Respiratory Fungal Infections

Molecular Diagnostic Tests

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KEYWORDS

• Assay • Fungal infection • PCR • Species

KEY POINTS

- A variety of polymerase chain reaction (PCR)-based assays have been developed for detection of *Aspergillus* spp. in respiratory specimens and in blood.
- Positive blood specimens suggest invasive infection and are less likely to be positive due to contamination.
- Ribosomal RNA gene is an effective target for the PCR assays and can be used to develop assays that distinguish species.
- At least 2 commercial assays are available outside of the United States. No assay has been approved by the Food and Drug Administration.
- Galactomannan detection is equally effective for diagnosis of aspergillosis.
- In general, the sensitivity and specificity of conventional PCR and real-time PCR assays for pneumocystis jirovecii pneumonia (PjP) are excellent.
- Real-time PCR allows quantification, which is potentially useful to distinguish PjP colonization from infection.
- Currently the decision of colonization versus infection based on molecular results must be driven by clinical information.

INTRODUCTION

Fungal infection of the respiratory tract can take several forms, the most common of which is pneumonia. Fungal infection can occur in the immunocompetent typically as a result of inhalation of a large inoculum of fungal elements (eg, histoplasmosis). However, the number of etiologic agents attacking immunocompetent individuals and causing significant infection is limited. A much larger menu of fungi is associated with respiratory disease in immunocompromised patients. The most common of these are *Aspergillus* species, *Pneumocystis jirovecii*, *Scedosporium* species, *Fusarium* species, *Candida* species, *Cryptococcus* species, and members of the *Mucorales* within the *Mucoromycotina*.[1]

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Given the high level of attributable mortality associated with these fungi in immunocompromised patients, the need for rapid diagnosis is self-evident. As reviewed in the previous article (by Lamoth and Alexander), traditional culture-based methods lack sensitivity and are generally too slow to provide useful information for therapeutic management. Nonculture-based methods, such as antigen testing, are helpful but lack specificity due to crossreactivity with various fungi and with nonfungal materials. To be useful, repeat testing may be required (eg, galactomannan). Nucleic acid–based diagnostics offer the potential for high sensitivity, high specificity, and rapid turnaround time. Despite such potential, there is currently no Food and Drug Administration (FDA)-approved nucleic acid–based test for diagnosis of fungal infection directly from a specimen. One assay that has received CE marking is the SeptiFast produced by Roche Diagnostics. This assay is LightCycler based and targets several *Candida* species and *Aspergillus fumigatus.* The performance of this assay in prospective clinical trials requires additional study. A second assay, MycAssay *Aspergillus* (Myconostica), is also CE-marked. This relatively new real-time PCR assay also awaits extensive prospective evaluation.

The most recent European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus documents regarding diagnosis of invasive fungal infections do not include the use of molecular assays as an adjunct for diagnosis due to the lack of standardization.[2] Interest in trying to incorporate PCR assays into the guideline is high.

Laboratories are faced with several challenges when considering the development of a molecular assay for diagnosis of respiratory fungal infections. For example, should the test target single or multiple specific pathogens or be a single target with broad specificity (eg, ribosomal DNA)? The answer to this is, of course, driven by the frequency of infection by different fungi, the patient population intended for the assay, the resources of the laboratory, and other factors. Also at issue in design of a molecular test is consideration for targeting organisms that are either part of the host microbiota or are common contaminants of the respiratory tract. Although *Candida* species are considered part of the normal microbiota of the oropharynx, other organisms, such as *Cladosporium, Aspergillus,* and *Fusarium* may also be commensals in some individuals.[3] Additionally, these organisms may simply be acquired through consumption of foods and be transient colonizers.

Another challenge for design of a molecular test is choice of specimen. Sputum specimens, which are relatively easy to collect and do not require invasive procedures, have the risk of poor sensitivity and poor specificity if the target is a common contaminant or part of the commensal population or if the specimen is not well produced. Bronchoalveolar lavage (BAL) may be preferable from the viewpoint of minimizing contamination but has a higher risk to the patient than collection of sputum and may be insensitive. Blood specimens may be insufficient for organisms that do not invade or have not invaded into the pulmonary vasculature, and for those that do invade, whether whole blood or a blood fraction provides highest sensitivity must be determined. Furthermore, some fungi causing respiratory disease may enter the blood stream but will not result in a positive blood culture (eg, *Aspergillus* spp.), making assessment of a molecular assay's performance characteristics for blood specimens difficult.

The design of molecular assays for fungal respiratory pathogens that are common contaminants or members of the normal respiratory microbiota must also consider whether the intent of the assay is for screening or for diagnosis. Within the concept of screening is the issue of whether patient sampling is a one-time event or occurs