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Review Article

Laboratory diagnostic methods for medically important fungi

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ABSTRACT

Fungi can cause a plethora of infections. They are often difficult to detect and identify. Various phenotypic and genotypic tests are there for fungal diagnosis. Here in this review article the author has tried to explain the extant culture and identification methods for various fungi of medical importance.

1. Introduction

Fungi are more commonly beneficial for us. Only a few pathogenic genera of fungi are there. Fungal infections cause different types of allergic and invasive infections.[1] Broadly fungal infections can be superficial, subcutaneous, deep and also toxin or mycotoxin mediated. Fungi can be morphologically of four broad types, yeasts, yeast-like, filamentous and dimorphic. They belong to either Phylum Ascomycota, Glomeromycota, Chytridiomycota or Basidiomycota.[2] This is based on the type of sexual spores they possess. They are eukaryotic. Filamentous bacteria like *Nocardia* and *Actinomyces* also are somewhat similar to molds or filamentous fungi in shape and size. There is very less of concise accurate information about laboratory identification of pathogenic fungi. Hence the author has tried to write this article. However this is not exhaustive and the reader is referred to standard textbooks for further reading.

2. Relevance of Fungal Infections in Predisposing Conditions

Conditions like HIV infection with low CD4 count, uncontrolled Diabetes mellitus and severe protein energy malnutrition can predispose to several disseminated fungal infections. These have to be kept in mind for proper selection of media and culture techniques for fungi. Moreover with the advent of nosocomial infections, azole-resistant fungi like *Candida auris* are assuming great importance.

2.1. Culture of fungi

Almost all the fungi can be cultured, except a few like *Rhinosporidium* spp. and *Pneumocystis jiroveci*. However, fungi take some time to yield visible growth or colonies. Yeasts grow within 1 week and molds may take 2 to 3 weeks to grow.

2.2. KOH mount

Before culture, laboratory scientists perform a 10% Potassium hydroxide mount from samples and examine it microscopically for detecting yeasts or hyphae. KOH damages intercellular junctions or desmosomes and keratin and makes the hyphae more visible. Usually, a small amount

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of Glycerol is also added to KOH solution so that the mount does not dry up easily and it can be examined next day also. For tissue and pus, 10% KOH is used. For hair, 20% KOH is used. Also, for detecting fungi in nails, 40% KOH mount is recommended. If KOH is not available, NaOH or Sodium hydroxide mount can be done instead.

2.3. India ink preparation

India ink preparation or mount is a type of negative staining. It is done to see capsulated fungi like *Cryptococcus neoformans* in samples like CSF or Cerebrospinal fluid. Its capsule does not take up India ink, which is actually colloidal Carbon, and is hence makes it apparent as a bright halo around the round large yeasts. Mucicarmine can be used for staining *Cryptococcus capsule* in tissue. It has aluminium that forma a chelating complex with Carmine. This changes the charge of the carmine molecule into a positive charge that allows it to bind with low-density acidic substrates like mucins.

2.4. Culture media for fungi

Generally Sabouraud's dextrose agar is preferred to other media for growth and cultivation of fungi.[3] It has glucose which suppresses the growth of saprophytic bacteria. Its pH is adjusted to about 5.6 so as to enhance the growth of fungi, particularly dermatophytes, and to slightly inhibit bacterial growth in all clinical samples.[4] In this media, yeasts appear as pasty and smooth colonies while molds or filamentous fungi appear as rough and powdery colonies. Other media like Corn meal agar and Potato Dextrose agar are also used frequently for fungal culture, identification and isolation.

2.5. Identification of yeasts

Many tests can be done for yeast identification. Corn meal agar or rice extract agar are useful for Dalmau technique for identification of yeasts and yeast like fungi. Dalmau plates or morphology agar need to be kept or incubated for a minimum time of 2 days or 48 hours. Surfactants like Tween 80 can be added to it to enhance diagnostic accuracy and pseudohypahe and chlamydospore formation. Corn meal agar usually gives best results when poured in glass Petri dishes and incubated at 25 degree C in the dark. For yeast identification germ tube test is of prime importance. *Candida albicans* and *Candida dubliniensis* are Germ tube positive. They can be distinguished by the fact that *C. albicans* is positive for lipase and can grow at 44 degrees C. These yield negative results for *C. dubliniensis.* Also, *C. dubliniensis* produces multiple terminal chlamydospores on Corn meal agar while *C. albicans* usually shows single terminal chlamydospores. Chlamydospores are readily formed on Rice extract agar also, or on Corn meal agar with 1% Tween-80.[5] Sugar fermentation, sugar assimilation or auxanogram and urease test are also done for yeast identification.[5] These media contain 2% sugar (weight/volume) in Peptone water, Durham's tube and Andrade indicator. *Candida krusei* ferments only glucose and forms a thick white pellicle over the broth. *C. albicans* ferments both glucose and maltose. Lactose is fermented only by *Candida kefyr*.

Bird seed agar can be used to identify colonies of *Cryptococcus* spp. by dint of its ability to produce melanin in this medium. This is by virtue of the phenoloxidase enzyme of *Cryptococcus neoformans*. Additionally, *Cryptococcus neoformans* is urease positive, negative for nitrate assimilation and grows at 37 degrees C.

Some yeasts like *Rhodotorula* produce carotenoid pigment and are inositol assimilating. This also helps in their identification.

Yeasts like *Malassezia* incriminated in dandruff or Ptyriasis versicolor, need to be grown on Modified Dixon agar or SDA with olive oil overlay, due to their lipophilic nature. They can also be distinguished by Tween 40, Tween 60 and Tween 80 assimilation pattern. Generally they are very small in size.

2.6. Identification of molds or filamentous fungi

Usually molds grow best at 25 to 30 degrees C. They can be identified easily by their conidial colour, colour of back or reverse and obverse side and microscopic morphology as in Lactophenol cotton blue mount. Usually fungi of Phylum Glomeromycota have aseptic or sparsely septate hyphae.

SDA with Voriconazole can be used for growing fungi of phylum Glomeromycota, like *Rhizopus* spp. and *Mucor* spp. This is because fungi of this phylum is resistant to Voriconazole while others are not.

SDA with chloramphenicol and cycloheximide (SDCC) is quite suitable for growing dermatophytes like *Trichophyton* spp. However in many studies the efficacy of SDA has been found to be the same as SDCC in growing dermatophytes.[6] Deep red colonies on Dermatophyte test medium can also help in detecting this group of fungi. *T.rubrum*, a common dermatophyte, also produces reddish pigment on Potato Dextrose agar. Typical morphology of macroconidia can help in identifying them. Furthermore, test like hair perforation test and rice adherence test can be useful for clinching the diagnosis of dermatophyte infections.

Simple tests like thermotolerance test can help in differentiating *Aspergillus fumigatus* from *Aspergillus flavus*. At 45 degree C, *A. fumigatus* grows well and not *A. flavus*. Moreover, *A. fumigatus* produces mousy-grey colonies on SDA and have smaller size of conidia, is uniseriate and phialides cover only one-third of the head. Aspergillus nidulans has hemispherical head and Hulle cells which plays an important role as genetic back-up material and also helps in identification.[7]

Media like BHI agar (Brain Heart infusion agar) can be used to show dimorphism in dimorphic fungi like *Sporothrix schenckii* and *Histoplasma capsulatum*. Pigment can also be seen in fungi, like red diffusible pigment in *Talaromyces marneffei* (formerly *Penicillium marneffei*).

PDA slant can be used to induce sporulation in molds. PDA square pieces can also be used for slide culture and incubated at 25 Degree C to induce conidiation in molds. Media like Oatmeal agar can be used to grow phaeoid or pigmented/dematiaceous fungi like Alternaria spp. and *Curvularia* spp.

List of dimorphic fungi is shown in table below

Table 1: List ofdimorphic fungi

Name	At 37 degrees C	At 25 degrees C
Sporothrix schenckii	Elongated yeasts with septa	Molds with flower like dental conidia
Histoplasma capsulatum	Yeasts	Hyphae with microconidia and tuberculate macroconidia
Talaromyces marneffei	Narrow yeasts with septa	Hyphae with red diffusible pigment
Blastomyces dermatitidis	Yeasts with broad based budding	Hyphae with microconidia
Paracoccidiodes brasiliensis	Yeasts with multipolar budding (mariner's wheel app[earance)	Hyphae with microconidia
Coccidioides immitis	Spherule with septa	Hyphae with microconidia

2.7. Stains for fungi

Gram stain can be done for fungi. Usually yeasts are Gram positive and molds are Gram negative. Grocott-Gomori's Methenamine silver stain can be done to identify Glomeromycota in tissue and *Pneumocystis jiroveci*. Giemsa stain can also be done for visualizing trophozoites of *Pneumocystis jiroveci*.

2.8. Serological tests for fungal identification

Various serological tests can be done to identify fungi. One of them is a latex agglutination test for *Cryptococcus neoformans*.[8] It can be done from CSF or serum samples. Basically it has monoclonal antibodies against capsular antigen of *Cryptococcus*, coated on latex particles. If done from serum samples, it should be heated to inactivate pronase.

Gel diffusion test can be done to detect antibodies against *Aspergillus* spp. in serum in invasive aspergillosis. It is a type of precipitation test and uses soluble antigens of *Aspergillus* spp.

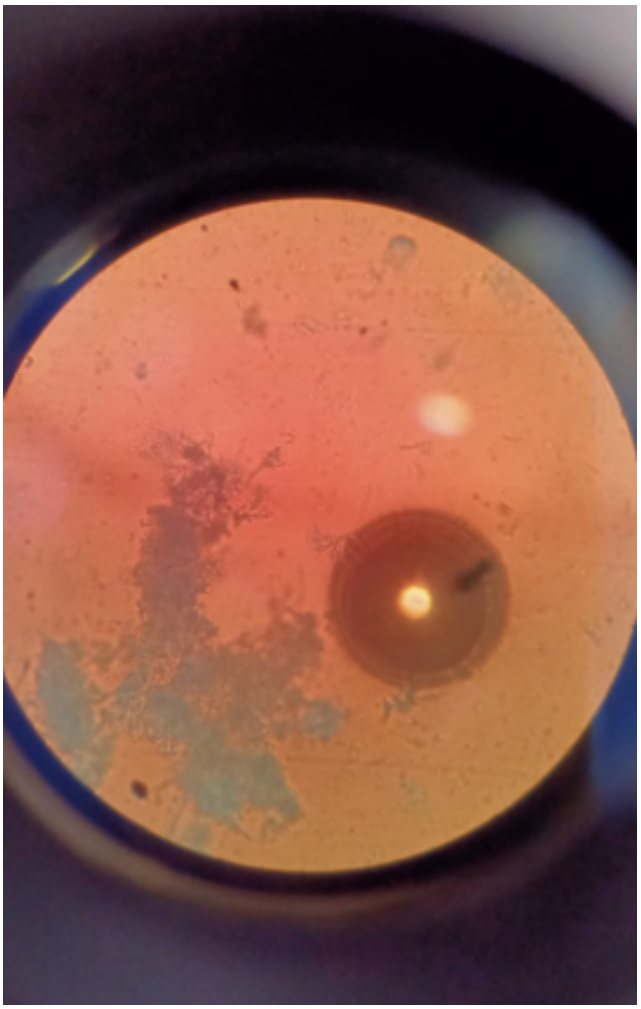

Fig. 1: LCB/LPCB mount showing microscopic morphology of Penicillium spp. (image: author)

Fig. 2: Powdery, cottony Mold colonies on SDA (image: author)

Galactomannan antigen of fungi can be detected in serum by ELISA test. It is a polysaccharide antigen and a component of fungal cell wall.[9] It is found mainly in *Aspergillus* spp. and seen in early or acute stages of infection by *Aspergillus* spp. Beta 1,3 d- Glucan is also an antigen found in *Candida* spp., *Aspergillus* spp. and *Pneumocystis jiroveci* and is used for serodiagnosis.[10]

Skin allergy test: Skin allergy testing can be done for diagnosis of invasive Aspergillosis, by injecting *Aspergillus* antigens intradermally and noting erythema and induration immediately and after sometime. Imaging tests like Chest X ray also helps detect Aspergilloma and Allergic bronchopulmonary aspergillosis (ABPA) by tram-track sign.

2.9. Metabolites for fungal infection diagnosis

Mannan, arabinitol and mannose can be indicator and markers of invasive or disseminated Candidiasis. This has also been proven in animal models.[11]

2.10. Molecular tests for fungi

Of late, PCR and MALDI-TOF has been used extensively for fungal diagnosis, especially for yeasts.[12] PCR is used from extracted DNA and MALDI-TOF MS is used for identification from colonies.

3. Discussion

More new techniques are coming up for fungal infection diagnosis. One needs to be updated with these tests or techniques. At the same time the old or phenotypic tests stand good and one needs to remember them.

4. Conclusion

Fungi can be diagnosed by conventional or molecular tests for better and accurate diagnosis.

5. Conflict of Interest

None.

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None.

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