# Comparisons Of Molecular Methods In The Diagnosis Of Pathogenic Fungi

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**Abstract:** Pathogenic fungi are responsible for high infectious morbidity and mortality in immunodeficient patients, a rapid and accurate identification of pathogenic fungi is critical for appropriate treatment. Recently, many molecular methods have been developed for diagnosis to improve the identification of pathogenic fungi. In this review we compared the advantage and disadvantage of five molecular methods that are widely used in the diagnosis of pathogenic fungi.

**Keyword:** pathogenic, fungi, diagnosis, molecular methods

## INTRODUCTION

Pathogenic fungi cause invasive disease [1, 2] and they are responsible for high infectious morbidity and mortality in immunodeficient patients such as patients with AIDS [3] or patients received immunosuppressive chemotherapy, Therefore, a rapid and accurate identification of pathogenic fungus is critical for appropriate treatment. In recent years, many molecular methods have been developed for diagnosis to improve the identification of pathogenic fungi [4, 5, 6, 7, 8] the performance of each method can be influenced by different setting such as genetic variation of the pathogen or host, as well as, the differences in characteristics of the infections agent. Five molecular methods are widely used in the diagnosis of pathogenic fungi, Fluorescent *in situ* Hybridisation (FISH), DNA Array Hybridization, Multiplex Tandem PCR (MT PCR), Mass Spectrometry MALDI-TOF, and Pyrosequencing. In this review we compared the advantage and disadvantage of five molecular methods that are widely used in the diagnosis of pathogenic fungi.

## FLUORESCENT *IN SITU* HYBRIDISATION (FISH)

Since discovery of ubiquitous fungi, understanding their biodiversity and abundance is an important point. PCR methods like fingerprinting or cloning do not make the difference between 'active' and 'dormant' fungi. The sample could be biased with the DNA extraction parameters due to the cell walls. Immune factors are also a difficulty for analysis. Working *in silico* for probes design can be useful: Searching for 'signatures' of each organism. Signatures are specific at phylogenetic level and depend of their variability. Probe is labelled with a fluorochrome. It will specifically anneal with complementary sequence and making a heteroduplex. Efficacy of FISH can be influenced because of sterical and electrostatical proprieties of DNA. But also feature of probes, fixation methods (even if FISH can be performed on non-fixed organisms too), buffers, stringency of probes, binding and incubation time. Accessibility of rRNA molecule for FISH is an important point. Study made by Inacio *et al.*[9] Presents 23 probes designed to target the 26S LSU of yeasts' RNA. They concluded that the specificity of probes is the main factor for the success of FISH. Limitation of FISH Technique includes substrate fluorescence, insufficient permeability of cell-walls but also non-specific probes or low ribosomes contents. Some fungi have an autofluorescence and it could be a false positive. Consequently it's important to check the autofluorescence of targets and non-specific fungi. To solve the problem of cell-wall permeability using a specific enzyme can be a solution (chitinases or glucanases).


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## DNA ARRAY HYBRIDIZATION

This technique is based on hybridization and amplification of labelled genome regions of interest. Nucleotides of genome regions are immobilized by spotting the experiment card. It's a powerful technique now for detection and identification of fungi and other microbes without the need of culture of organisms. Design of group-specific nucleotides determines the specificity and the sensitivity of this test. The length of the oligonucleotide, number, type and position of SNP (single-nucleotide polymorphism) which contain a nucleotide, fix the discriminatory potential. A desirable nucleotide sequence should have the good melting temperature, contains as many polymorphic sites as possible close to center or in the 3' half and have less chance to form dimer or hairpin. DNA array have been developed based on a single region for the detection of one taxonomic group: 16S rRNA is used for bacteria, ITS1 and various proteins regions are used for fungi. Microarrays are effective diagnostic tools for bacteria, fungi, viruses or nematodes. This technique is sensitive as most of PCR techniques. With multiple capacities form accommodation of nucleotides on membrane and its reusability, DNA array have a lower cost. It could make multiple detections for one plate. It's possible to detect 24 genera fungi know in a single test of cranberry. [10]

## MULTIPLEX TANDEM PCR (MT PCR)

Multiplex PCR has been developed to identify simultaneously multiple fungal pathogens in a single reaction. This molecular method provides a reliable alternative to conventional methods for rapid identification

of pathogenic fungi directly from clinical sample. Recently, Many studies demonstrate that numerous fungi can be detected such as *Aspergillus fumigates, Cryptococcus neoformans*, 11 species of *Candida* , and others [11,12]. Primers were designed using the variation of the ITS1 sequence. Multiplex PCR was initially evaluated on 70 blood cultures. Yeast was seen in Gram's stain as well as 200 bacterial blood cultures and 30 samples which did not flag positive. The Sensitivity and specificity of assays was 100%. Fungi identification was always correct, no interference was observed in cases of polyfungal infection but some rare disease-casing yeast was not identified by MT PCR. This study demonstrates the diagnostic usefulness of MT PCR for detecting pathogenic fungi without hours of sample culture (48-96h required for culture).

## IDENTIFICATION BY MASS SPECTROMETRY MALDI-TOF

Identification with mass spectrometry emerges as a reliable tool for diagnostics. It has many advantages and could be a threat for IS Pro Fungi. This method has been introduced for differentiation of microscopic fungi. MALDI-TOF* gives a unique mass spectrometric profile. Since the development of MS* for identification, numerous techniques basing on the cell surface have emerged. MALDI-TOF is one of the fastest and reliable tool in MS techniques. In 2013, two reviews appeared about this technique: they emphasized the use of MALDI-TOF for diagnostic of fungi in clinical microbiology laboratories. Limits of standard biological techniques are filled by this new approach of identification. [13,14]. The advantages of MALDI-TOF identification are: small amount of material are required (for example less than 100ng), measurement and data interpretation are easy and fast. Enough peptides (or proteins) can generate a signal usable to make a specific fingerprint. Failed identifications were associated with missing spectrums on the database. During MALDI-TOF measurement, mass spectra are obtained by the laser ablation of the cell wall structures of spores because of the acidity of matrix solution. For different isolates of yeasts, a study compared MALDI-TOF identification and conventional techniques. Species identification by MALDI-TOF was observed in 85.6% of yeasts [15]. For blood samples, the analysis necessit only centrifugation and protein extraction. The complete removal of blood cells by a lysis solution is essential for obtaining high capacity of detection. This method is fast and economic: the cost is less 1$ per sample. But the method's weakness is its poor performance for polyfungal bloodstream infections, they occur rarely fortunately.

## SEQUENCING AND PYROSEQUENCING

Pyrosequencing is an alternative to Sanger sequencing and allows *de novo* DNA sequencing. Each incorporation is followed by the release of pyrophosphate (PPi) in a quantity similar to the amount of incorporated nucleotide and each peak, or light signal, is proportional to the amount of dNTP incorporated. It could be used on liquid and solids samples. The technique has application on many domains: profiling, identifying or classification. It can be utilized in typing of fungi from immunocompromised patients. General consensus primers are utilized for 18S and ITS regions. Amplified DNA fragments are sequenced up to 40 bases to be identified. Sequence data obtained by Pryosequencing suggests that 18 to 32 bases are sufficient for identification. [16].Sample and single strand DNA preparation are very rapid: about 15 minutes, while sample preparation takes almost four hours for Sanger sequencing. Pyrosequencing has a high throughput and needs low quantities of sample and reagents. But it's still an expensive technique, it has short reads for phylogenetic interferences and has a high error rate (0.0098). During the reaction, long fusion primers may include bias to the analysis.

## DISCUSSION

The variation in the quantity of DNA extracted with different commercial kits demonstrates how DNA extraction methods are important in molecular diagnosis. In fact, the Different methods of DNA extraction that are available in the market have their own specificity which isn't the same with all fungi species. For example, If we use a DNA extraction kit for *Candida albicans* we obtain high quantity of DNA, whereas, if we use the same kit for *Aspergillus fumigates* we can obtain low quantity of DNA. In addition, using Simple lysis procedures, like hot detergents and proteases, are not effective to obtain high DNA quantity. Alternative methods for fungal cell wall lysis such as agitation with microspheres, digestion with enzymes or grinding cells with liquid nitrogen, work well for the large preparations of DNA cultures. But these methods are not practical for a clinical microbiology use. Panfungal PCR proposes a new approach for identification of pathogenic fungi directly from samples, [18]. Panfungal PCR is the most effective technique to identify common clinical fungal isolates compared to conventional methodes.

## CONCLUSION

Mass Spectrometry techniques and MALDI-TOF are effective method for fungal identification and shows good results [17].These results made MALDI TOF as a serious competitor method ;it is fast, economic and it could be used in routine in medical laboratories. Pyrosequencing can also be an important competitor method because of its high throughput. In fact, the cost of test per simple is cheap but the price of machine and equipments is really expensive.

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