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# A Review on Diagnosis of Fungal Diseases: Classical to Modern Technologies

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ABSTRACT

Incidences of fungal infections are increasing day by day with the increase in use of drugs, development of immune-compromised conditions and dreadful conditions like diabetes. Under such circumstances there is increase in incidences of fungal diseases and development of resistant of pathogenic fungi .The diagnosis of such infection has been developed from quite early times but are time consuming. Seeing the chronic developmental phases in the fungal diseases, a need for fast and appropriate diagnostic techniques is persisting. Several novel techniques have been developed and the persistent techniques have been modified for better, fast and specific diagnosis of fungal infections.

## INTRODUCTION

As the number of patients with profound immunosuppression continues to rise, the morbidity and mortality burdens attributed to invasive fungal infections are increasing. In the case of invasive fungal infections, expedient identification of the offending organism is essential for optimal patient management and the best clinical outcomes. As the antifungal susceptibility profiles for many fungi (both yeasts and molds) are predictable, organism identification frequently is sufficient to expedite appropriate empirical antifungal therapy. This has been demonstrated both to reduce the overall length of hospitalization and to maximize favorable clinical outcomes. The methods for identification of yeasts in the diagnostic clinical-microbiology laboratory have improved significantly over the past several decades, with methods ranging from simple manual biochemical assays to automated biochemical methods, to sophisticated nucleic acid-based assays. While these advancements in methodology have greatly enhanced our ability to identify yeasts, the limitations of these methods include cost, turnaround time, and, in some instances, the need for considerable expertise. Additionally, the accuracy of identification for some less-common species is not optimal for some of the methods.

## REVIEW

The persistent techniques available for fugal diagnosis are generally based on Culture-based detection methods, Antigen-based detection methods and Molecular-based detection methods. Cuenca et al 2008 found that despite the development of new techniques and new antifungal agents, diagnosis of invasive fungal infection (IFI), still relies upon a combination of clinical observation and laboratory investigation and remains a challenge especially for immune-compromised patients. From the reports of Dismukes, 2003 it is evident that superficial and subcutaneous fungal infections often produce characteristic lesions that suggest the diagnosis, a thorough knowledge of potential causative organisms is yet required to aid the diagnostic process, mainly in situations where systemic fungal infection is suspected but the clinical presentation is nonspecific and then ascribable to a wide range of infections, underlying illnesses, or complication of treatments. Perea, S. and Patterson, T.F. 2002; Rodriguez-Tudela et al 2008 reported that the exact identification of the infecting organism is essential in light of the increased use of prophylactic schedules that predispose the patient not just to fungal infection, but also to the selection of fungal

species such as non-*albicans Candida* (e.g., *C. glabrata* and *C. krusei*), *Aspergillus terreus*,*Scedosporium* species, and *Zygomycetes*, many of which are intrinsically resistant to the available antifungal agents.

Laboratory diagnosis of IFI remains based on conventional approaches, such as the direct microscopically detection of the etiologic agent in clinical specimen and the isolation and identification of the pathogen in culture, and non-culture based methods involving detection of a serologic response to the pathogen or other marker of its presence such as fungal antigens or metabolites. (Dismukes 2003).

Singh N and Paterson,2005 found that visual examination of fungi in tissue samples allows presumptive identification based on cellular morphology and staining properties, but it should be appreciated that invasive procedures necessary to obtain biopsies may be precluded in haematological patients. It should be noted that microbiological cultures are often insensitive or of limited use, since even with modern blood cultures systems candidaemia can be transient and not detected, or *Aspergillus* cannot be cultured from a significant proportion of sputum or bronchoalveolar samples from patients with invasive aspergillosis.

Filamentous fungi can be identified only by visualization of macroscopic (colonial form, surface colour, and pigmentation) or microscopic (spore-bearing structures) morphologic characteristics, following to sub-cultivation of a mould isolate to encourage sporulation,a process that takes days to weeks. Persing et al, 2004 insisted that in addition to the use of genetic probes for the culture confirmation of dimorphic systemic fungal pathogens (e.g., *Histoplasma capsulatum*), an alternative and useful approach to the detection and identification of fungi in clinical specimens involves a broad-range polymerase chain reaction (PCR) followed by nucleic acid sequencing, After which the nucleic acid sequence is compared with known sequence database and identification is based on DNA homology. However, these methods are expensive and time-consuming, and they are not currently suitable for routine identification.

Although a variety of culture media and incubation conditions may be required for recovery of fungal agents, chromogenic primary isolation media (e.g., CHROMagar Candida medium) can be employed for the presumptive identification of the most medically important *Candida* species, including *C. albicans, C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. Sanguinetti et al 2007 in their work reported that most yeasts isolated from clinical samples can be identified using one of the numerous commercial identification systems, such as API 20C AUX, VITEK 2, and RapID Yeast Plus.

Lars F et al 2013 presented the results of a multicenter study, evaluating the Vitek MS system for identification of clinically relevant yeasts. Identification of yeasts using the Vitek MS is faster and more accurate than phenotypic identification systems currently employed in clinical microbiology laboratories and affords accuracy comparable to that of more laborious and costly molecular methods. Implementation of this methodology should streamline yeast identification in the laboratory, positively affect patient care, and reduce health care-associated costs.

The International Sub-commission on Fungal Barcoding and the CLSI (MM18-A) 2008 have proposed the ITS region as the default region for species identification. Currently, sequence-based identification represents the gold standard because it generally grants a methodical and dependable approach to fungal identification; it is accurate and yields objective data, provided sequences of ensured quality are selected for comparisons Accurate identification of bloodstream isolates like S. boulardii and Malassezia furfur to the species level is also of epidemiological significance, since it can contribute to the early detection of iatrogenic S. boulardii ICU and neonatal ward M. furfuroutbreaks. In that respect, no identification would be preferable, since it would alert the clinical laboratory and encourage referral of the isolate for molecular identification. Pappas P. G., et al 2009 expressed that users should be aware of potential rare species misidentifications exhibited by these commercial systems, among which Vitek 2-YST demonstrated the highest rates. Also, in some cases, as in that of C. lusitaniae isolates, chromogenic media can facilitate screening for rare species, increasing alertness for likely ambiguous results.

Meletiadis et al 2011 evaluated the commercial yeast identification systems API ID32C, Auxacolor, and Vitek using 251 molecularly identified bloodstream isolates and 2 reference strains, representing a total of 35 species (6 common and 29 rare). Correct identification rates were higher for common species (Auxacolor, 95%; API ID32C, 94%; Vitek, 92%) than for rare species (Auxacolor, 43%; API ID32C, 56%; Vitek, 64%). All systems performed equally among the former, and Vitek performed best among the latter.

Among commercially available assays, the Fungitell, which is also an ELISA technique described by Odabasi et al 2004, is widely used to detect serum BG concentrations as low as 1 pg/mL. The use of Platelia Candida, an ELISA that combines the detection of mannan antigen and anti-mannan antibodies in serum, led to earlier diagnosis of *Candida* infection when compared with blood cultures.( Sendid Bet al . 2002). A recent prospective evaluation of serial PCR assays against or along with GM and computed tomography was carried out by Suarez et al, 2008 and Cuenca-Estrella. 2009 in haematological patients, thus showing acceptable sensitivity and specificity. A total of 750 clinical yeast isolates were evaluated by two identification systems, VITEK 2 and RapID Yeast Plus, using sequence analysis of the rRNA gene internal transcribed spacer regions as the reference method. The VITEK 2 and RapID systems correctly identified 737 (98.2%) and 716 (95.5%) isolates, respectively. **Putignani, L et al**2008 came to a conclusion from their sudy that Gene sequencing is highly accurate but is expensive, time-consuming, and technically demanding.

Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is becoming a reliable method for identification of microorganisms. Fenselau 2001and Santos et al 2010 concluded from their study that the remarkable reproducibility of the methodology is based on the measurement of constantly expressed and highly abundant proteins such as ribosomal molecules. Some recent studies have shown the potential of the MALDI-TOF technique to identify fungal clinical species such as *Aspergillus* and *Fusarium*. Recently, the MALDI-TOF technology provided a fast and accurate identification of common and unusual species

of *Aspergillus* when tested on 124 clinical and 16 environmental isolates. With regard to *Fusarium* species, a recent case report bySeyfarth F et al 2008 supported the usefulness of MALDI-TOF analysis in diagnosing an infection due to *Fusarium proliferatum*, which is a very infrequent pathogen within this genus.

Lindsay G et al 2010 evaluated the use of MALDI-TOF analysis for the identification of clinically important yeast species. Traditional biochemical testing for yeast identification is inexpensive but time-consuming and sometimes inaccurate. His results were in accordance with results obtained by **Freydiere, et al** 2001**; Sanguinetti, et al** 2007 and **Verweij et al ,** 1999. The aim of this study was to determine if MALDI-TOF would be suitable for rapid yeast identification. Working with 109 reference and type strains representing 44 species in 8 genera, we created a MALDI-TOF spectral library and then challenged the library with 197 clinical isolates. Three clinical isolates gave no spectral score and could not be identified because they were not in the spectral library. Two organisms (*C. rugosa* and *C. neoformans*) gave consistently low spectral scores and could not be identified. Thus, of the 194 isolates (23 species in 6 genera) included in the spectral library, 192 (99.0%) were identified correctly at the species level by MALDI-TOF. These results compare favorably with the studies of Marklein et al. 2009 and van Veen et al. 2010. Marklein et al. evaluated 18 reference strains representing 8 species of yeast and 267 clinical isolates All reference organisms and 92.5% of their clinical isolates were identified correctly. The organisms that were not identified were not in the database library. When appropriate reference organisms were added to the database, all clinical isolates were identified correctly. Van Veen et al. evaluated the accuracy of MALDI-TOF for the identification of 80 yeast isolates (14 species in 7 genera). A total of 97.5% of isolates were identified correctly at the genus level, with 87.5% identified correctly at the species level, with most of the cases of unidentified organisms being due to insufficient entries in the database and to species-level identification being accepted only if the MALDI-TOF spectral score was >2.0.

For studies validating a new identification system, it is important that the database be constructed with a comprehensive collection of accurately identified reference strains. Although we selected type and reference strains from the American Type Culture Collection and the University of Alberta Microfungus Collection, 11 ATCC reference organisms were identified incorrectly, as determined by gene sequencing (Table 1). Ten of these organisms were identified correctly by MALDI-TOF, with no acceptable spectra obtained for one isolate, *Cryptococcus luteolus* (*Cryptococcus podzolicus*) ATCC 42279. No acceptable MALDI-TOF spectra were obtained for four additional organisms (*C. luteolus* ATCC 42279, *Geotrichum candidum* ATCC 10834 and ATCC 74169, and *Malassezia sympodialis* ATCC 96803). This was most likely due to the rigorous standards used for inclusion of a reference strain in the MALDI-TOF database (refer to Materials and Methods), because both clinical isolates of *G. candidum* were identified correctly.

Spectra of clinical isolates were analyzed using the reference strain database and MALDI BioTyper software (BioTyper Library v 2.0.4; Bruker Daltonics), a proprietary algorithm for spectral pattern matching resulting in a logarithmic score of 0 to 3. The results reported in this study demonstrate that all organisms identified at the species

level had a spectral score of 1.8 or greater. Five clinical isolates were not identified in this study: two organisms had unacceptably low spectral scores, 1.3 and 1.5, and three organisms had spectral scores of 0 because the species were not in the reference library database.

All isolates were tested in quadruplicate, that is, each sample preparation was spotted four times on the steel target plate. Because 89.6% and 97.4% of the isolates were identified with the first spot and the first two spots, respectively, we believe that testing should routinely be performed in duplicate, with retesting in duplicate if the spectral scores are <1.8. This is a practical approach to integrating this technology into the routine laboratory because there are no additional reagent costs for duplicate testing, the analysis of individual spots requires less than 5 min, and the steel plates are washed and reused.

## CONCLUSION

Although new techniques (e.g. galactomannan detection) and new antifungals have appeared, these opportunistic infections remain difficult to diagnose and have a high mortality. New diagnostic techniques could improve this outlook, although they are far from becoming available in daily practice. Vitek MS is faster and more accurate than phenotypic identification systems currently employed in clinical microbiology laboratories and affords accuracy comparable to that of more laborious and costly molecular methods. Implementation of this methodology should streamline yeast identification in the laboratory, positively affect patient care, and reduce health care-associated costs.

Molecular detection methods, combined with additional microbiological and clinical information, have the potential not only to accurately and rapidly identify fungal pathogens, but also to indicate whether the pathogen is likely to respond to conventional antifungal treatment. Inclusion of these methods in a diagnostic surveillance strategy to exclude IFI in high-risk patients with haematological malignancy should result in improved clinical management, thus allowing more rational use of antifungal drugs.

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