95/95 BY 2025

ENSURE THAT BY 2025 95% OF PEOPLE WITH SERIOUS FUNGAL DISEASE ARE DIAGNOSED AND 95% TREATED = 95-95

Appendix 4

Fungal Disease Diagnosis and portfolio of diagnostic tests in Mycology Reference Laboratories

Fungal Disease Diagnosis

Some visible fungal infections can be diagnosed clinically with no laboratory support (i.e. oral candidiasis), but the vast majority of infections require a laboratory diagnostic test for confirmation and separation from similarly presenting conditions. Increasingly non-culture based tests are used, as culture is slow and often insensitive. The most serious infections often require several sophisticated diagnostic tests such as CT scans and/or biopsy or antigen/antibody tests or fungal DNA detection by means of PCR (Table 4.1). A modern laboratory offering testing for fungal diseases needs a core set of non-culture tests as shown in table 4.1.

Other diagnostic expertises are also critical to making rapid and accurate diagnoses of many fungal diseases, especially histopathology and radiology. The greatest need for radiology expertise is in the lungs, brain and sinuses. Histopathology expertise will define the tissue response to infection and may be able to identify causative pathogens, supported by molecular identification of unusual fungi from tissue.

A detailed summary of all diagnostic tests for fungal disease is provided on the LIFE website here: www.life-worldwide.org/fungal-diseases/diagnostics

Reference Laboratory capabilities

GAFFI is calling for at least one expert in fungal diagnosis in each country, and one person per state or province in larger countries.The majority of these people would be based in laboratories that offer at least the core set of tests in Table 4.1. Once this portfolio of tests is offered, the laboratory can legitimately call itself a Mycology Reference Laboratory.

Mycology Reference Laboratories are preferably sited with existing diagnostic facilities with an immediate patient base that they can serve. Many of the capabilities are generic (ELISA, PCR, antibody detection) and some automated platforms serve multiple departments.

Each Mycology Reference laboratory will have the following functions:

1 Provision of a comprehensive diagnostic test portfolio

2 Training of laboratory and clinical staff for the region and country

3 Surveillance of specified nationally agreed fungal diseases and antifungal resistance rates

4 Research related to epidemiology and diagnostic testing.

Major referral hospitals and Mycology Reference Laboratories serving large communities need to develop a complete portfolio of diagnostic tests, allied with expertise in fungal infection to guide clinicians. Depending on the population to be served, including geographical variations of fungal disease, a slightly different test portfolio may be required. Rapid testing using microscopy, antigen, antibody and/or molecular assays overcomes the insensitivity of culture, and usually is faster than culture.

Professor Juan Luis Rodriguez Tudela from GAFFI, explaining fungal disease issues to colleagues.

GAFFI seeks to improve existing health capacity and calls for at least one expert in fungal disease diagnosis in each country to provide the combination of critical mass, scale for economy and quality, surveillance data and a training focus. Strong clinical links are very important for hospital integration and education.

Appendix 4/2

Fungal Disease Diagnosis and portfolio of diagnostic tests in Mycology Reference Laboratories

A modern mycology laboratory offers the following portfolio of tests and these will form the core diagnostic capabilities of each newly constituted laboratory. These core tests should be present in any large mycology laboratory. Additional testing and the complete portfolio of testing for a Mycology Reference Laboratory is described in the text below.

Table 4.1 Core diagnostic tests for a modern mycology laboratory

Test	Infection	Diagnostic sensitivity	Turnaround time*
Direct microscopy	Invasive infections, skin, hair and nails, VVC	30-90%	2 hours
Antigen	Cryptococcal meningitis	99%	2 hours
PCR on respiratory samples	*Pneumocystis* pneumonia	98%	1 day
Antigen (ELISA) on serum and respiratory samples	Invasive aspergillosis (histoplasmosis◊)	80%	2 days
Glucan detection	Most fungal infections, high NPV allowing therapy to be stopped	65-77%	2 days
Aspergillus IgG antibody	Chronic pulmonary aspergillosis	80-95%	2 days
Aspergillus IgE	Screen for ABPA in asthma	>95%	2 days
Fungal culture and identification	All except *Pneumocystis*	10-50%	3-14 days
Molecular identification from histopathology positive, culture negative	All, especially mould infections	50-60%	7 days
Itraconazole, voriconazole and posaconazole blood levels.TDM	Aspergillosis	100%	3 days

* Turnaround time includes transport to laboratory, test time (including batching), reporting and assumes a normal working day.
◊ *Aspergillus* antigen cross-reacts with *Histoplasma* antigen **NPV** - negative predictive value

For the most common fungal diseases, the following tests are essential. For rarer diseases, combinations of direct microscopy, culture, histopathology and molecular identification of fungi are required. Adaptation over time to accommodate changing circumstances, outbreaks, technology improvements and research findings is required, and no doubt this listing will be out of date in a few years. Clinical links and surveillance are also critically important functions (see below).

Nail infections
Microscopy, culture, fungal identification and molecular detection (PCR) for the most common cause of nail infection *Trichophyton rubrum.*

***Pneumocystis* pneumonia**
Pneumocystis molecular detection (PCR) or immunofluorescence microscopy, 1,3 beta-D-glucan detection in blood.

Cryptococcal meningitis Cryptococcal antigen, fungal culture and molecular identification, flucytosine blood levels.

Histoplasma capsulatum
disseminated and pulmonary infection: *Histoplasma* antigen testing, blood culture, *Histoplasma* antibody testing, itraconazole blood concentrations.

***Talaromyces marneffei* disseminated infection (in South East Asia)**
Blood culture, *Aspergillus* galactomannan (antigen) testing, itraconazole blood concentrations.

Candidaemia, invasive candidiasis, *Candida* peritonitis and oesophageal candidiasis
Blood and other cultures, rapid identification of *Candida* species (ie Chromagar or equivalent, PNA FISH, PCR), complete yeast identification and fluconazole and echinocandin susceptibility testing.

Chronic pulmonary aspergillosis:
Aspergillus IgG testing (Siemens Immunolite 2000 is best), fungal culture of respiratory samples, with identification of moulds, susceptibility testing of *Aspergillus* spp., itraconazole and voriconazole blood concentrations.

Continued overleaf.

Fungal Disease Diagnosis and portfolio of diagnostic tests in Mycology Reference Laboratories

Recurrent vulvovaginal candidiasis
Microscopy and fungal culture, *Candida* species identification.

Invasive aspergillosis
Aspergillus galactomannan (antigen) testing, *Aspergillus* molecular detection (PCR), fungal culture of respiratory samples, with identification of moulds, itraconazole and voriconazole susceptibility testing of *Aspergillus* spp., itraconazole and voriconazole blood concentrations.

Tinea capitis (and other cutaneous fungal infections) Microscopy of skin, hair and nails, fungal culture and/or *Trichophyton rubrum* PCR.

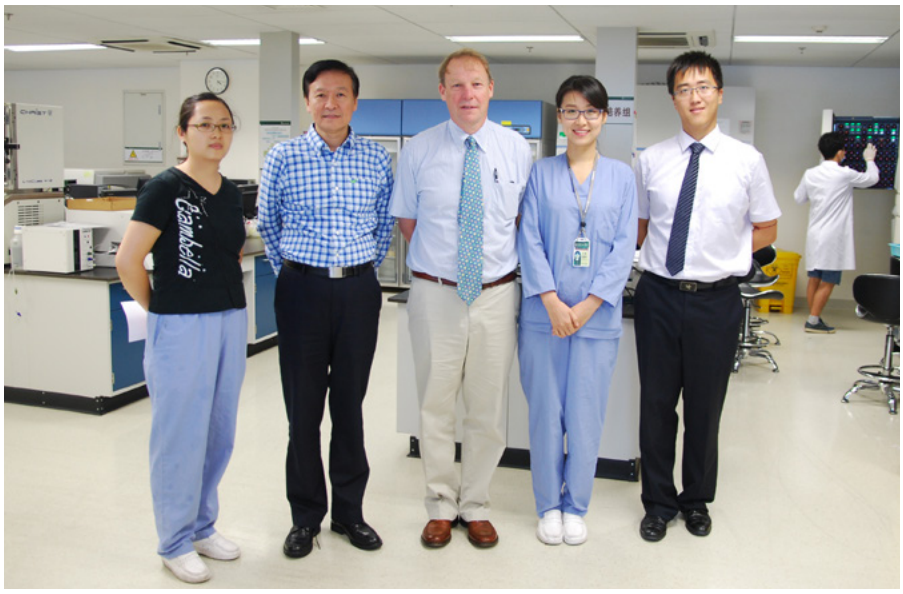
Fungal keratitis
Microscopy of corneal scraping, fungal culture, direct and molecular identification of moulds.

Severe asthma with fungal sensitization (SAFS) and allergic bronchopulmonary aspergillosis complicating asthma (ABPA)
Total IgE, fungal specific IgE (*Aspergillus, Alternaria, Cladosporium, Candida, Penicillium, Trichophyton*), itraconazole blood concentrations.

Treatment monitoring
The triazoles itraconazole, voriconazole and posaconazole should be monitored, if possible. Low concentrations are associated with clinical failure, and occur in patients not taking medication, too low a dose, when there is a drug interaction (notably rifampicin), some patients with HIV infection because of intestinal disease and with some generic formulations of itraconazole. Amphotericin B, fluconazole, terbinafine, caspofungin, micafungin and anidulafungin do not require therapeutic drug monitoring. Flucytosine at currently recommended doses (especially in neonates and in those with renal failure) does need monitoring, although if doses can be lowered, or duration of therapy shortened, this may no longer be advised.

Clinical links
Excellent clinical links greatly strengthen the effectiveness of both the laboratory and patient care, but clinical expertise in fungal infection and allergy covering several specialties is rare. In well-developed countries, diagnostic mycology laboratories tend to be the repository of knowledge about fungi. Institutions that deliver the best care have well-recognized clinical experts in fungal disease who advise others, and work closely with the laboratory and radiologists.

Surveillance of fungal disease
Almost all fungal diseases require laboratory confirmation and so accurate diagnosis is a necessary component of surveillance. Many fungal diseases require only one test for diagnosis, such as cryptococcal antigen or *Pneumocystis* PCR, and so collection of laboratory data will provide a reasonable approximation to the full burden of infection for that population. Others are more complex, such as fungal sinusitis or invasive aspergillosis, and more complicated and costly surveillance studies are required to get an accurate picture of the burden.



Professor Denning visiting the Department of Clinical Laboratory, Peking Union Medical College Hospital, Graduate School, Peking Union Medical College, Beijing. This laboratory provides identification and susceptibility testing for yeasts isolated from blood for 100 hospitals throughout China, in a research time frame.

Prof Ying-Chun Xu (second from the left)
Dr Meng Xiao (first from the right)