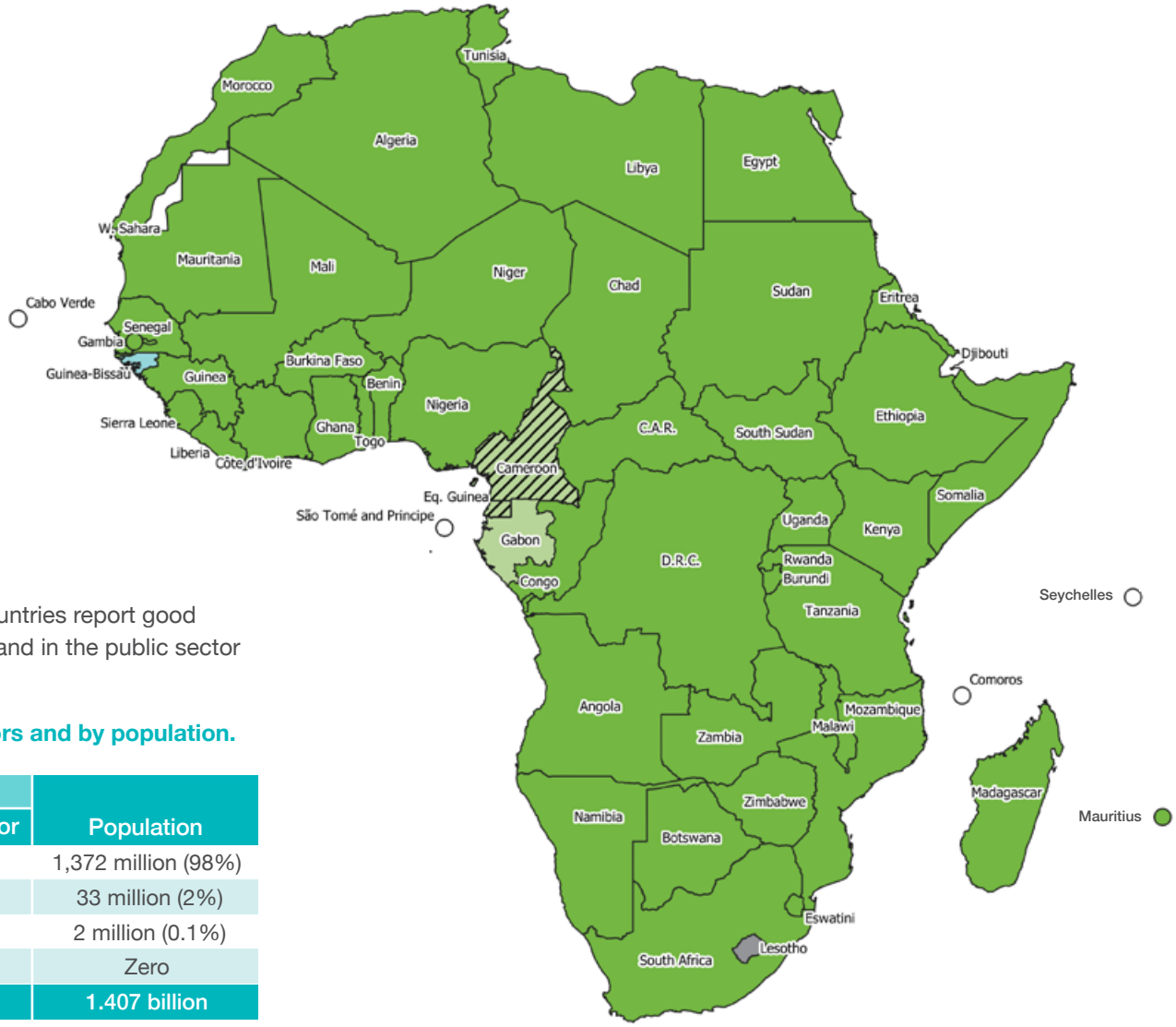
As digital images are in the computer, they are also amenable to machine learning algorithms for interpretation, although the chest X-ray is probably the most complex image to use for this purpose, because of the large number of differential diagnoses.





# Availability of chest X-ray by country

- No Data
- Not publicly available / used rarely
- Publicly available / used rarely
- Privately available / used rarely
- Publicly available / used occasionally
- Privately available / used occasionally
- Publicly available / used often
- Privately available / used often
- Population < 1 million




Access to chest X-rays in Africa by country. Note that all countries report good access except Guinea Bissau and limited access in Gabon and in the public sector in Cameroon.

**Availability of chest X-ray in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 44 (92%) | 33 (69%) | 1,372 million (98%) |
| Occasional | 3 (6%) | 4 (8%) | 33 million (2%) |
| Rarely | 1 (2%) | 3 (6%) | 2 million (0.1%) |
| Never | 0 | 8 (17%) | Zero |
| **Totals** | **48** | **48** | **1.407 billion** |

# Availability of chest X-ray in hospitals

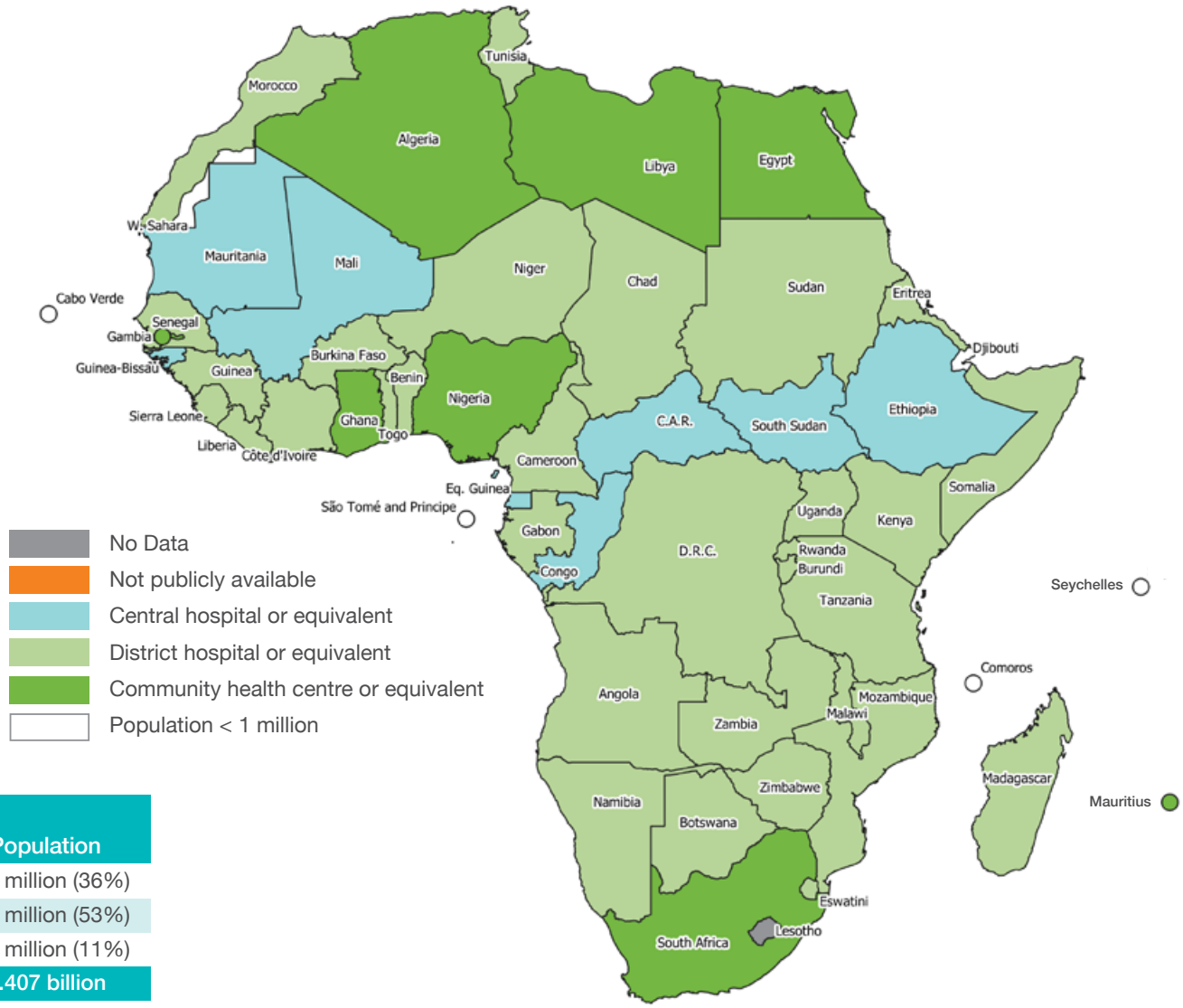
Access to chest X-rays is only available at teaching or central hospitals in several countries, notably: Mauritania, Mali, Equatorial Guinea, Republic of Congo, Central African Republic, South Sudan and Ethiopia (population 135 million).

## Costs and ease of use

The actual cost of a chest X-ray is under $10, and often as little as $4, depending how costs are charged. However the cost of a radiologist report in addition pushes the total cost up to $15 to $200, typically in the $15 - $40 range.

## Guidelines

Chest X-ray is an integral part of almost all TB and respiratory disease guidelines. It is an important component of the work up of several lung pulmonary diseases, including pneumonia ((i.e. *Pneumocystis* pneumonia) and chronic pulmonary aspergillosis (Denning, 2018). It is a key screening tool for pulmonary TB (WHO, 2021). It is also a key initial investigation for many other conditions including haemoptysis (Kang, 2018).




| **Lowest-level** facility providing CXR or equivalent | Countries surveyed: Public sector | Population |
|---|---|---|
| Community health centre | 9 (19%) | 507 million (36%) |
| District hospital | 32 (67%) | 747 million (53%) |
| Only at central hospital | 7 (15%) | 153 million (11%) |
| **Totals** | **48** | **1.407 billion** |

# CT scanning (computed tomography)

CT scanning is used for many purposes and is a workhorse imaging modality in many medical specialities. It is often perceived that its primary role is to assist in the diagnosis of cancer, but a large number of non-malignant diagnoses can be established with a CT scan. With respect to fungal disease, the main organs needing visualization with a CT scan are the lungs, the sinuses and the brain. If available, the brain is better visualized with an MR scan in immunocompromised patients.

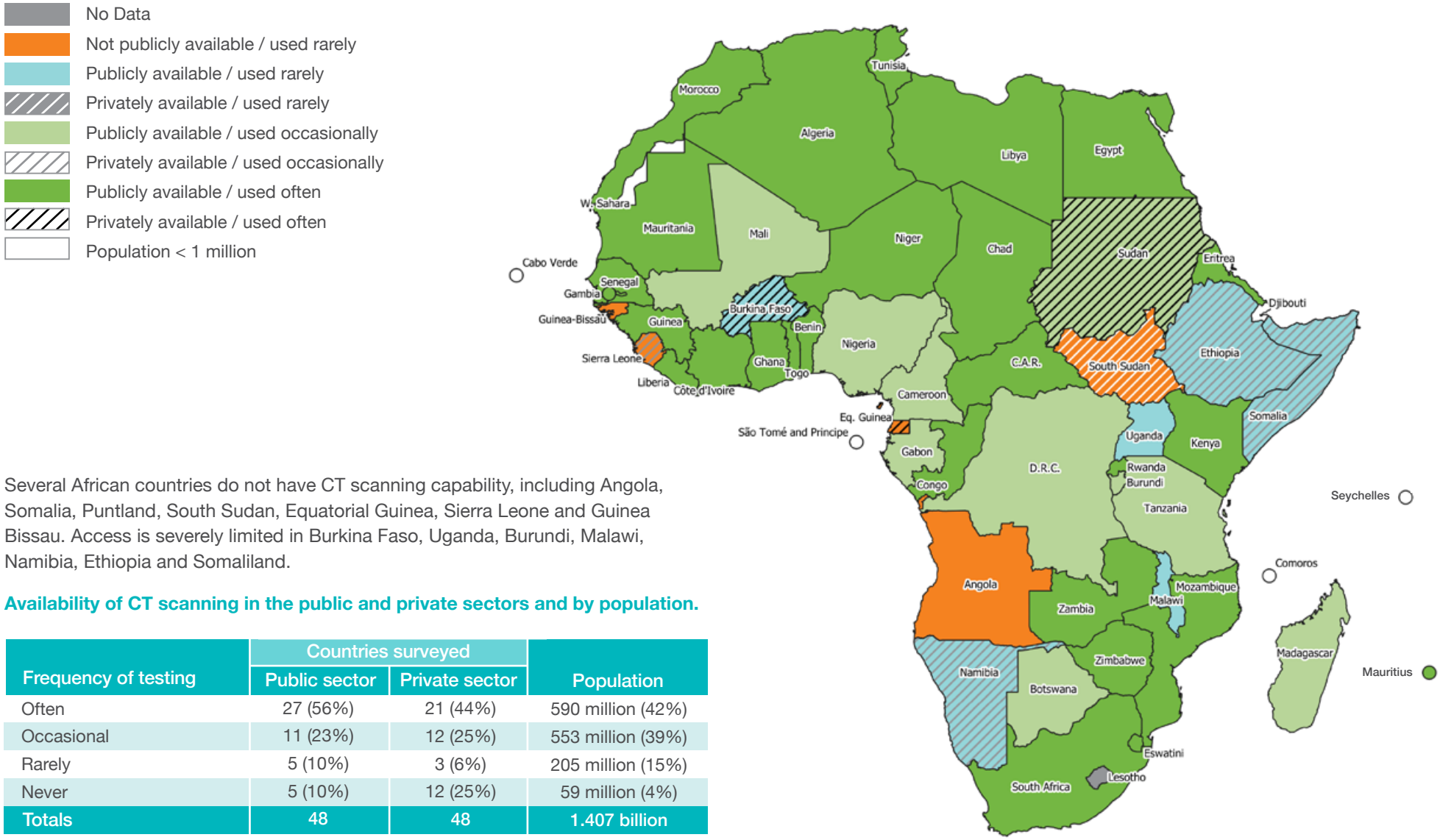
Many different CT scanners are on the market with the major differences being the speed of scanning and definition (how many slices). Use of contrast is important for some indications, especially brain imaging. Radiographer training is critical to successful CT scanning.

Both chest X-rays and CT scans are complex investigations to interpret. High quality clinical care depends in part on accurate interpretation of abnormalities. The survey asked about the frequency of expert radiological interpretation of images of all kinds.

**Figure Below** Selected examples of fungal diseases where CT scanning is critical to optimal care.

| CT scan | Disease | Context | Comment |
|---|---|---|---|
| Lungs | Chronic pulmonary aspergillosis | Possible TB relapse | Identifies cavities with material within.<br>Defines extent of disease and bronchiectasis |
| | *Pneumocystis* pneumonia | HIV or immunosuppressed | Highly characteristic ground glass appearances, bilateral |
| | Invasive aspergillosis | Leukaemia or HIV | Typical nodules or cavities.<br>More sensitive than chest Xray |
| Sinuses | Maxillary sinus fungal ball | Normal people | Cure with surgical removal |
| | Mucormycosis | Diabetes, leukaemia | Critical for diagnosis and surgical planning |
| Brain | Fungal brain abscess | Immunocompromised | Critical for diagnosis and neurosurgical intervention |

# Availability of CT scanning (computed tomography) by country

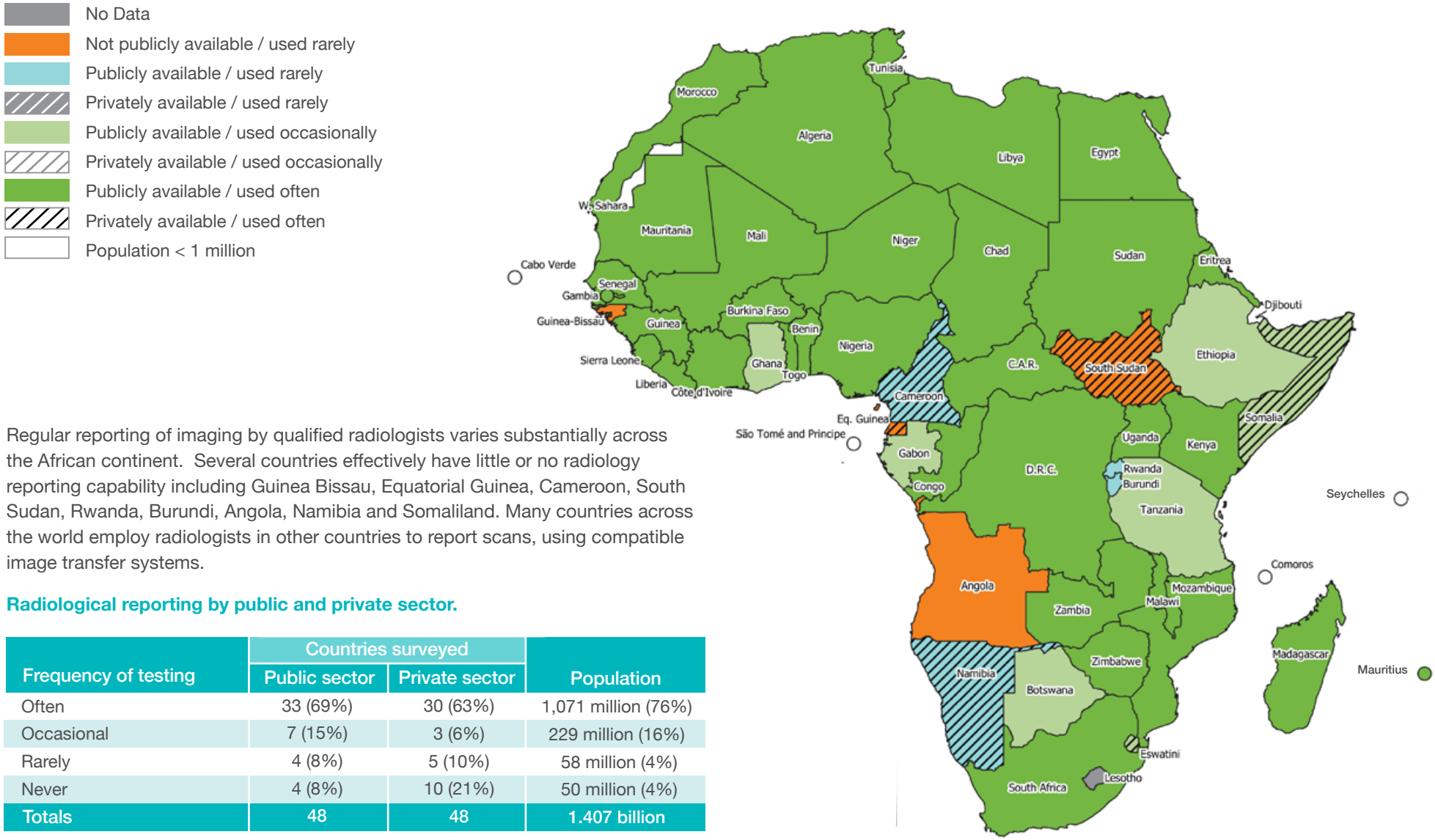



Several African countries do not have CT scanning capability, including Angola, Somalia, Puntland, South Sudan, Equatorial Guinea, Sierra Leone and Guinea Bissau. Access is severely limited in Burkina Faso, Uganda, Burundi, Malawi, Namibia, Ethiopia and Somaliland.

**Availability of CT scanning in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 27 (56%) | 21 (44%) | 590 million (42%) |
| Occasional | 11 (23%) | 12 (25%) | 553 million (39%) |
| Rarely | 5 (10%) | 3 (6%) | 205 million (15%) |
| Never | 5 (10%) | 12 (25%) | 59 million (4%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# Availability of image reporting by a radiologist in each country




Regular reporting of imaging by qualified radiologists varies substantially across the African continent. Several countries effectively have little or no radiology reporting capability including Guinea Bissau, Equatorial Guinea, Cameroon, South Sudan, Rwanda, Burundi, Angola, Namibia and Somaliland. Many countries across the world employ radiologists in other countries to report scans, using compatible image transfer systems.

### Radiological reporting by public and private sector.

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 33 (69%) | 30 (63%) | 1,071 million (76%) |
| Occasional | 7 (15%) | 3 (6%) | 229 million (16%) |
| Rarely | 4 (8%) | 5 (10%) | 58 million (4%) |
| Never | 4 (8%) | 10 (21%) | 50 million (4%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# CT scanning (computed tomography). Additional information

Several companies are working on machine learning approaches (artificial intelligence, or computer-aided diagnosis) and currently distinguishing normal from tuberculosis is possible with a high degree of accuracy. Addition of other diagnoses are being proposed, but the AI solutions tend to be X-ray manufacturer specific, at present. weblink

### Costs and ease of use

CT scanning, with or without contrast, is usually $200-250 including radiologist interpretation. However in some settings, costs can be as low as $15-$80, but in others as high as $300.

### Guidelines

CT scanning of the thorax is an intrinsic, critical component of the diagnostic workup for many chest conditions, including invasive and chronic pulmonary aspergillosis (Schlenz, 2015; Denning, 2016; Ullmann, 2018), mucormycosis (Cornely, 2019), lung cancer, idiopathic pulmonary fibrosis, bronchiectasis (Hill, 2019), pulmonary nodules (Callister, 2015), blunt and penetrating chest trauma and many cardiac and mediastinal conditions.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. CT scans are primarily done to evaluate brain, paranasal sinus, chest or abdominal disease. Both trauma and potential cancer evaluations are common reasons for CT scans. In the case of lung disease, a CT scan is superior to chest X-ray for fungal infection whether acute invasive, chronic or allergic. MR scanning of the brain is superior to CT scanning for most infections, especially in HIV-infected people.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | Some |
| Skin rash, or lumps | Some |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Usually |
| Leukaemia and neutropenia / corticosteroids | Yes |
| Critically ill and ICU | Usually |
| Fever and sepsis | Some |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing – separating imaging from where the radiological image is interpreted and reported. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below give an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

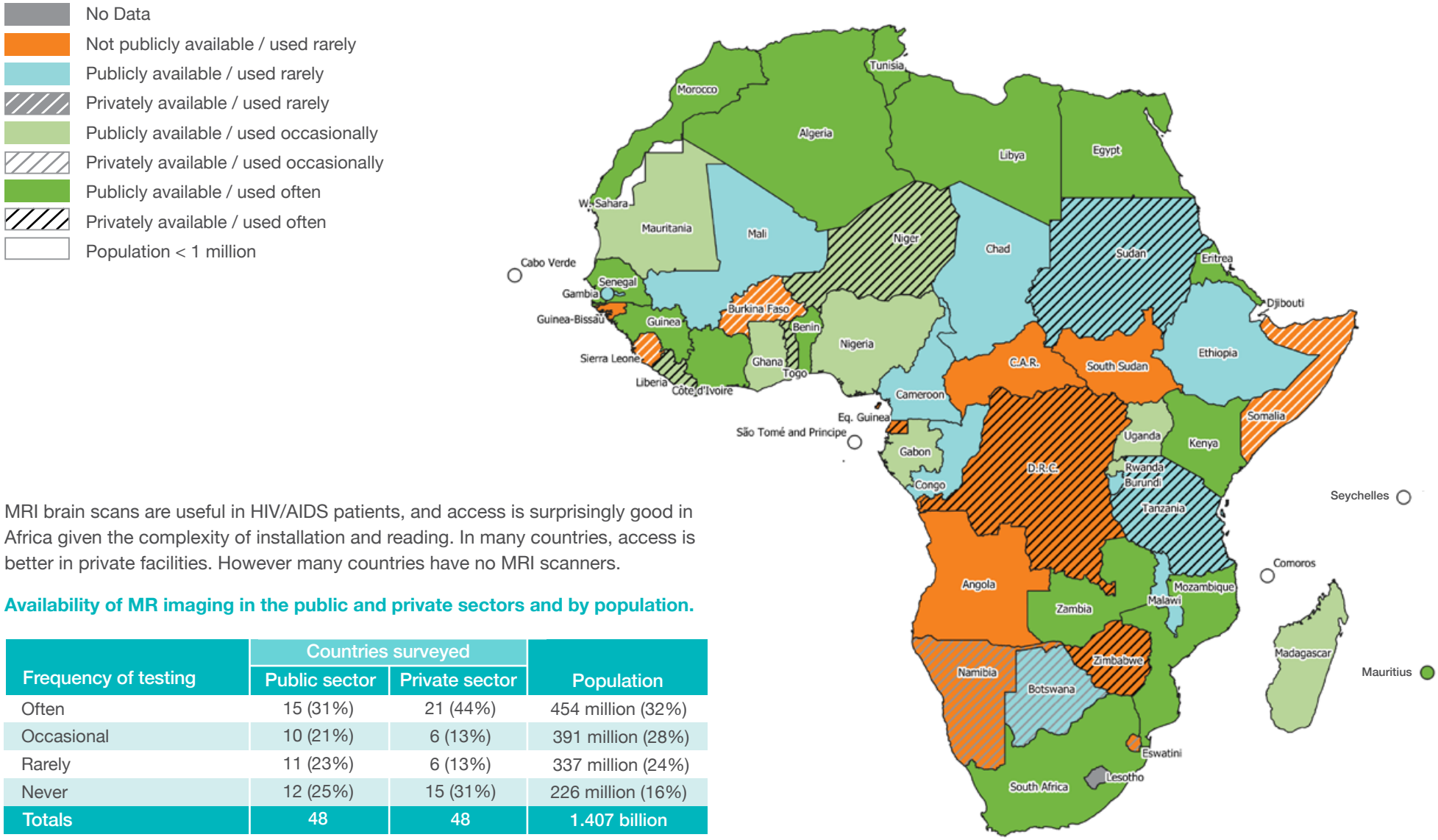
| Where to test | CT scanner location | Radiologist interpretation |
|---|---|---|
| Community services | No | No |
| Local TB clinics | Possibly | Possibly |
| Local HIV clinics | No | No |
| Local hospitals | Possibly | Preferably |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

# MR imaging (magnetic resonance)

MRI scanners are especially useful for imaging soft tissues, spinal cord and brain. They have one advantage over CT scans, in that they do not use ionizing X-ray radiation. The brain, spinal cord and nerves, as well as muscles, ligaments, and tendons are visualised more clearly with MRI.

In the context of HIV infection, MRI clearly differentiates white matter and grey matter and is especially useful for diagnosing cerebral toxoplasmosis and other intracranial infections such as progressive multifocal leukoencephalopathy caused by the JC virus or primary cytomegalovirus encephalitis. CT scanning is not very sensitive for cerebral toxoplasmosis and lesions (which are usually several) are often missed, which they are not with MRI.

# Availability of MR imaging (magnetic resonance) by country




MRI brain scans are useful in HIV/AIDS patients, and access is surprisingly good in Africa given the complexity of installation and reading. In many countries, access is better in private facilities. However many countries have no MRI scanners.

**Availability of MR imaging in the public and private sectors and by population.**

| Frequency of testing | Countries surveyed | | Population |
|---|---|---|---|
| | Public sector | Private sector | |
| Often | 15 (31%) | 21 (44%) | 454 million (32%) |
| Occasional | 10 (21%) | 6 (13%) | 391 million (28%) |
| Rarely | 11 (23%) | 6 (13%) | 337 million (24%) |
| Never | 12 (25%) | 15 (31%) | 226 million (16%) |
| **Totals** | **48** | **48** | **1.407 billion** |

# MR imaging (magnetic resonance). Additional information

### Costs and ease of use

In several African countries, an MRI scan including reporting fee is $200-300 but can be as high as $600, or as low as $100.

### Guidelines

Summary guidelines on neurological complications of HIV and AIDS were provided by a European task force in 2004 (Portegies, 2004). These assumed both CT and MRI imaging are available and articulate the key diagnostic value of both positive features and negative findings. The WHO Advanced HIV guidelines are silent on recommendations for imaging.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. MRI scans are excellent for evaluating brain disorders and most bone and soft tissue conditions. In particular, MRI scans are very useful for brain disorders in HIV patients, as well as other immunocompromised patients.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | Some |
| Skin rash, or lumps | |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing – separating imaging from where the radiological image is interpreted and reported. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below give an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | CT scanner location | Radiologist interpretation |
|---|---|---|
| Community services | No | No |
| Local HIV clinics | No | No |
| Local hospitals | Possibly | No |
| Referral hospitals | Yes | Preferably |
| Teaching/specialist hospitals | Yes | Yes |

# HEALTH SYSTEMS DIAGNOSTICS PLANNING

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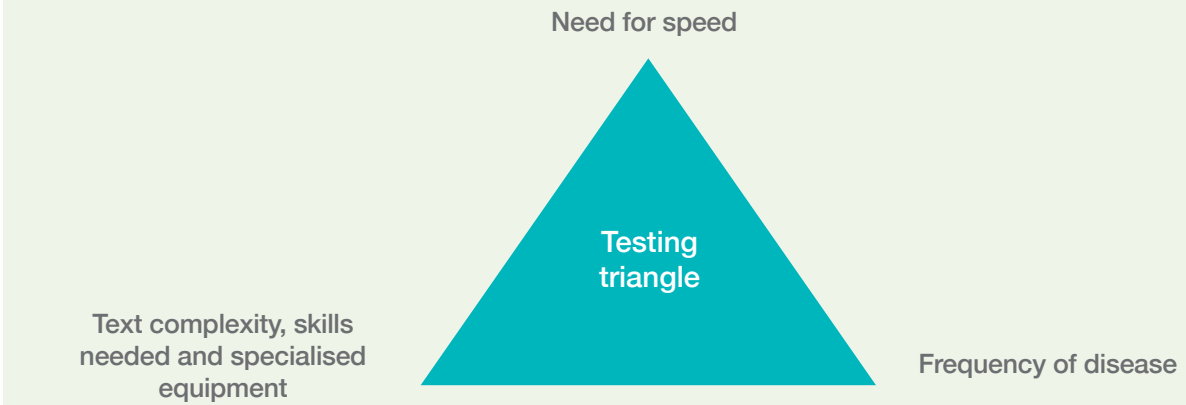
# Health systems diagnostics planning

Some fungal infections are common, at least in certain patient groups, others uncommon or rare. Some diagnostic tests are simple to do and interpret, others require specialised equipment and/or skills to perform. Some fungal diseases are immediately life-threatening, others more indolent, with less need for immediate treatment. These are the main factors that should determine where diagnostic tests should ideally be performed. A structure for health systems planning and implementation for fungal diseases was described in 2017 (Cole et al), and followed up with a major meeting on the topic for Latin America (GAFFI, 2019).

A distinction needs to be drawn between sample acquisition and where the test is done or interpreted. Not everything must be local. So, chest X-rays are done in many facilities, but interpretation by a radiologist can be done remotely, if the image is digital and can be conveyed to distant centres. For other fungal diseases, the proximity of sample acquisition and handling should be close, a good example being corneal scraping done by an ophthalmologist and the laboratory for microscopy and culture.

From a planning perspective, many of the facilities required for TB diagnosis are similar to those required for fungal disease, with the major addition of antigen and antibody tests for fungal disease. Cryptococcal antigen testing is best performed immediately and as close to the patient as possible. In some institutions the agar plates are supplied to the clinics and inoculated there and then transported to the laboratory, with some material for microscopy.

**Different fungal disease diagnostics require different response times, equipment and training.**




# Health systems diagnostics planning

| Test | Complexity, including equipment required | Skill level required to complete testing and issue a report |
|---|---|---|
| **Antigen testing** | | |
| *Cryptococcus* | Low | Low |
| *Aspergillus* | Moderate (sample handling) | Moderate |
| *Histoplasma* | Low to moderate | Low to moderate |
| **Microscopy** | | |
| Skin, hair and nail samples | Low to moderate | High |
| Vaginal samples | Low | Moderate |
| Deep and eye samples | Moderate | High |
| Histopathology | Moderate to high | High |
| **Culture** | | |
| Blood culture | High | Moderate |
| Respiratory samples | High | Moderate to high |
| Vaginal samples | Moderate | Moderate |
| Skin, hair and nail samples | Moderate | Moderate to high |
| **PCR & molecular tests** | | |
| *Pneumocystis* PCR | High | Moderate |
| Identification of unusual fungi | High | Moderate to high |
| **Antibody testing** | | |
| *Aspergillus* IgG antibody | Low to moderate | Low to moderate |
| *Aspergillus* IgE and total IgE | Moderate | Moderate |

**Summary of diagnostic tests complexity and skill level which determines the minimum level of infrastructure support required**

# Health systems diagnostics planning

Given all these considerations, the schematic on the next page describes one approach to distributing high quality diagnostic testing equitably across any healthcare system, given the considerations of relative fungal disease frequency, test complexity and skill requirements and need for rapid results. Sample transport systems and rapid results delivery are key components for success, as is fiber-optic IT connectivity for radiology reporting for remote populations.

GAFFI has supported a national Diagnostic Laboratory Hub (DLH) in Guatemala integrating rapid testing for HIV patients for TB, cryptococcal disease and histoplasmosis (Samayoa, 2020). The focus of the DLH is immediacy of providing test results, with fast transport systems and internet links for immediate transmission of results to local centres. Over the years 2017-2019, mortality dropped by 8% among about 2,300 screened patients in 13 centres (11.6%) (Medina, 2021). Fewer cases of tuberculosis were diagnosed and relative survival increased by 14.9%, and conversely more cases of histoplasmosis were diagnosed, again with improved survival (Medina, 2022).

A longer summary of this program is provided on page 82.

Multiple free educational resources about fungal disease are online at: www.fungaleducation.org

**RAPID turnaround time**
(1-2 days, less if possible)

- Cryptococcal antigen
- *Aspergillus* antigen*
- *Histoplasma* antigen
- Direct microscopy of CSF, bronchoscopy and eye samples
- CD4 cell counts*
- *Pneumocystis* PCR
- Blood culture for yeasts
- *Aspergillus* IgG antibody screen
- Beta D glucan
- Histopathology on immunocompromised or hospitalized patient samples
- Radiology reporting on immunocompromised or hospitalized patient images*

**SHORT turnaround time**
(2-5 days)

- Fungal culture
- Fungal identification (if possible)
- Microscopy of skin, hair, vaginal and nail samples
- Histopathology
- Radiology reporting*
- Therapeutic antifungal level monitoring*

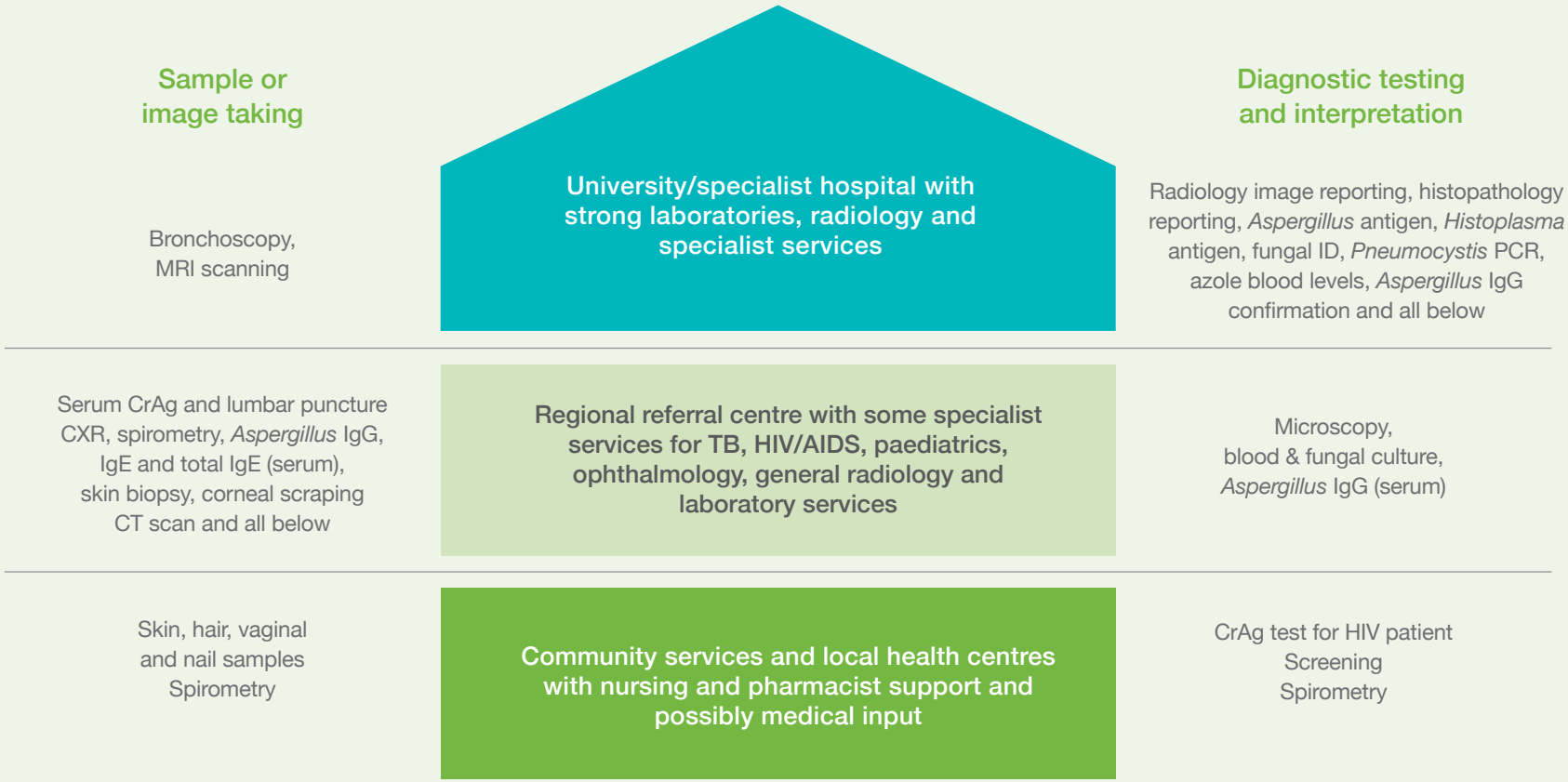
**LONGER turnaround time**
(5-10 days)

- *Aspergillus* IgG antibody quantification and other fungal antibody tests*
- *Aspergillus* and other fungi IgE antibody*
- Total IgE*
- Fungal identification of rare fungi
- Fungal susceptibility testing

* repeat testing often required to monitor therapeutic response.

# Tests for different lab and hospital complexity

**In general terms, the closer to the patient that testing can be done and interpreted, the faster the result will be available for clinicians. Local healthcare delivery arrangements, skill availability, resource and transport and IT networks are all important in providing excellent services for patients.**



# Aspiring to excellence in fungal disease management

### Clinical links

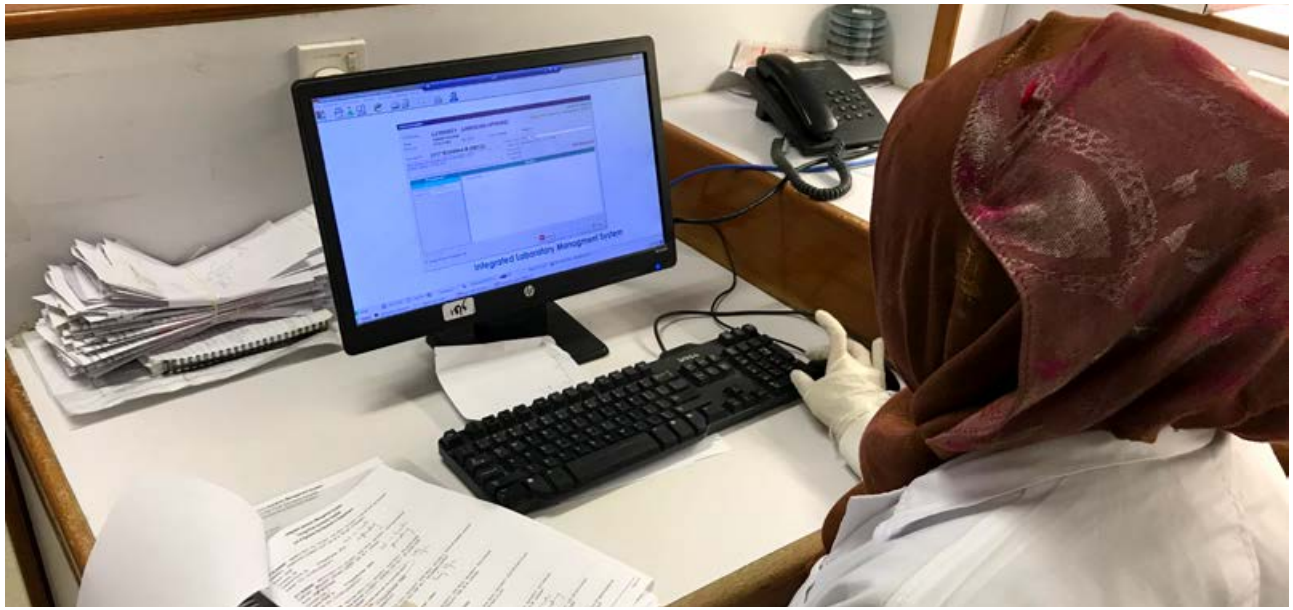
Strong links between the laboratory and clinical teams greatly strengthen the effectiveness of both the laboratory and patient care. Clinical expertise in fungal infection and allergy is patchy, but can be grown with regular contact and external engagement. Most advanced medical centres have mycology experts, usually in infectious diseases, occasionally haematology, critical care or respiratory medicine. Institutions that deliver the best care have well-recognized clinical experts in fungal disease who advise other clinicians and they work closely with the laboratory and radiologists.

### Fungal disease surveillance and outbreak investigation

Accurate diagnosis is a necessary component of surveillance. Many fungal diseases require a single test for diagnosis, such as cryptococcal antigen or *Pneumocystis* PCR, and for these infections laboratory-based surveillance provides a reasonable approximation of the full burden of infection for that population. Other fungal diseases are more complex to diagnose, such as fungal sinusitis or invasive aspergillosis – data collection is more costly and so surveillance studies are required to monitor incidence and trends over time. Outbreak investigations require a heightened approach to diagnosis to detect cases that might otherwise be missed.

### Other diagnostic expertise

Radiology and histopathology are critical to making rapid and accurate diagnoses of many fungal diseases. Radiology expertise is particularly necessary for lung, brain and sinus imaging. Not only can histopathology expertise spot fungi in tissue, the tissue response can be delineated, which is important. Most fungi cannot be identified to species or genus level on histopathology staining, so molecular identification from tissue may be definitive, if culture is negative. Combinations of direct microscopy, culture, histopathology and molecular identification are necessary for rarer fungal diseases.

# Financing diagnostics

Apart from histopathology, we have not attempted to collect data on the costs of laboratory tests. The acquisition costs of the tests varies somewhat, mostly related to the number of tests ordered, shipping and customs clearance costs. As a general statement lateral flow assays cost from $4-12 each, ELISA antigen or antibody tests $6-25 each (partly related to the number of samples run together), *Pneumocystis* PCR $30-50, inclusive of extraction. The material costs of direct microscopy and culture are very low (under $3), but the time taken on microscopy and positive cultures, including identification procedures is considerable and requires a skilled technologist. In Lagos, as an example, the direct microscopy charge is $13.

Some countries provide complete or almost complete healthcare coverage for their citizens, notably Algeria, Libya, Malawi, Mauritius and South Africa. Others provide some support, including Morocco, Egypt, Guinea, Benin, Gabon, Uganda, Kenya, Burundi, Angola, Zambia, Mozambique, Madagascar, Zimbabwe, Botswana, Namibia and Eswatini. In these (and a few other countries) certain services are fully provided at no (or minor) cost to the patients, notably outpatient HIV care, including CD4 cell counts, antiretroviral therapy, fluconazole and cotrim, and TB care, including an initial chest X-ray. Some countries provide free maternity and baby care. Some countries also provide free or highly subsidised hospital care for those with HIV, if externally funded.

Substantial numbers of patients benefit from healthcare supported by charitable organisations, sometimes on an ongoing basis, sometimes as focussed intermittent services (i.e. cataract surgery). The individual country profiles appended to this report give summary detail of healthcare organisation and funding (Appendix 4).

# Financing diagnostics

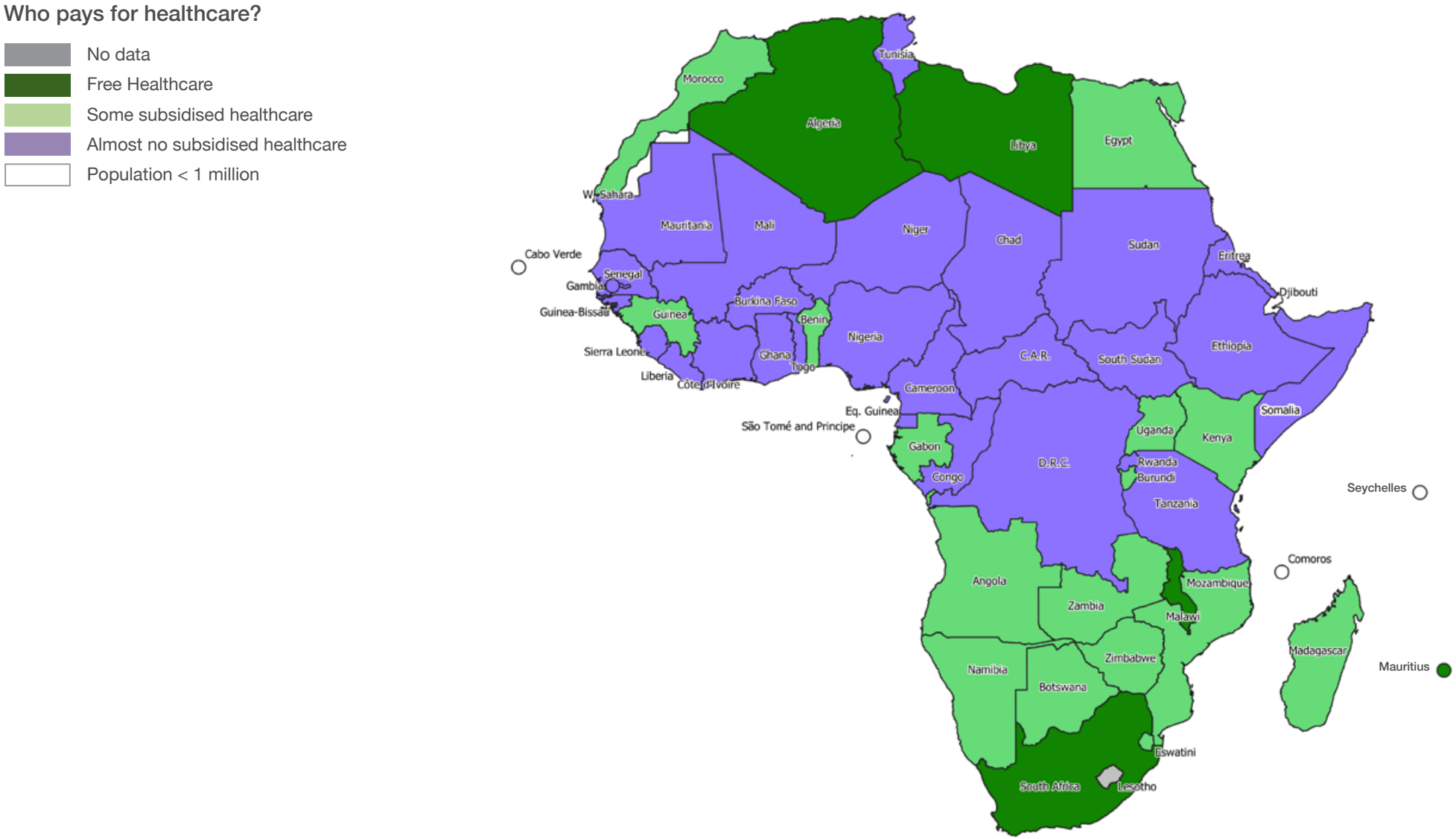
### Examples of diagnostics cost variations

There is wide variation in the costs of diagnostic procedures, imaging and laboratory tests in different countries. Some of this variation may be related to accounting approaches (i.e., whether institutional overheads are or are not included), whether delivered in the public sector, by charitable institutions or privately; in addition, in some countries (such as Eritrea) the prices listed are what is charged to patients, not a 'fully loaded' cost to the health sector. There is also substantial variation within countries, even in the public sector. Examples of variations are shown in the table below (converted to USD).

| Country | Skin biopsy | Corneal scraping | Bronchoscopy | Spirometry | Histopathology | Chest X-ray + report | CT scan + report | MRI scan + report |
|---|---|---|---|---|---|---|---|---|
| Uganda | $27 | $19 | $81 | $27 | $41 | $4 | $81 | $216 |
| Nigeria (Lagos) | $100 | Free | ND | $13 | $28 | $13 | $145 | $225-300 |
| Mali | $15 | ND | $30 | ND | $25 | $35 | $20 | ND |
| Liberia | $100-150 | $50-100 | $50-100 | $50-100 | $50-150 | $25-50 | $50-100 | $100-200 |
| Mozambique | $50 | $100 | $40 | $20 | $50 | $50 | $70 | $250 |
| DRC | $20 | $100 | $350 | $20 | $80 | $40 | $250 | $350 |
| Morocco | $10-100 | $20-100 | $60-300 | $40-100 | $15-45 | $15-30 | $150-300 | $400-600 |
| Puntland | ND | ND | ND | ND | ND | $200-300 | $200-300 | $200-300 |
| Eritrea | $3 | $5 | $10 | $5 | $20 | $15 | $15 | $15 |

ND = not done

# Who pays for healthcare?



# Donor funding

Substantial funding for sub-Saharan Africa HIV/AIDS care and TB care comes through the Global Fund for AIDS, TB and Malaria (GF) and the US President's Emergency Plan for AIDS Relief (PEPFAR). A recent publication documents this by country, as well as domestic funding, and its impact on deaths and other parameters of programmatic success (Granich, 2022).

There is a moderate correlation between antiretroviral therapy and expenditure (R-square 0.42), but almost none between expenditure and AIDS death rate (R-square 0.178) (see figure below). There are many possible reasons for this significant difference including domestic spending (especially in South Africa), hospital capability in terms of caring for sick patients with AIDS (diagnostics, drug availability, monitoring for drug toxicity, quality of care etc.), distance between patients, homes and hospitals and general community awareness about the life-threatening nature of opportunistic infections in AIDS. What is clear is that the emphasis on ART rollout is somewhat impactful, but this expenditure is not adequately addressing saving lives from AIDS.

# RESEARCH NEEDS

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# Research needs and opportunities

This report is focused on access to currently available diagnostics. This should remain the primary focus for this broad subject area.

### Implementation studies

However, the optimal implementation of diagnostics into a hospital, community or country that has never before had access to that technology and information is a topic of uncertainty and therefore research. Examples:

- The CrAg lateral flow assay is simple enough to be done by a conscientious and trained nursing assistant or pharmacist. If positive, how is that critically important result conveyed into the permanent patient's medical record and also acted upon by physicians?
- A locally taken chest X-ray showing upper lobe shadowing may never be reported by a radiologist or seen by an experienced physician. It may represent fungal lung disease, not TB, and incorrect treatment given.

The actions following on from positive tests need managing in a health system. How positive and negative test results are actioned is a ripe area of research to ensure optimal utilisation of new diagnostics.

### Cost-effectiveness and diagnostic utility in different settings

The frequency of different fungal diseases varies widely by age, underlying disease and its status, geography, occupation and co-morbidities. In particular, screening for serious infections may not be clinically worthwhile. For example, disseminated histoplasmosis in northern Tanzania has an incidence of <1% in febrile HIV patients admitted to hospital, in contrast to the area around Ibadan in central Nigeria where it is >10% (Oladele, 2022). Research studies need to be conducted to establish the local incidence or prevalence of disease (especially endemic mycoses) to decide whether to apply tests as standard screens or as part of a diagnostic algorithm. Doing these small-scale research projects also provide the laboratory technicians (or other HCWs) with experience in conducting the tests and integrating them into the work flow and establishing SOPS for conducting the test and reporting the result.

### Specific examples of studies required include:

- *Histoplasma* exposure or antigen testing in advanced HIV disease mapping to identify hot spots where antigen testing is required
- The utility and yield of *Aspergillus* antibody testing of PCR and smear negative pulmonary TB cases
- The clinical response rate and longevity of response of fungal asthma to antifungal therapy

### Integration of diagnostics into stewardship programs

Given the growth in AMR and the need to actively address it, rapid fungal diagnostic testing which can rule diagnosis in or out is important in reducing empirical antibacterial and antifungal therapy (Denning, 2017). A key component of this is a rapid turnaround time. Stewardship tools, probably mobile phone-based, could greatly supplement clinical decision-making, with test performance data integrated into a decision tree framework. Such tools are beginning to be developed, but very few are applicable to LMICs where paper-based algorithms from national or international guidelines predominate. Development and testing of such tools will be an important contributor to addressing AMR.

# Research needs and opportunities

### Artificial intelligence contributions to healthcare worker shortages

A shortage of expertise is endemic across the world in all healthcare systems except the most highly supported and specialised university or research institutes. This may be manifest as a lack of direct microscopy skills, in histopathology or radiology reporting, or in the clinical arena with complex or unfamiliar cases. Some artificial intelligence solutions could help with specific examples being:

- Computer-aided reading of chest X-rays and CT scans to highlight possible fungal infection
- Interpretation of direct microscopy
- Recognition of skin disease to provisionally diagnose ringworm or other skin fungal disease that should be further investigated by biopsy
- Distinguishing bacterial from fungal keratitis

In most cases, the AI report would be an aid to diagnosis, in the hands of an experienced practitioner, but in the hands of relatively untrained staff, for example community healthcare workers, could recommend first - and second - line therapy and triage those patients who need specialist diagnosis and care, rapidly.

### Improved and accurate diagnosis opens the door to the study of new medications

The vast majority of new medicines are trialled in advanced economies. Improvement of diagnostic capability and general medical infrastructure, opens the door to an expansion of clinical trials for new medicines into less privileged economies. This would offer benefits to the local citizens, high quality jobs for workers, faster recruitment for companies at modest cost and earlier registration of valuable new medicines. Accurate diagnosis linked with data collection builds cohorts of patients suitable for studies. Unusual adverse event profiles in these less privileged communities would be highlighted early and in controlled conditions, prior to market release.

# CASE STUDIES

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# Nigeria improves cryptococcal meningitis care through multisite training

### Baseline situation in 2018

The 2017 WHO Advanced HIV guidelines include CrAg testing followed by pre-emptive fluconazole for CrAg-positive patients without cryptococcal meningitis. The Nigeria HIV treatment guidelines reflect the WHO recommendations but were far from routine. There was poor awareness of national recommendations, including the value of CrAg screening by health care providers, poor uptake of lumbar punctures with a lack of necessary equipment and limited equipment for diagnosis.

### Training and awareness program

A training curriculum was developed to raise awareness and improve clinical skills of healthcare professionals (HCPs) in the recognition, diagnosis, management, and prevention of HIV-associated cryptococcal meningitis (Oladele, 2020), including course materials from the www.fungaleducation.org website. weblink Modules covered the following topics; 'Overview of cryptococcal meningitis', 'What is *Cryptococcus* and cryptococcosis?', 'Recognizing signs and symptoms of cryptococcal disease', 'How to perform a lumbar puncture', 'Diagnosing cryptococcal disease', 'Treating cryptococcal meningitis', 'Antifungal drug interactions', 'Preventing cryptococcal meningitis', 'Decision-making guide for cryptococcal screening' and 'Your role as a health care provider' and 'The way forward (focus group discussions)'. Training was conducted in 13 sites with 761 participants.

### Outcomes

After training, healthcare professionals correct questionnaire responses rose from 60% to 91% (14 key questions). Focus group discussions revealed that many of the HCPs were not aware of the CrAg screening and pre-emptive treatment recommendations in Nigerian guidelines and said they had not seen or managed any cases of cryptococcal meningitis. Respondents highlighted challenges with CrAg screening due to a lack of access to CD4 testing and CrAg test kits. They endorsed the need for similar trainings across all tiers of care in Nigeria.

# Getting rapid results for patients - the Diagnostic Laboratory Hub (DLH)

### Baseline situation

In 2015, care of newly presenting and hospitalised people with HIV relied mostly on clinical judgement with little diagnostic testing, throughout Guatemala. Histoplasmosis is hyperendemic in Guatemala and lethal in AIDS, but only in the capital Guatemala City was testing with the most sensitive assay (urinary antigen) done based on clinical suspicion. Over 45% of new HIV diagnoses were made late, with advanced immune suppression. In 2016, deaths from AIDS were 1,600 among an estimated 46,000 infected people, with only 59% coverage of antiretroviral therapy.

### Enhancing diagnostics and training through a new Diagnostic Laboratory Hub (DLH)

Through the well-equipped NGO Asociación de Salud Integral, GAFFI supported improved diagnosis and patient care via several coordinated actions:

- Enhanced 'free' diagnostics for 13 of 16 HIV units in Guatemala (60% of the nation's patients with HIV) for TB (smear, culture, PCR), histoplasmosis (urine antigen, PCR, culture) and cryptococcosis (antigen, culture)
- Rapid sample transport system
- Internet ordering and results delivery between each centre and the DLH.
- Extensive online and in-person training of clinicians and laboratory staff.

A screening approach was taken for all HIV patients, regardless of CD4 count for TB, histoplasmosis and cryptococcosis.

### Outcomes

From 2017-2019, there were 2,127 newly diagnosed HIV patients assessed and 21% had one or more of these life-threatening infections. Histoplasmosis was the most frequent infection (7.9%), followed by TB (7.1%); 94.4% of these infections occurred in patients with a CD4 < 350 cells/mm3. Dual or triple infections were seen in 8.1% highlighting the importance of the screening approach. Overall mortality decreased from 34% to 27%, 54% dying within the first month. The number of TB diagnoses went down and survival increased by 14.9%, indicative of mis-diagnosis of TB early in the program. Histoplasmosis mortality fell from 32.8% in 2017 to 21.2% in 2019. Overall, 6366 patients were enrolled, and 7.4% had histoplasmosis, with an increase from 2017-2019 from 6.5% to 8.8%, with improved screening coverage.

The success of the program is such that the costs of the diagnostic tests are now covered by the Ministry of Public Health and Social Assistance and expanded.

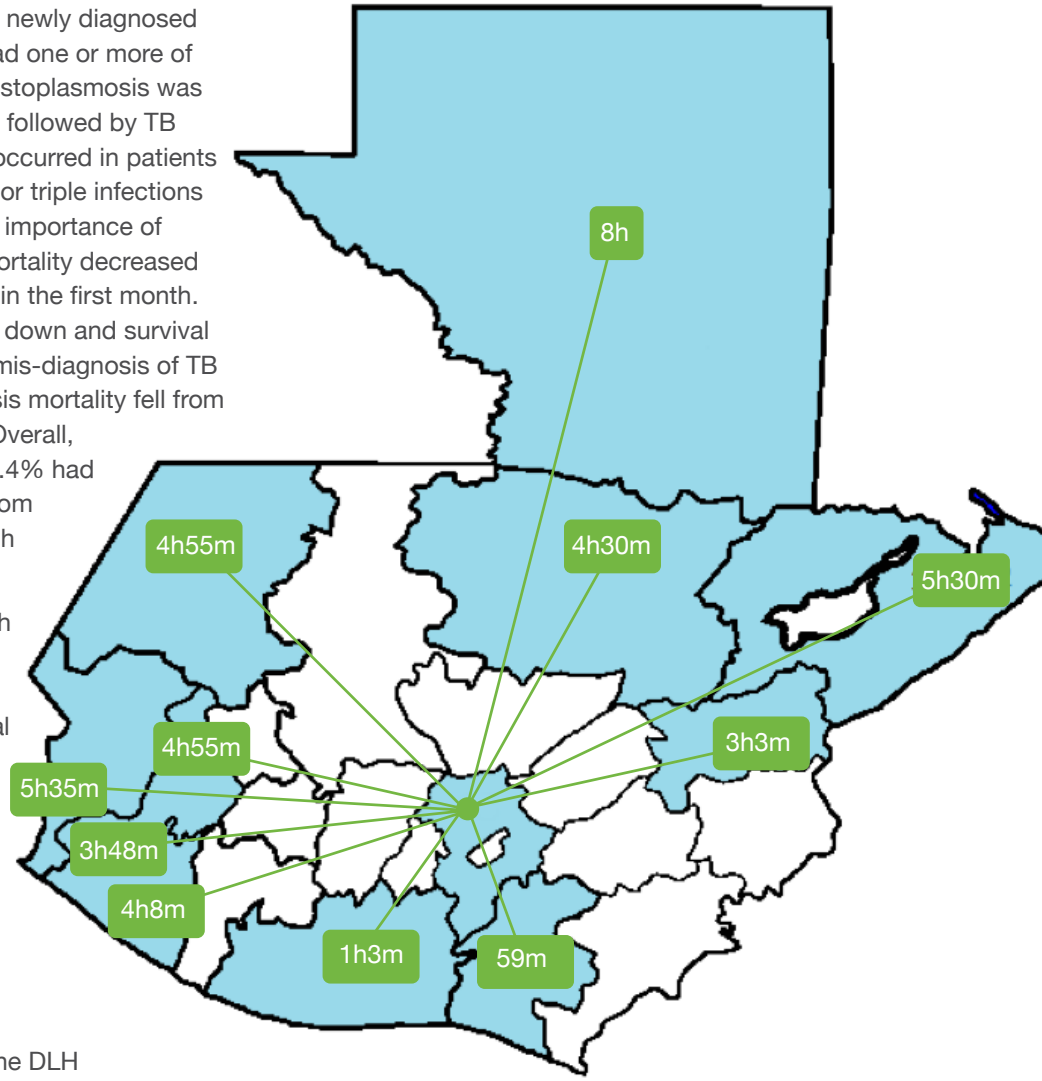



**Figure Right** Distance by car to the DLH

# Diagnosing chronic pulmonary aspergillosis in a TB endemic area: the Ugandan experience

### Baseline situation in 2016

A 2015 paper on the burden of all fungal diseases in Uganda estimated a prevalence of chronic pulmonary aspergillosis (CPA) following TB of 436 - 3347 patients (Parkes-Ratanshi, 2015), but the diagnosis had barely been made previously. This paper noted that fungal diseases were underestimated and under-diagnosed in Uganda. In 2013 and then 2016, two Ugandans (Richard Kwizera and Felix Bongomin) completed an MSc in Medical Mycology at the University of Manchester.

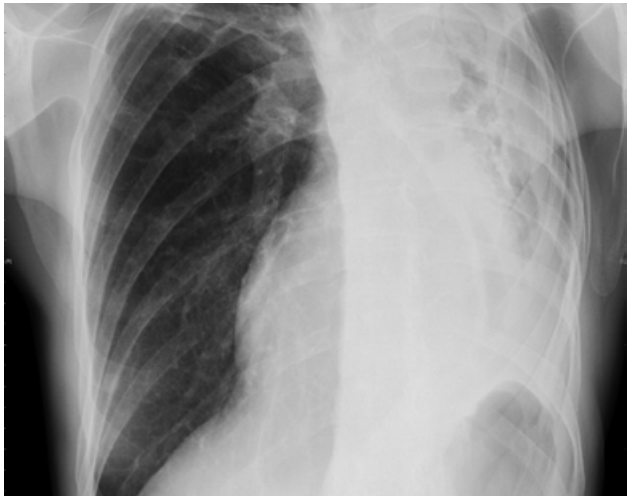
### Research studies done

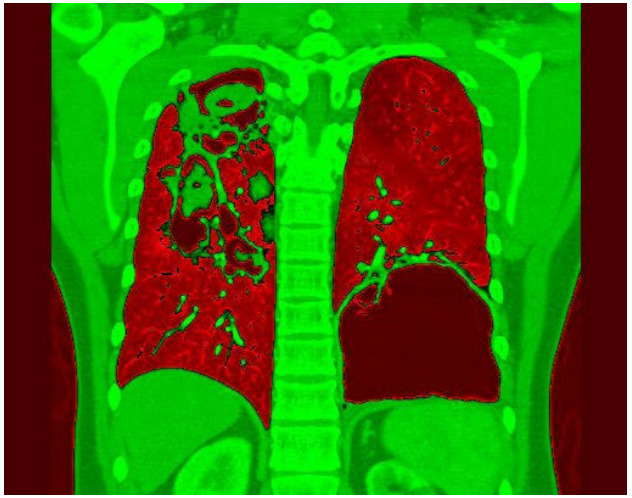
Multiple studies of aspergillosis were undertaken in Uganda:

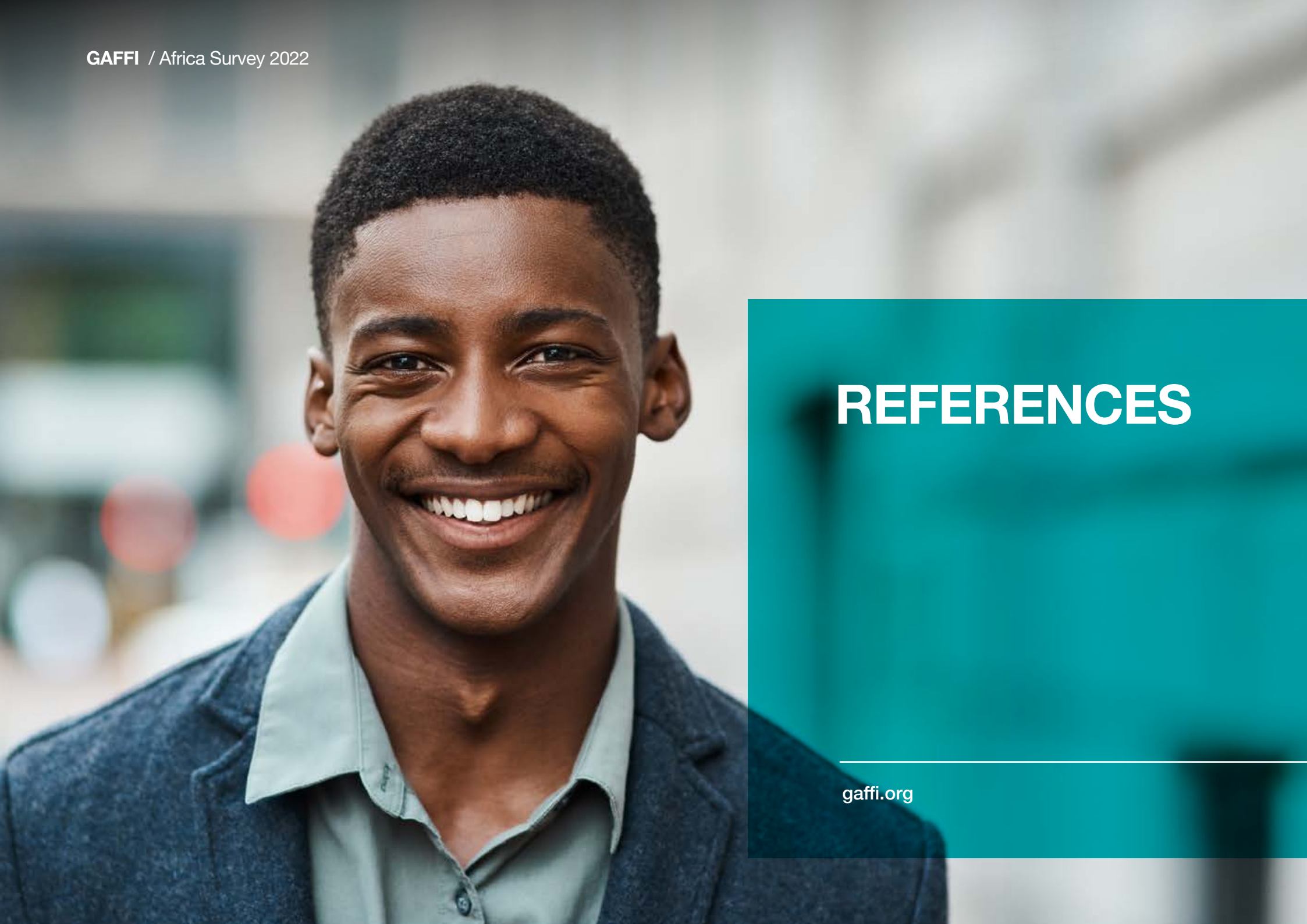
- At the end of TB treatment, 10% of the patients had elevated *Aspergillus* IgE or *Aspergillus* sensitization (linked to worse lung function) and *Aspergillus* IgG antibodies were elevated in 9% of the patients (Kwizera, 2017).
- From 2015-2017, a prospective survey of 400 people cured of TB in Gulu (northern Uganda), with and without HIV infection, found 6.5% each year to develop CPA if they had a residual TB cavity and 0.2% if they did not (Page, 2019).
- In 2018, the new *Aspergillus* IgG/IgM lateral flow device (LFD) from LDBio Diagnostics was introduced and used to actively seek CPA cases on the pulmonary and infectious disease wards at Mulago national referral hospital. Six case reports were published; some having been treated four times for pulmonary TB without improvement (Kwizera, 2021).

### Outcomes

The index of clinical suspicion for CPA was heightened. Case discussions, CME's and talks led to increasing awareness among the junior medical officers and laboratory technologists. Free testing using the Asp-LFD donated from the UK. Anti-TB medication was safely discontinued in patients with a negative TB work up who met the diagnostic criteria for CPA and instead they were commenced on antifungal therapy with an overall good outcome. 'Simplified notes about CPA' for clinicians in resource-limited settings was published (Bongomin, 2020). Funding from Forgarty has enabled a cross-sectional study to determine the prevalence and factors associated with CPA among pulmonary TB patients in Kampala, explicitly designed to provide more data on the *Aspergillus* LFD for possible inclusion on the Ugandan list of essential diagnostics for CPA.







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# APPENDIX 1
# Questionnaire Development

# Questionnaire development

The questionnaire consisted of seven sections.

## Respondent

This covered the respondent(s), including their role, facility and whether their country had a BSL3 lab with or without protocol for handling pathogenic fungi.

## WHO-Recommended Essential Diagnostics

This covered the availability of the WHO-recommended list of essential fungal diagnostics, classified in two ways: type of facility providing the diagnostic, and regularity of use.

The five levels of facility used were: Not available anywhere; Private centres; Specialist/university centres; District hospitals; Community health centres.

For each diagnostic, respondents were asked to select how often it was performed at each type of facility: Often; Occasionally; Rarely.

This provided a granular, multi-dimensional view of the availability of each diagnostic. There were also two further fields to provide additional context regarding availability of diagnostics and frequency of use: one for any comments, including reasons that procedures are not performed regularly (e.g., broken equipment, lack of trained personnel); and one asking about payment, using four classifications, of which respondents were asked to list any that apply including: Patient pays; Insurance pays; Government/ health service pays; Charity/foundation pays.

## CD4 counts

This consisted of two multiple-choice questions about CD4 count policy: which patients have counts taken, and which assay is used.

## Essential Clinical Procedures

This covered essential clinical procedures and radiology and used the same tabular structure as for WHO-recommended essential diagnostics, with facilities, regularity of use, payment and a comments section.

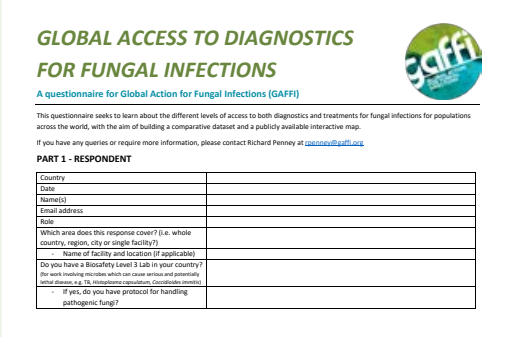
## Costs of tests and procedures

Respondents were asked for approximate costs of several diagnostics/procedures.

## Other fungal diagnostic tests

Respondents were asked for any additional fungal diagnostics used in their country

## Any other comments



GLOBAL ACCESS TO DIAGNOSTICS FOR FUNGAL INFECTIONS

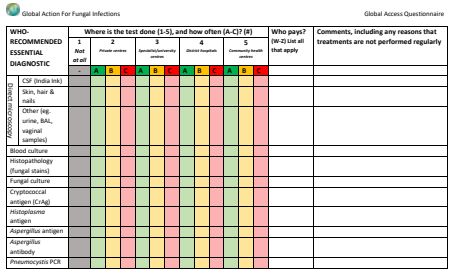
A questionnaire for Global Action for Fungal Infections (GAFFI)

This questionnaire seeks to learn about the different levels of access to both diagnostics and treatments for fungal infections for populations across the world, with the aim of building a comparative dataset and a publicly available interactive map.

If you have any queries or require more information, please contact Richard Penney at rpenney@gaffi.org

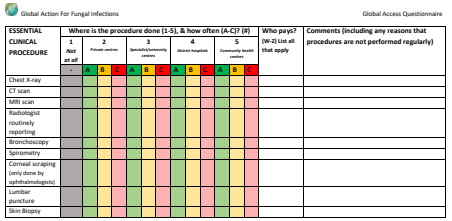
**PART 1 - RESPONDENT**

| | |
|---|---|
| Country | |
| Date | |
| Name(s) | |
| Email address | |
| Role | |
| Which area does this response cover? (i.e. whole country, region, city or single facility?) | |
| - Name of facility and location (if applicable) | |
| Do you have a Biosafety Level 3 Lab in your country? (for work involving microbes which can cause serious and potentially lethal disease, e.g. TB, *Histoplasma capsulatum*, *Coccidioides immitis*) | |
| - If yes, do you have protocol for handling pathogenic fungi? | |



Global Action For Fungal Infections — Global Access Questionnaire

| WHO-RECOMMENDED ESSENTIAL DIAGNOSTIC | Where is the test done (1-5), and how often (A-C)? (#) | | | | | | | | | | | | Who pays? (W-Z) List all that apply | Comments, including any reasons that treatments are not performed regularly |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 Not at all | 2 Private centres | | 3 Specialist/university centres | | | 4 District hospitals | | | 5 Community health centres | | | | |
| | - | A | B | C | A | B | C | A | B | C | A | B | C | |
| DIRECT MICROSCOPY | CSF (India Ink) | | | | | | | | | | | | | |
| | Skin, hair & nails | | | | | | | | | | | | | |
| | Other (eg. urine, BAL, vaginal samples) | | | | | | | | | | | | | |
| Blood culture | | | | | | | | | | | | | | |
| Histopathology (Fungal stains) | | | | | | | | | | | | | | |
| Fungal culture | | | | | | | | | | | | | | |
| Cryptococcal antigen (CrAg) | | | | | | | | | | | | | | |
| *Histoplasma* antigen | | | | | | | | | | | | | | |
| *Aspergillus* antigen | | | | | | | | | | | | | | |
| *Aspergillus* antibody | | | | | | | | | | | | | | |
| *Pneumocystis* PCR | | | | | | | | | | | | | | |



Global Action For Fungal Infections — Global Access Questionnaire

| ESSENTIAL CLINICAL PROCEDURE | Where is the procedure done (1-5), & how often (A-C)? (#) | | | | | | | | | | | | Who pays? (W-Z) List all that apply | Comments (including any reasons that procedures are not performed regularly) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 Not at all | 2 Private centres | | 3 Specialist/university centres | | | 4 District hospitals | | | 5 Community health centres | | | | |
| | - | A | B | C | A | B | C | A | B | C | A | B | C | |
| Chest X-ray | | | | | | | | | | | | | | |
| CT scan | | | | | | | | | | | | | | |
| MRI scan | | | | | | | | | | | | | | |
| Radiologist routinely reporting | | | | | | | | | | | | | | |
| Bronchoscopy | | | | | | | | | | | | | | |
| Spirometry | | | | | | | | | | | | | | |
| Corneal scraping (only done by ophthalmologists) | | | | | | | | | | | | | | |
| Lumbar puncture | | | | | | | | | | | | | | |
| Skin Biopsy | | | | | | | | | | | | | | |

# APPENDIX 2
## Biosafety Level 3 Laboratories

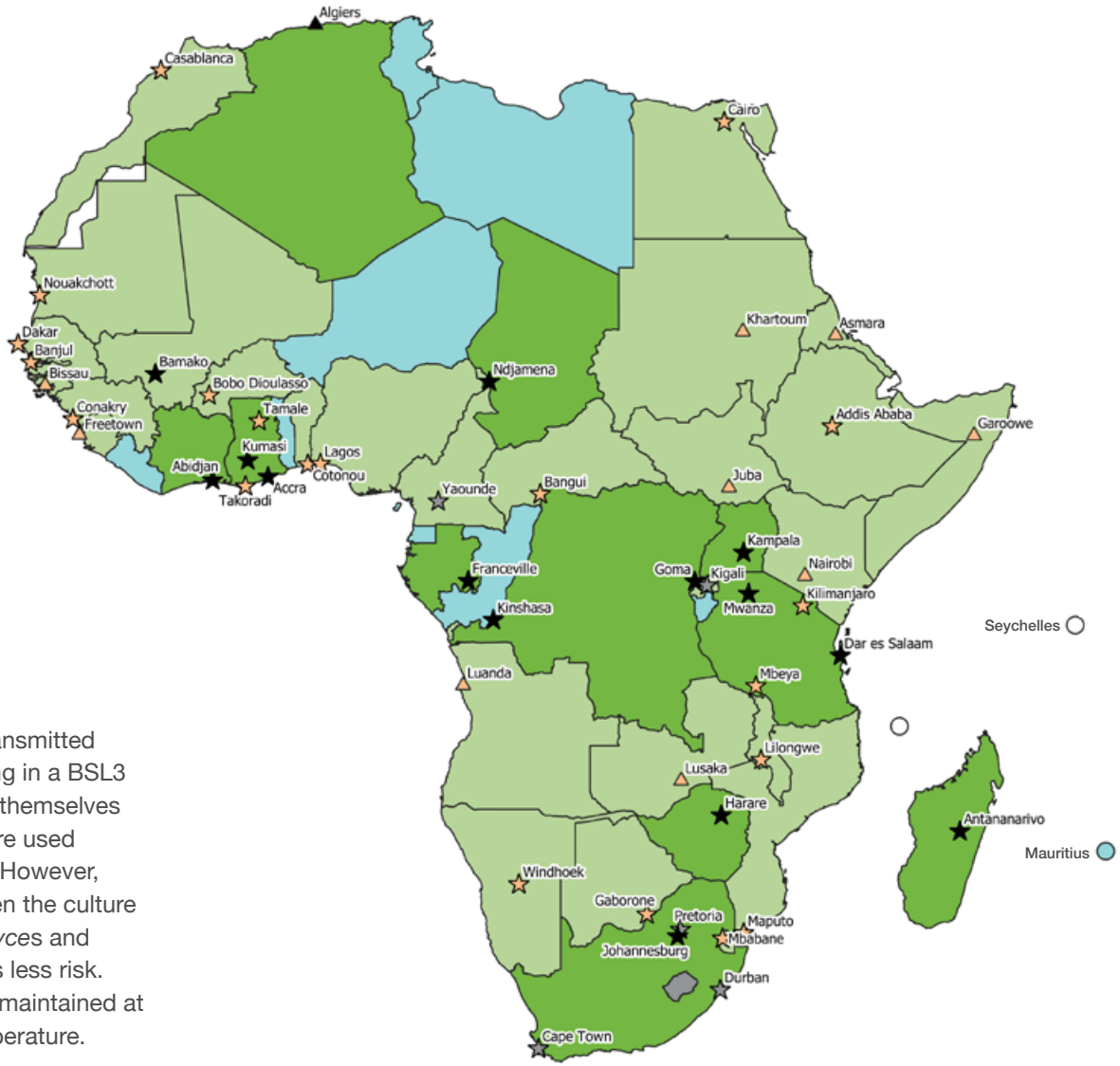
# Fungal culture. Biosafety Level 3 labs

### Country status

- No Data
- BSL-3 lab(s) with a protocol for handling pathogenic fungi
- BSL-3 lab(s) without a protocol for handling pathogenic fungi
- No BSL-3 lab
- Population < 1 million

### Lab location and fungal protocol

- Lab location confirmed
- Lab location unconfirmed
- Protocol for handling pathogenic fungi
- Fungal protocol unconfirmed
- No protocol for handling pathogenic fungi




### Biological safety level 3 fungi (BSL3)

A small number of uncommon or rare fungi can cause laboratory–transmitted infection and are therefore classified as requiring culture and handling in a BSL3 laboratory (like *Mycobacterium tuberculosis*). The patients' samples themselves are not infectious to laboratory staff if standard safety precautions are used Good Microbiological Practice and Procedure (GMPP) (WHO 2020). However, culture increases the risk because airborne conidia may escape when the culture is manipulated. Temperature dimorphic pathogens such as *Blastomyces* and *Histoplasma* spp. are yeasts at 35-37°C, and this growth form poses less risk. However, care must be taken to ensure that such yeast cultures are maintained at 35-37°C and not permitted to revert to the mould form at room temperature.

# Fungal culture. Biosafety Level 3 labs

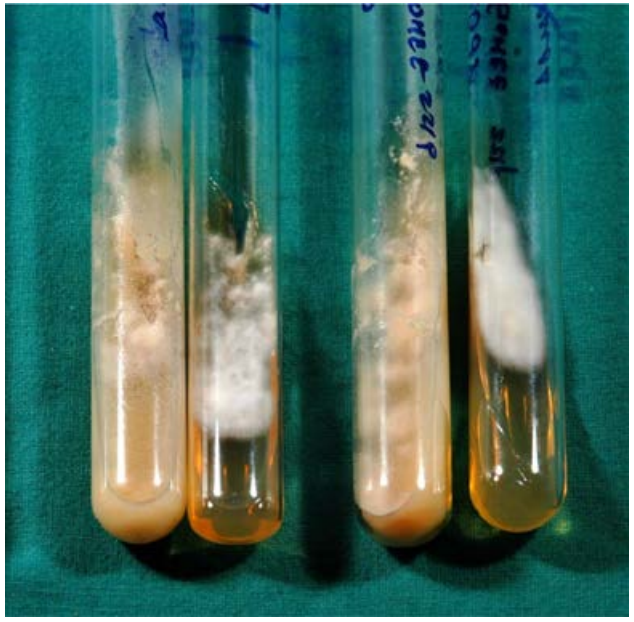
**Hazard category 3**
**human pathogenic fungi**

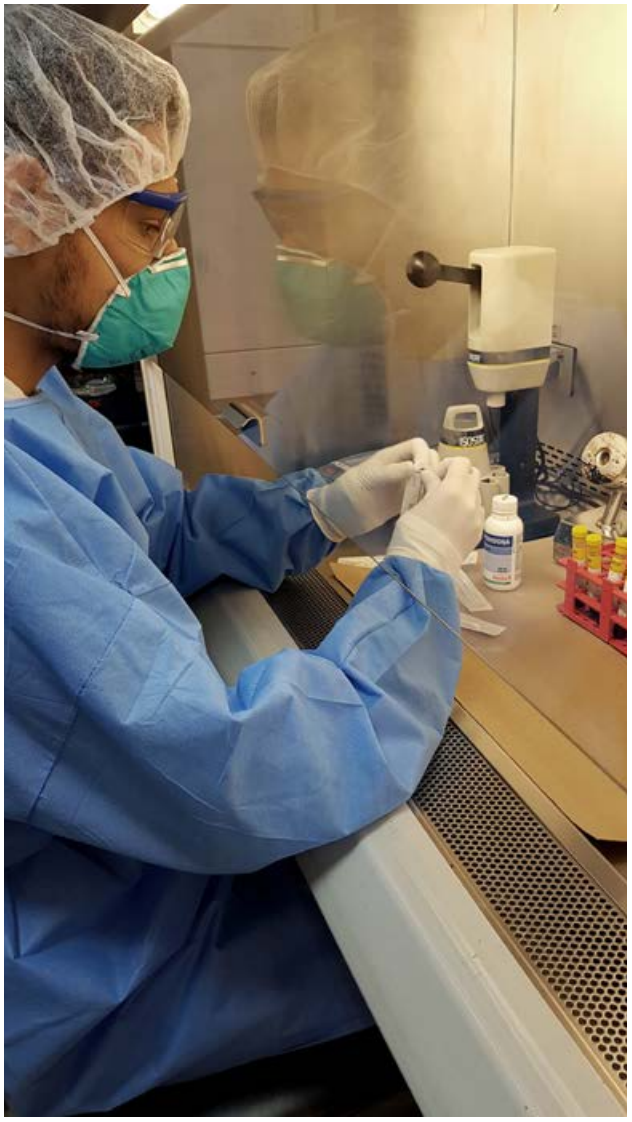
*Blastomyces* spp.*
*Cladophialophora bantiana**
*Coccidioides* spp.
*Histoplasma* spp.*
*Paracoccidioides brasiliensis*
*Talaromyces marneffei*
*Rhinocladiella mackenziei* #
*Emergomyces* spp.*†

* endemic in Africa
\# endemic in the Middle East and Morocco

† Not clearly defined as hazard category 3 – closely related to *Histoplasma*, yet no documented cases of laboratory infection and infection only been described in immunocompromised hosts (with HIV infection or other underlying conditions).

No specific guidance is available for low resource settings which undertake fungal culture from diagnostic samples about handling of possible BSL3 fungi. To minimise possible laboratory-transmitted infection (especially if a technician may be HIV-infected or otherwise immunocompromised), demonstration of dimorphism in *Blastomyces* and *Histoplasma* spp. by culture at 30ºC is not recommended and molecular means of fungal identification preferred. Training in safe handling of fungal cultures is fundamental. Transfer of cultures between laboratories should be done with appropriate safety precautions and according to IATA Dangerous Goods regulations.





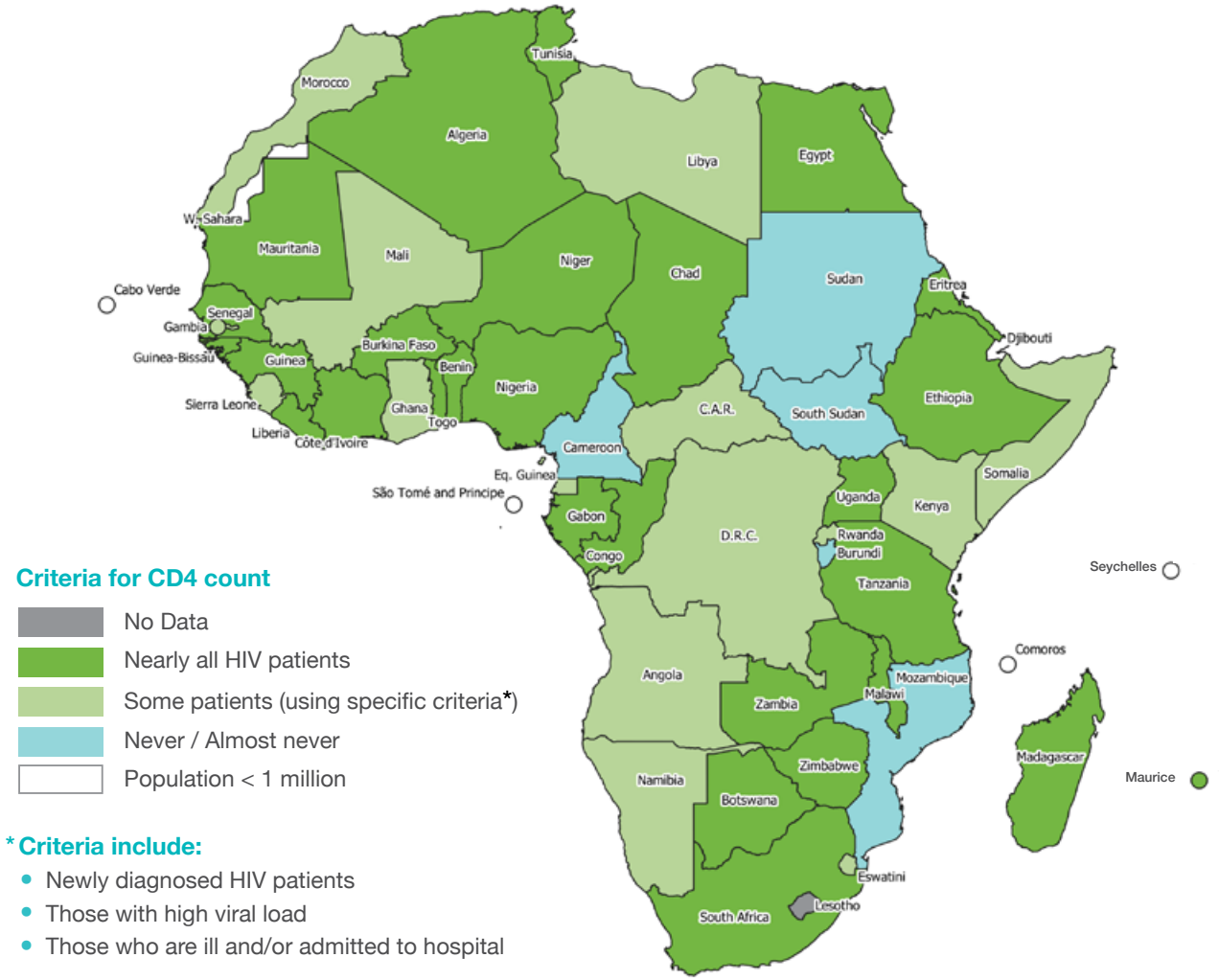
# APPENDIX 3
# CD4 Counts

# CD4 counts

During the course of HIV infection, progressive impairment of a person's immune system occurs, typically in adults over 5-7 years. This falling immune status can be tracked by measuring T helper cell counts in the blood otherwise known as CD4 cells. The earliest clinical signs of immunodepression are seen when the CD4 count falls below 350 cells/mm³ (i.e. skin fungal infections, herpes zoster and lymphadenopathy), and below 200 cells/mm³ oral and oesophageal candidiasis and life-threatening infections become increasingly common.

For newly presenting adults with HIV infection, a CD4 count has a major impact on patient assessment and infection risk. Those with CD4 counts under 200 cells/mm³ should be screened for life-threatening infection, and arguably all those with counts under 350 cells/mm³, although the likelihood of some infections is much less. Stable patients on ART with low or undetectable viral load do not usually need a CD4 count measuring. Newly hospitalised patients or those returning to care off ART for some months need a CD4 count assessment.

CD4 counting in patients with HIV/AIDS has been a cornerstone of risk assessment and immune function stratification for at least 3 decades. It is still widely available across Africa, especially in major urban centres. Countries have different guidelines for CD4 count measurement, as this map shows.




**Criteria for CD4 count**

- No Data
- Nearly all HIV patients
- Some patients (using specific criteria*)
- Never / Almost never
- Population < 1 million

***Criteria include:**

- Newly diagnosed HIV patients
- Those with high viral load
- Those who are ill and/or admitted to hospital

**CD4** cell enumeration was accepted as an Essential Diagnostic by the WHO in 2018. It is recommended for staging advanced HIV disease and to monitor response to antiretroviral therapy (in settings where quantifying viral load is not available) as a disease-specific IVD for use in clinical laboratories (EDL 3, Section II.b).

World Health Organization

# CD4 count testing. Additional information

### Availability of testing - public sector / by country

| Testing regimen | Countries | Population |
|---|---|---|
| Nearly all HIV patients | 29 | 963 million |
| Some patients according to specific criteria* | 14 | 311 million |
| Never/ almost no testing | 5 | 133 million |

***Criteria include:**
New patients / Those with high viral load /
Those who are ill and/or admitted to hospital.

### Cost and ease of use

CD4 counts cost about $10, whether as an LFA or by machine. Most CD4 counts have been measured by fluorescence-activated cell sorting (FACS) using relatively complex equipment and reagents.The recent introduction of the point of care lateral flow assay Visitect (Accubio) allows simpler and faster measurement of CD4 counts. The current Visitect assays determine above and below either 350 cells/ $mm^3$ or 200 cells/$mm^3$ counts.

### Guidelines

CD4 counts have been done since the earliest days of the HIV epidemic, but latterly less so, in favour of measuring HIV viral load. The WHO advanced HIV disease guidelines (2007) advocates their routine use for all adults and older children with HIV, unless they are stable on ART and have recovered their CD4 count to above 200 cells/mm3. CD4 counts are not helpful in children <5 years old.

**Who to test:** Clinical expertise and country guidelines will determine which patients should be tested and at what stage of disease. New HIV patients, newly hospitalised patients with HIV and those who are unwell are the primary group needing testing. Patients stable on ART, with suppressed viral load do not need testing. Young children (i.e. under 5 years) may have high CD4 counts, yet also advanced HIV disease.

| Who to test | Test |
|---|---|
| Headache, neurological symptoms | Yes if HIV+ |
| Skin rash, or lumps | Yes if HIV+ |
| Eye symptoms with corneal abnormality | |
| Persistent lung symptoms | Yes if HIV+ |
| Leukaemia and neutropenia / corticosteroids | |
| Critically ill and ICU | |
| Fever and sepsis | Yes if HIV+ |

**Where to test:** Each health system will have different approaches to diagnostic testing and ensuring that results reach patients in a timely fashion. The table below sets out a general approach to diagnostic testing - separating sample acquisition from where the testing is actually performed. Many factors impact sampling and testing locations including test complexity, frequency of disease, cost, sample transport logistics and need for rapid results. The table below gives an indication of how this cascade of testing could be arranged and emphasises that Essential Diagnostics should be available at teaching/specialist hospitals in all countries, as a minimum standard.

| Where to test | Where samples can be taken | Where tests may be best performed |
|---|---|---|
| Community services | Possibly | Preferably |
| Local TB clinics | Yes | Preferably |
| Local hospitals | Yes | Preferably |
| Referral hospitals | Yes | Yes |
| Teaching/specialist hospitals | Yes | Yes |

For more information visit: **www.gaffi.org**

**Diagnostics Survey** Africa 2022

# DIAGNOSTICS FOR FUNGAL DISEASE IN AFRICA

## A GAFFI SURVEY 2022

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