**Filamentous Fungi**

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**ABSTRACT** Filamentous mycoses are often associated with significant morbidity and mortality. Prompt diagnosis and aggressive treatment are essential for good clinical outcomes in immunocompromised patients. The host immune response plays an essential role in determining the course of exposure to potential fungal pathogens. Depending on the effectiveness of immune response and the burden of organism exposure, fungi can either be cleared or infection can occur and progress to a potentially fatal invasive disease. Nonspecific cellular immunity (i.e., neutrophils, natural killer [NK] cells, and macrophages) combined with T-cell responses are the main immunologic mechanisms of protection. The most common potential mold pathogens include certain hyaline hyphomycetes, endemic fungi, the Mucorales, and some dematiaceous fungi. Laboratory diagnostics aimed at detecting and differentiating these organisms are crucial to helping clinicians make informed decisions about treatment. The purpose of this chapter is to provide an overview of the medically important fungal pathogens, as well as to discuss the patient characteristics, antifungal-therapy considerations, and laboratory tests used in current clinical practice for the immunocompromised host.

**INTRODUCTION**

Fungi are ubiquitous, eukaryotic organisms found throughout the environment. These microorganisms exist as either saprophytes or parasites, with the former obtaining nutrients from decaying organic matter and the latter sequestering nutrients from a living host. As such, fungi can be both plant and animal pathogens with significant agricultural and medical impact. A limited number of fungi are primary pathogens. These organisms have the ability to cause disease in both immunocompetent and immunosuppressed individuals. In contrast, the majority of filamentous fungi causing disease in humans are opportunistic and require specific host conditions be met before infection can occur. Immunocompromised patients are at highest risk for the development of invasive infection with opportunistic organisms. The focus of this chapter is on the medically important filamentous (i.e., molds) and dimorphic fungi known to cause disease in immunocompromised hosts.

**TERMINOLOGY AND TAXONOMY**

**Morphology**

Fungi can be unicellular or multicellular and, depending on the organism, can take on multiple morphologic forms based on mode of cellular division and growth. In general, fungi grow as either yeast or filamentous fungi. Yeast divide by either budding or fission, whereas molds grow by apical extension of their filaments, known as hyphae (singular = hypha). This hyphal growth can occur with or without cell-wall separation of cellular compartments, which is known as septation. Continued growth results in a complex network of hyphae referred to as a mycelium. Whether these hyphal branch while growing or not, and the characteristics of this branching if it does occur, is one of the many characteristics used for the differentiation of groups of fungi. Mode of growth can also be an important factor contributing to the virulence of potentially pathogenic fungi, as both
biofilm formation and tissue invasion have been shown to contribute to pathogenesis (1, 2).

Morphologic characteristics are used to identify fungi in tissue specimens and culture. Most clinically significant fungi can be grouped into larger, non-phylogenetic categories based on their appearance and manner of growth. For example, dimorphic fungi, including pathogens such as *Histoplasma capsulatum*, are those that take a yeast or yeast-like form while infecting a human host, but are filamentous in the environment and *in vitro* under standard clinical laboratory culture conditions. The hyaline hyphomycetes, such as *Aspergillus fumigatus*, are septate molds that lack pigment in their hyphae, whereas dematiaceous fungi are yeast or filamentous fungi that are darkly pigmented.

**Reproductive Structures**

In addition to the vegetative forms of yeast or hyphae, fungi can also produce sexual and asexual reproductive structures. Conidiophores or sporangiophores are asexual structures that produce propagules by division or redistribution of nuclei without nuclear fusion (3). Depending on the genus, these asexual propagules may be called conidia or spores. In contrast, sexual structures such as cleistotheca, perithecia, basidia, or zygosporangia produce propagules after the union of two compatible haploid nuclei (3). Depending on the phylum, these sexual spores are either contained within sacs, called asci (phylum Ascomycota), or are external (phyla Basidiomycota or Zygomycota). There are some fungi, the “fungi imperfecti” for which no sexual reproductive cycle has been identified; these are classified within the phylum Deuteromycota.

Documenting the presence (or absence) and appearance of asexual and sexual structures under appropriate conditions is a useful tool for identification to the genus and, sometimes, species level. Assigning the appropriate binomial nomenclature for fungi has historically depended on whether an asexual (anamorph) or sexual (teleomorph) state is observed, with each state assigned its own unique name. To confuse matters further, when a fungal organism had more than one anamorph, these multiple asexual states have been referred to as synanamorphs and have had unique binomial nomenclature designations as well. For example, the teleomorphic pathogen *Pseudallescheria boydii* has two asexual states/anamorphs (or synanamorphs) referred to as *Scedosporium apiospermum* and *Graphium eumorphum*.

In 2011, the International Botanical Congress voted to discontinue the practice of dual nomenclature for pleomorphic fungi (4). While this amendment will ultimately clarify discussions and reporting of clinically significant fungi, the process of change is slow and not all synonyms will be discarded. It is important for clinicians and laboratorians to be aware of the dual nomenclature used to refer to potential fungal pathogens. The anamorph and teleomorph designations for applicable fungal pathogens discussed in this chapter are listed in Table 1.

**ETIOLOGIC AGENTS OF INVASIVE DISEASE**

Approximately 1.5 to 5.0 million fungal species exist on the planet (5), but only several hundred have been associated with human disease (6). General characteristics of the most common pathogenic filamentous and dimorphic fungi, along with some rare and emerging fungal etiologic agents of invasive disease, are described in this section. While some key macroscopic and microscopic features that assist with the identification of certain fungi in a clinical setting will be noted here, the authors direct readers to additional texts dedicated to morphological identification of clinically significant fungi for more complete descriptions (7, 8).

**Dimorphic Pathogens**

The dimorphic fungi are highly infectious. As a result, this group of primary pathogens is classified as Biosafety

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**TABLE 1** Dual nomenclature of pathogenic pleomorphic fungi

<table>
<thead>
<tr>
<th>Category</th>
<th>Anamorph (asexual)</th>
<th>Teleomorph (sexual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimorphic fungi</td>
<td><em>Histoplasma capsulatum</em></td>
<td><em>Ajellomyces capsulatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Blastomyces dermatitidis</em></td>
<td><em>Ajellomyces dermatitidis</em></td>
</tr>
<tr>
<td></td>
<td><em>Emmonsia parva</em> (synonym <em>Chrysosporium parvum</em>)</td>
<td><em>Ajellomyces crescens</em></td>
</tr>
<tr>
<td>Hyaline hyphomycetes</td>
<td><em>Aspergillus fumigatus</em></td>
<td><em>Neosartorya fumigata</em></td>
</tr>
<tr>
<td></td>
<td><em>Paecilomyces</em></td>
<td><em>Byssochlamys</em></td>
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<tr>
<td></td>
<td></td>
<td><em>Chromocleista</em></td>
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<tr>
<td></td>
<td></td>
<td><em>Talaromyces</em></td>
</tr>
<tr>
<td>Dematiaceous fungi</td>
<td><em>Scedosporium apiospermum</em> (synanamorph <em>Graphium eumorphum</em>)</td>
<td><em>Pseudallescheria boydii</em></td>
</tr>
</tbody>
</table>
Level (BSL) 3 organisms. At a minimum, processing of clinical specimens for fungal culture should be done in a BSL 2 facility under a class II biosafety cabinet (9, 10) and when dimorphic fungi are suspected, all isolates should be maintained on slants to prevent aerosolization (10). When grown in culture in filamentous forms, BSL-3 practices should be used when manipulation of isolates is required (9, 11).

**Histoplasma capsulatum**

There are two variants of *H. capsulatum* that are known to cause the disease histoplasmosis. Both *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii* are found in the environment in nitrogen-rich soil, such as that contaminated with bird or bat feces (12, 13). *H. capsulatum* var. *duboisii* is endemic in areas of sub-Saharan Africa and infection typically involves the skin, subcutaneous tissue, lymph nodes, and bones (13). *H. capsulatum* var. *capsulatum* is endemic in the Ohio and Mississippi River Valleys of the United States, but studies have also identified infections in patients living outside these traditional mycosis-endemic areas with no reported prior exposure (14). Depending on the host and environmental exposure, infections can range from asymptomatic to life-threatening disease.

As a thermally dimorphic fungus, *H. capsulatum* takes on two different forms; at temperatures up to 37°C, *H. capsulatum* is in its mold form, but transitions into a yeast form at temperatures greater than or equal to 37°C. Therefore, when seen infecting tissue or blood specimens obtained from a patient, the yeast form is observed. *In vivo*, *H. capsulatum* var. *capsulatum* appears as oval 2- to 4-μm yeasts that may show narrow-based buds (15), while *H. capsulatum* var. *duboisii* is larger (8 to 15 μm) and thick-walled (8, 13). The yeast forms are often observed within host immune cells, as *H. capsulatum* is capable of surviving within macrophages following phagocytosis (16). Normal culture conditions practiced in the clinical microbiology laboratory induce filamentous growth of *H. capsulatum* (17). Observation of characteristic macroscopic growth and eventual production of tuberculate macroconidia assist with the identification and differentiation of *H. capsulatum* from the morphologically similar *Blastomyces dermatitidis* (7). During mycelial growth, the two variants of *H. capsulatum* appear macroscopically and microscopically similar (8, 13).

**Blastomyces dermatitidis**

*B. dermatitidis* is also a thermally dimorphic fungus that transitions from a yeast state at temperatures greater than or equal to 37°C to a mycelial form at temperatures less than 37°C (17). This fungus is found primarily in the river estuaries from Minnesota to Mississippi in the United States, in the Canadian provinces bordering the Great Lakes of North America, and in scattered areas around the world (18). It is found in soil and infection can occur following inhalation of conidia during occupational or recreational exposure. *B. dermatitidis* is the etiologic agent of blastomycosis, which can present as either a local infection or a disseminated disease (19). In tissue, *B. dermatitidis* appears as broad-based budding yeast of varying diameters (ranging from 8 to 30 μm) with thick, refractile cell walls (20). In contrast to *H. capsulatum*, these yeast forms are not intracellular. Under standard laboratory conditions, which include incubation at 25°C to 30°C, *B. dermatitidis* produces vegetative hyphae and conidiophores with microconidia that are similar in appearance to an early culture of *H. capsulatum*. Therefore, care must be taken when making the identification of an immature colony (8).

**Coccidioides species**

There are two species of *Coccidioides* (*C. immitis* and *C. posadasii*) that cause human disease. They have similar phenotype and pathogenicity, but differ in genotype and geographic distribution (21). *C. immitis* is localized to California and northern Mexico while *C. posadasii* is found throughout the endemic areas of the southwestern United States (Arizona, California, Nevada, New Mexico, Texas, and Utah) (22). They are the etiologic agents of coccidioidomycosis, also referred to as Valley Fever, which ranges from asymptomatic infections to pneumonia and severe disseminated disease (23). These organisms are found in the soil, especially in low-moisture environments. Preventing exposure can be difficult due to the ubiquitous risk of dust inhalation by individuals living in endemic areas. Moreover, because of the route of exposure and highly infectious nature of the fungus, outbreaks of coccidioidomycosis may occur following wind storms that disrupt soil (21).

*Coccidioides* species (spp.) take a unique, yeast-like form in the tissue of human hosts. During this parasitic lifecycle, these structures are called spherules which contain endoconidia that are released when the spherule ruptures. The endoconidia then proceed to form additional spherules (24). During vegetative growth *in vitro*, however, the fungus grows as a mold. In contrast to the thermally dimorphic fungi, temperature alone is not a sufficient environmental factor to induce changes in
mold found in soil, plants, and decaying vegetation. 

Invasive infections caused by other Penicillium species pose significant risk to the immunocompromised host (39). Infections with these species cause a wide range of disease, collectively referred to as aspergillosis. Invasive aspergillosis (IA) is the presentation associated with the highest morbidity and mortality. The lung and respiratory tract are the most common sites of infection, but dissemination can occur and include the central nervous system (CNS), heart, kidney,
and gastrointestinal tract. *Aspergillus* species are rarely isolated from the blood (41).

*Aspergillus fumigatus* is the most common species isolated from patients and is the leading cause of IA (42). *A. fumigatus* is a thermotolerant organism, with the ability to survive temperatures up to 70°C (43). In vitro studies, however, demonstrate that *A. fumigatus* germinates most rapidly at temperatures between 37°C to 42°C (44). This ability to grow rapidly at body temperature has been shown to contribute to the pathogenesis of this organism (45, 46). *A. fumigatus* also relies on a complex network of attributes such as stress responses, metal-ion homeostasis, and nutrient-acquisition mechanisms, which likely facilitate growth in its natural environment as well as infection in a human host (47, 48).

Other *Aspergillus* species, such as *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus* can also cause disease in humans (49–52). These organisms can be readily identified in the laboratory based on the appearance of their asexual conidiophores, color of the colony front, which is a by-product of conidial pigmentation, and in combination with other features, such as arrangement of conidia around the vesicle and presence or absence of metulae (7).

**Fusarium**

Many species of the genus *Fusarium* are primarily soil saprophyles or plant pathogens (53) that can have detrimental effects on crop production, especially grains (54). Certain species within this genus including the *Fusarium solani* complex, *F. oxysporum* complex, and *Gibberella fujikuroi*, can also be opportunistic pathogens of humans. *Fusarium* infections can be superficial, locally invasive, or disseminated depending on host state and site of entry. The most frequent organs involved in disseminated disease include the heart, kidney, and spleen, with isolation from the bloodstream also being common (41). Attributes that are thought to contribute to the pathogenesis of these infections include the production of mycotoxins (55) and adventitious conidia (generation of spores in tissue) with concomitant hematogenous dissemination.

Colonies of *Fusarium* appear as a variety of colors based on conidia pigmentation, with shades of red being the most frequently observed in culture. *Fusarium* spp. can be differentiated from similar-looking organisms based on the production of multicellular, fusoid macroconidia (7). Identification to the species level is difficult because this group of organisms is morphologically similar, but phylogenetically quite diverse. Multilocus DNA-sequence analysis (56) or, potentially, matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (57) is required to confidently differentiate the medically important *Fusarium* species. However, species-level identification is not essential because drug-susceptibility patterns are variable and cannot be predicted based on species designations alone.

**Rare and emerging species**

There are multiple other hyaline hyphomycetes that can cause disease in humans, albeit on a less-frequent basis. These include some *Paecilomyces* spp., *Purpureocillium lilacinum* (previously named *Paecilomyces lilacinus*), *Scopulariopsis* spp., *Acremonium* spp., and *Trichoderma* spp. All of these fungi are environmental molds, found in soil and decaying plant matter, and therefore can be contaminants in culture. Given the appropriate host and clinical context, however, they can also be rare etiologic agents of disease. The extent of infection depends on host immune status, with more severe disease occurring in immunocompromised patients. *Pseudallescheria boydii* is a potential human pathogen that also produces hyaline hyphae; its anamorph, *Scedosporium apiospermum*, however, is a darkly pigmented mold and this organism is therefore discussed in the dematiaceous fungi section.

**Mucorales**

Infections caused by organisms in the order *Mucorales*, formerly called zygomycoses in reference to the phylum *Zygomycota*, are rapidly progressive and angioinvasive (58). Mucormycosis is caused primarily by species within the genera of *Mucor*, *Rhizopus*, or *Rhizomucor*. These organisms are characterized by broad, ribbon-like hyphae with little to no septation and irregular branching. They grow rapidly in culture, maturing in three to five days, and often produce abundant aerial hyphae that give the colonies a cottony appearance in vitro (7).

*Mucorales* are commonly found in decaying vegetation, food stuffs, fruit, soil, and animal excreta. For this reason, they can often be isolated as culture contaminants. Inhalation of sporangiopores released from the sporangiophore can result in rhinocerebral, pulmonary, and/or disseminated disease in the appropriate host (59). Cutaneous disease may develop as a result of direct inoculation of spores into traumatized skin. The rapid growth rate results in progressive infection with vascular invasion that can cause fulminating tissue damage and necrosis (60). Early recognition and therapeutic intervention for at-risk patients is key; but even with prompt therapy, outcomes may be poor.
Dematiaceous Fungi
The dematiaceous fungi are darkly pigmented because of their melanin-containing cell walls. This heterogeneous group of molds is ubiquitous in the environment and can be isolated as culture contaminants (61, 62). Infection with a dematiaceous fungus can be classified as phaeohyphomycosis, chromoblastomycosis, or mycotic mycetoma depending on the observed morphology of the organism in tissue. Phaeohyphomycosis refers to tissue invasion by pigmented, septate hyphae. Phaeohyphomycosis can be further divided into clinical forms ranging from superficial to invasive infections, with different etiologic agents having a propensity for specific forms of disease (62). In the case of chromoblastomycosis or mycotic mycetoma, sclerotic bodies or grains are observed, respectively.

Scedosporium
Scedosporium spp. are commonly found in the environment as inhabitants of soil, polluted water, and animal excreta. The two major species that are considered pathogenic in the appropriate human host are Scedosporium apiospermum and S. prolificans. Pseudallescheria boydii, a hyaline hyphomycete, is the teleomorph of S. apiospermum, which also has a synanamorph called Graphium eumorphum. S. prolificans has neither a known sexual state, nor a synanamorph. Both S. apiospermum and S. prolificans can cause invasive disease (63), whereas only S. apiospermum is known for causing mycetoma (64, 65).

Additional dematiaceous fungi
Species within the genera of Alternaria, Bipolaris, Exserohilum, and Curvularia are common saprophytes or plant pathogens, but can also be causes of mycotic keratitis, sinusitis, cutaneous and subcutaneous infections, pulmonary nodules, and disseminated disease (66). Within the genus Exserohilum, there are three species that have been identified as potential human pathogens: Exserohilum rostratum, E. longirostratum, and E. mcginnisii. E. rostratum is the most common of these three species and was recently associated with an outbreak of cerebral phaeohyphomycosis due to contamination of injectable steroids (67). All of these genera form characteristic conidia, the appearance of which can help with identification to the genus level. Further identification to the species level, however, is difficult using morphology alone.

There are also certain species of darkly pigmented fungi that are neurotropic, which make them more likely to cause cerebral phaeohyphomycosis (68). These include Cladophialophora bantiana (formerly Cladosporium bantianum) (69), which is the most commonly isolated cause of cerebral phaeohyphomycosis, Scolecobasidium gallopavum (formerly Ochroconis gallopavum) (70), Ramichloridium mackenziei (71), and Exophiala dermatitidis (formerly Wangiella dermatitidis) (72, 73).

IMMUNOLOGY OF INVASIVE MOLD INFECTIONS
Antifungal immune responses are complex and dynamic processes that vary with respect to organism, morphotype (i.e., conidia versus hyphae), and site of infection. Similarly, the risk for developing an invasive-fungal disease (IFD) also differs depending on the fungal species, environmental exposure, and which component of the innate and/or adaptive immune system is impaired. The goal of this section is to provide a general overview of the critical components of antifungal immunity and highlight the ways in which different forms of immunosuppression predispose to common IFDs.

Innate Fungal Immunity
The skin and mucosa are the first lines of host defense, with breaches in these physical barriers increasing susceptibility to IFD. The primary mode of exposure to molds is via inhalation of spores or conidia. If these fungal elements are not promptly cleared by the immune system, invasive disease of the sinus or lung may ensue. Direct inoculation of spores into skin structures is also a potential route of transmission, again resulting in both local and disseminated infections depending on the effectiveness of the immune response.

Innate immune-effector cells
Neutrophils, monocytes, and macrophages are essential immune-effector cells. Not only does this group of phagocytes mediate intra- and extracellular killing of fungi; they also impede fungal growth through the secretion of antimicrobial peptides and other mediators that deprive these pathogens of essential nutrients (74). Monocytes and macrophages are able to ingest fungal spores, with killing accomplished by opsonin-independent, nonoxidative methods (75). Once conidia have germinated, neutrophils and monocytes can also kill and/or damage hyphae. Since hyphae are often too large to be completely phagocytosed, damage is typically achieved by extracellular means. Both oxidative mechanisms and defensins appear to be involved (76).

Impairments of neutrophil number and function are major risk factors for IFD. The most common cause of
Filamentous Fungi

profound neutropenia is cytotoxic chemotherapy, but it may also result from underlying diseases such as acute leukemia, myelodysplastic syndrome, aplastic anemia, advanced HIV infection, or autoimmune disease. Corticosteroid use is an important cause of impaired neutrophil function. These drugs impede the oxidative burst, reduce lysosomal activity, and decrease immune-cell mobilization (77, 78). The importance of oxidative killing is also illustrated by the natural history of chronic granulomatous disease, a hereditary condition marked by reduced production of oxidative intermediates and high rates of IA.

Pattern-recognition receptors

Mammalian hosts have evolved an arsenal of soluble and cell-associated pattern-recognition receptors (PRRs) that recognize conserved structures, called pathogen-associated molecular patterns (PAMPs), on the surface of invading microorganisms. Fungal PAMPs are comprised of cell-wall polysaccharides, proteins, and nucleic acids. PRRs are expressed on macrophages and dendritic cells, but are also in various “nonprofessional” immune cells like epithelial cells, endothelial cells, and fibroblasts.

Currently, four different PRR families have been identified and include the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Of these, the TLRs and CLRs are associated with the innate-immune recognition of fungal pathogens. TLRs are a family of 10 different transmembrane-receptor types, with TLR-2, -3, -4, -6, and -9 known to be involved in fungal interactions at the cell surface and/or inside the phagosomes of innate-immune cells. TLRs that recognize fungi have been reported to collectively sense β-glucan, O-linked mannans and fungal nucleic acid (79). Interestingly, TLRs on the surface of immune-effector cells may activate intracellular signals that result in opposing biological functions. For example, macrophages produce the pro-inflammatory cytokines tumor-necrosis factor (TNF)-α and interleukin (IL)-1 in response to Aspergillus conidia through TLR 4-dependent mechanisms, while hyphae stimulate production of the anti-inflammatory cytokine IL-2 via TLR-2 pathways (80). Thus, activation of specific cell-surface receptors during germination and hyphal extension may allow Aspergillus to circumvent host defenses (81).

The CLR superfamily comprises a large group of proteins divided into soluble as well as transmembrane-associated receptors (82). Members of this group of PRRs that are involved in fungal immunity include Dectin-1, Dectin-2, dendritic cell (DC)-specific intracellular-adhesion molecule (ICAM)3-grabbing non-integrin (SC-SIGN), macrophage-inducible C-type lectin (mincle), and the soluble mannose-binding lectin (MBL) receptor. CLRs interact with N-linked mannans and chitin, as well as α and β-glucan (79).

The innate-immune system link to adaptive fungal immunity

Dendritic cells (DCs) play an instrumental role in linking innate and adaptive immune responses for a variety of fungal infections (83). DCs capture and present fungal antigens, express lymphocyte costimulatory molecules, migrate to lymphoid organs, and secrete cytokines (84). The signals transmitted by DCs vary depending on the fungal antigen presented, with resultant differences in adaptive T-cell responses and antibody production.

Adaptive Fungal Immunity

T-helper cells

Differentiation of CD4+ T cells along a T-helper type 1 (Th1) or Th17 pathway leads to a pro-inflammatory cellular immune response with antifungal activity (85). Although multiple cytokines are involved, the action of Th1 cytokines interferon (IFN)-γ and TNF-α and/or a Th17 response predicated on IL-17 and IL-22 are integral to antifungal protection (86). A predominance of Th1/Th17 responses over the anti-inflammatory Th2 response (e.g., IL-4, IL-5, and IL-10) promotes fungal clearance and is linked to protection against a diverse array of important filamentous fungal pathogens including A. fumigatus (87, 88) and the dimorphic fungi (89, 90).

T-cell function is essential for antifungal immunity. Certain treatment regimens for cancer and autoimmune disease, as well as the current antirejection therapies used for solid-organ transplant (SOT) and hematopoietic stem-cell transplant (HSCT), significantly impair T-cell responses and/or reduce lymphocyte numbers. Pharmacologic agents with significant impact on cellular immunity include the anti-neoplastic purine analogs (e.g., fludarabine) and antibodies directed against T cells (e.g., anti-CD52 monoclonal antibodies, anti-thymocyte globulins, anti-CD3 monoclonal antibodies). These drugs induce prolonged lymphopenia and have lasting effects on T-cell function (91). Similarly, TNF-α blockade has emerged as a useful therapy for graft-versus-host disease (GvHD) and some autoimmune diseases. Anti-TNF-α agents have also been linked to IFD risk across a range of host groups (92).
Antifungal-antibody responses

Although a diminished number and/or function of B cells does not generally lead to an increased susceptibility for IFD, multiple studies have shown that fungus-specific antibodies do confer some degree of protection. In vitro studies demonstrating antibody-mediated killing through opsonization and complement activation as well as enhancement of T-cell activity provide supportive evidence for protective antibody-based immunity (93). Furthermore, there is some evidence to suggest the possibility of antifungal-vaccine protection predicated on humoral immunity (94). The majority of protective antibodies described to date recognize cell-surface molecules, but no common target has been identified across fungal pathogens. Rather, organism-specific antigens have been identified that elicit protective antibody-mediated responses (95–97).

EPIDEMIOLOGY OF FILAMENTOUS MYCOSES IN SELECTED IMMUNOCOMPROMISED GROUPS

Recognition of the patient groups at highest risk for IFD is essential for informing preventative, diagnostic, and clinical-management strategies. Table 2 summarizes the most important clinical-risk factors affecting selected groups of immunocompromised patients.

Host Genetic Factors

An increasing number of studies have reported associations between host genetic variants (e.g., single-nucleotide polymorphisms [SNPs] or variable tandem-number repeats) and risk for IFD. In particular, polymorphisms in the TLR-1, TLR-4, and TLR-6 genes have been associated, albeit with varying strengths, with IA in HSCT recipients (98, 99). Genes that encode cytokines have also been shown to influence IFD risk. For example, mutations that confer overproduction of IL-10 have been associated with the development of IA and SNPs that reduce production appear protective (100, 101). Furthermore, TNF-α production is also thought to have a genetically determined component in that variability in TNF-α production may contribute to IA risk across similar patients who undergo equally immunosuppressive-treatment regimens (102). It is important to note, however, that many gene-association studies are inherently limited by statistical power and the number of SNPs considered in the analysis. Larger studies will be required to confirm the clinical significance of many of the SNPs reported to date. These findings are expected to have important implications for clinical practice in the future because genetic profiles could conceivably be used for risk stratification and individualization of preventative antifungal strategies.

Hematologic Malignancy and Hematopoietic Stem Cell Transplantation

Certain subsets of patients with hematologic malignancy (HM) and HSCT are at significant risk for the development of IFD. The highest-risk groups include patients with acute myeloid leukemia (AML), especially during induction cycles of chemotherapy (103), and allogeneic

### TABLE 2 Common invasive mold infections stratified by risk group and predisposing factors

<table>
<thead>
<tr>
<th>Disease</th>
<th>Highest-risk groups</th>
<th>Patient-specific risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td>Acute myeloid leukemia, Allogeneic HSCT</td>
<td>Induction chemotherapy, neutropenia, advanced age, iron overload, refractory cancer, Mismatched donor, unrelated donor, cord-blood stem cell source, T-cell-depleted graft, neutropenia, GVHD, prolonged steroid use, TNF-α inhibitor use, CMV infection, advanced age, iron overload, donor TLR or cytokine polymorphisms</td>
</tr>
<tr>
<td></td>
<td>Lung or heart-lung SOT, Heart SOT, Liver SOT</td>
<td>Airways colonization, prolonged steroid use, allograft rejection, CMV infection, renal impairment, re-transplantation or fulminant liver failure as an indication for transplant (liver SOT only)</td>
</tr>
<tr>
<td></td>
<td>Chronic granulomatous disease</td>
<td>None identified</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>Diabetes</td>
<td>Neutropenia, Hyperglycemia ± ketoacidosis, Iron overload, Trauma, Prolonged steroid use, Severe neonatal prematurity</td>
</tr>
<tr>
<td></td>
<td>Acute myeloid leukemia, Allogeneic HSCT</td>
<td></td>
</tr>
<tr>
<td>Endemic Mycoses</td>
<td>HIV/AIDS</td>
<td>Environmental exposure, T-cell immunosuppression/deficiency, TNF-α-inhibitor therapy</td>
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<td></td>
<td>Solid-organ transplant</td>
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<td></td>
<td>Autoimmune disease</td>
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</tbody>
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*Adapted from Herbrecht et al. (26), with permission.

*HSCT, hematopoietic stem cell transplant; GVHD, graft-vs-host disease; TNF, tumor necrosis factor; CMV, cytomegalovirus; TLR, toll-like receptor; SOT, solid-organ transplant; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.
(allo)-HSCT recipients, particularly those with unrelated or mismatched stem-cell donors and/or cord blood as a stem-cell source (104).

Bodey et al. first reported that the strongest predictor of infection in patients with acute leukemia was the duration of neutropenia (105). If neutropenia persisted for 3 weeks, infection risk including IFDs was 60%, with a further rise to 100% when the neutrophil count dropped to levels of <0.10 × 10^9/L. Not only was the incidence of infections related to neutrophil count, but the outcome of bacterial and fungal infections was also dependent on the depth of neutropenia, with the highest mortality observed among patients with long-standing profound neutropenia (<0.10 × 10^9/L) (105). Recent studies have corroborated these classic observations (106). Additionally, persistent lymphocytopenia combined with the T-cell-immunosuppressive therapies utilized for the prevention and treatment of GvHD are also important drivers of risk in the months following allo-HSCT (107).

Two large multicenter studies, the Transplant-Associated Infection Surveillance Network (TRANSNET) and Prospective Antifungal Therapy (PATH) Alliance Registry, have recently characterized the epidemiology of IFDs in adult HSCT and HM patients (108–110). In both registries, IA was the most common mycelial infection followed by invasive candidiasis and then mucormycosis. A. fumigatus was the most common single species isolated (111, 112). Twelve-month cumulative incidence estimates for any IFD after HSCT was relatively low (3.4%), but rates varied significantly by geographic region and individual transplant center (range, 0.9% to 13.2%) (110). Importantly, the overall incidence of IFD did not decrease during the 3-year TRANSNET study despite routine use of antifungal prophylaxis (110).

Multiple reports have described an increasing incidence of non-Aspergillus invasive mold infections, particularly mucormycosis, among HM and HSCT patients receiving antifungal drugs with activity against Aspergillus (113, 114). Invasive infections caused by the Mucorales and the hyaline hyphomycetes (e.g., Fusarium spp.), and some dematiaceous fungi (e.g., Scedosporium spp.) are of particular concern because of their rapid and aggressive clinical course combined with intrinsic resistance to many of the currently available antifungal drugs. During the TRANSNET surveillance period, 124 cases of Fusarium spp., Scedosporium spp., and Mucorales infections were identified out of 983 total cases of proven or probable IFD in HSCT recipients (63). The total number of cases varied by transplant center and region; in general, the estimated cumulative incidence of mucormycosis increased among HSCT subcohorts. The lower-respiratory tract and sinuses were the most common sites of infection, similar to the patterns of disease caused by Aspergillus species. These observations highlight the difficulty in differentiating mold pathogens based on clinical symptomatology or patterns of organ involvement alone. It is imperative that clinicians and laboratorians work together to select the appropriate fungal tests to make the correct diagnosis.

**Solid-Organ Transplantation**

IFDs are also an important infectious complication of SOT as a result of the long-term immunosuppressive therapy required to prevent and treat allograft rejection. In the PATH Alliance and TRANSNET registries, invasive candidiasis was the most common IFD followed by IA, cryptococcosis, non-Aspergillus molds (other than Mucorales), and then the endemic mycoses (115, 116). The 12-month cumulative incidence estimate for any IFD after SOT was 3.4% (115). Again, A. fumigatus was the most frequently isolated mold species, with most cases of IA limited to the lungs. The majority of endemic fungal infections were due to H. capsulatum with geographic restriction to transplant centers located in endemic areas. This group of infections was uncommonly encountered overall but when they did occur, dissemination involving multiple organ systems was observed (117).

Important differences in IFD risk and epidemiology exist across SOT groups owing to differences in the net state of immunosuppression, intensity of environmental exposures, and the organ transplanted. Lung-transplant recipients are at highest risk for invasive mold infections, in part because the allograft is exposed to the environment and inhalation of fungal spores is continuous. Clinical manifestations in this patient group may range from asymptomatic airway colonization, which is common, to tracheobronchitis, invasive parenchymal disease, empyema, and disseminated disease. Differentiating airway colonization from invasive infection can be quite difficult using current microbiologic and radiographic tools. New diagnostic approaches are urgently needed for this population in particular.

Donor-derived mold infections have rarely been reported. Twenty-three cases were recently reviewed (118). The majority (91%) of these infections occurred in kidney-transplant recipients and Aspergillus was the most common organism (71%) identified. Risk factors for donor-derived mold infections include an immunosuppressive state in the donor, near-drowning events, and transplant-tourism practices.
Human Immunodeficiency Virus (HIV)
Although combination antiretroviral therapy (ART) has dramatically changed the face of the HIV epidemic, many patients present for care with advanced HIV-related immunosuppression. Advanced HIV infection (AIDS) results in significant declines in CD4+ T-cell counts, which has historically been a reliable predictor of opportunistic-infection risk. There is now increasing recognition that HIV infection also has complex effects on both myeloid and lymphocytoid lineages, which also contributes to increased susceptibility to IFDs (119).

Globally, Pneumocystis pneumonia, cryptococcosis, and mucocutaneous candidiasis are the most common IFDs affecting HIV-positive patients (120). In the North American PATH Alliance study, cryptococcosis, candidiasis, and IA were most frequent (108). Aspergillosis is not considered to be an AIDS-defining opportunistic infection and is relatively uncommon in this patient population. Data analyzed from the Adult and Adolescent Spectrum of HIV Disease (ASD) project revealed an overall incidence of 3.5 IA cases per 1,000 person-years (121). The traditional risk factors, including neutropenia and corticosteroid use, are associated with IA in HIV-positive patients (122). Invasive pulmonary disease and a syndrome of bronchial-obstructing aspergillosis have been observed in HIV-positive patients (123).

In contrast to aspergillosis, extrapulmonary histoplasmosis and coccidioidomycosis are AIDS-defining illnesses (124). Similarly, T. marneffei is an important opportunistic infection affecting AIDS patients in Southeast Asia, India, and China (125). In general, patients with CD4 cell counts <100 cells/μl are at greatest risk for developing these infections (126), which may result from recent exposure or reactivation of latent infection (127).

Each endemic mycosis presents with a unique set of clinical features. Patients with progressive disseminated histoplasmosis, the most common form of disease among HIV-infected persons, usually have fever, malaise, and weight loss over a period of weeks. Diagnosis is established by isolating the fungus from respiratory secretions, blood, or bone marrow and/or by detecting Histoplasma antigens in serum and/or urine. Most HIV-infected patients with coccidioidomycosis seek treatment for pneumonia. The sites of dissemination frequently seen in non-HIV patients (i.e., bone, joint, and meninges) appear less commonly involved in those with HIV (128). Penicilliosis (caused by T. marneffei) is associated with a characteristic papular rash that includes lesions with a central umbilication reminiscent of molluscum contagiosum. Diagnosis is usually established by examining Wright-stained samples of bone-marrow aspirates or touch smears of skin-biopsy specimens (129, 130).

Diabetes Mellitus (DM)
As a result of impaired innate and adaptive immunity, hyperglycemic patients are at risk for a variety of IFDs. Hyperglycemia causes altered neutrophil chemotaxis, impaired transmigration through vascular endothelium, and reduced superoxide production (131). Adaptive immunity is also impaired. For example, poorly controlled type-1 diabetics have been shown to have diminished inflammatory-cytokine production, including reductions in IL-1, IL-6, TNF-α, and IFN-γ (132).

Mucocutaneous candidiasis is the most common infection affecting diabetic patients while mucormycosis is the most feared. Reviews of the literature have reported DM as a predisposing factor in 36% to 88% of cases of mucormycosis (133). Furthermore, in a large review of 929 mucormycosis cases, rhinocerebral disease was seen more frequently in patients with DM (33%) as compared to patients with HM where pulmonary involvement was most common (59). Overall, Rhizopus spp. were the most frequently isolated Mucorales.

Patients with diabetic ketoacidosis (DKA) are at the greatest risk for Mucorales. In addition to the phagocyte dysfunction described above, DKA leads to elevated free-iron serum concentrations that supports the growth of Rhizopus oryzae at acidic but not at alkaline pH. (134). Furthermore, adding exogenous iron to serum allowed R. oryzae to grow profusely at acidic conditions but not at pH ≥7.4 (134).

Primary Immunodeficiencies
An increasing number of congenital immunodeficiencies have been linked to recurrent or severe filamentous mycoses in children and young adults (135). Inborn errors of the phagocyte NADPH-oxidase complex (chronic granulomatous disease) (136), autosomal-dominant hyper-IgE syndrome (137), severe congenital neutropenia (138), and autosomal-recessive leukocyte-adhesion deficiency type I (CD18 deficiency) (139) predispose to pulmonary aspergillosis. More rarely, patients with idiopathic CD4+ lymphopenia (140) or mutations affecting the IL-12/INF-γ axis (141–143) have developed disseminated endemic fungal infections.

Critical Care Settings
While IFD in critically ill patients is most commonly due to Candida species, Aspergillus spp., Mucorales, Fusarium spp., and Scedosporium spp. have also been shown to infect this patient population (144). In addition to
the patient characteristics discussed in the previous sections, chronic obstructive-pulmonary disease (COPD) is a major risk factor for IFD in critically ill patients in intensive-care units (ICU) (104, 145). In particular, COPD is a risk factor for invasive pulmonary aspergillosis (IPA), especially in conjunction with prolonged steroid treatment (145). Aspergillus tracheobronchitis is also of concern in this patient population. While these infections are infrequent (approximately 5%), they are an often fatal complication of COPD in the ICU (146).

**LABORATORY TESTS**

The etiologic diagnosis of infections due to filamentous fungi is complex and multiple challenges remain. Many factors complicate the attribution of a pathogen to a disease process, especially in specimens collected from nonsterile sites such as the respiratory tract, where IFD is most common. Immunosuppression, anatomic abnormalities, and/or underlying COPD promotes colonization of the airways with molds, thus leading to challenges in interpretation of positive laboratory results. An additional challenge for the diagnosis of IFD of the lung is the difficulty in obtaining adequate noninvasive specimens from the site of infection. Many patients do not expectorate sputum (147). Bronchoscopy can be helpful for obtaining specimens in these patients (148), especially if tissue biopsy can be performed. Unfortunately, however, bronchoscopy carries additional risk of bleeding and iatrogenic infection in immunocompromised patients due to many factors including thrombocytopenia, neutropenia, and mucositis. For these reasons, diagnosis is often based on a composite probability derived from host risk factors, clinical signs and symptoms, microbiologic studies, and radiology. Combinations of diagnostic studies are the rule rather than the exception for IFD.

**Microbiology and Molecular Techniques**

**Direct staining of clinical materials**

Staining of specimens collected from sites suspected to be infected with invasive fungi can facilitate the detection of these pathogens. Respiratory specimens as well as other specimen types, such as ocular scrapings and tissue, can be stained directly, whereas dilute specimens, including bronchoalveolar lavage (BAL) fluid, must be centrifuged to concentrate the sample before staining.

Direct staining of clinical specimens is rapid and inexpensive, but overall this approach has limited sensitivity. Methylene blue stains most microorganisms but has low sensitivity due to heavy background staining. Use of a fluorescing stain such as calcofluor white improves sensitivity by increasing contrast against background material. Calcofluor binds to beta-glucans, chitin (149), other cell-wall polysaccharides, and cellulose. Chitin is present in the cell walls of most fungi as well as arthropod structures.

**Histopathology**

Detection of fungal elements in tissue remains the diagnostic “gold standard” for IFD. Furthermore, the host immune response directed at the pathogen can also be visualized, which may help identify the presence of an infectious process. Immunocompromised patients and the critically ill, however, are often poor candidates for the invasive procedures that are required to obtain a tissue sample for histopathology.

The utility of histopathology to identify invasive fungi beyond generalized terms varies based on the organism in question (15). The dimorphic molds have characteristic structures in tissue, such as the spherules seen in cases of coccidioidomycosis and the budding-yeast forms of histoplasmosis and blastomycosis. Dematiaceous molds can often be distinguished by use of additional stains that bind melanin pigment such as Fontana-Maisson (15), whereas agents of mucormycosis have broad, ribbon-like, and nonseptate hyphae that often have a distinct appearance on histopathology. In contrast, hyaline molds with broadly varying antifungal-susceptibility profiles are indistinguishable from one another in tissue. Although Aspergillus is the most common cause of IFD, describing all septe hyphae with dichotomous branching as being “consistent with Aspergillus” is misleading for the clinician. In a single center 10-year review (150), only 79% of specimens with concurrent fungal culture were correctly identified based on morphologic features in histologic and/or cytologic specimens. There were 10 discrepant diagnoses (21%) that included misidentification of septate and nonseptate hyphal organisms and yeast forms. Errors resulted from morphologic mimics, use of inappropriate terminology, and incomplete knowledge in mycology.

Despite these difficulties, correctly characterizing fungal elements into one of these four broad categories is still clinically useful. An amphotericin formulation is the drug of choice for Mucorales spp., whereas an extended-spectrum triazole such as voriconazole may be preferred for the hyaline hyphomycetes. Thus, early discrimination to this level helps clinicians make more informed and appropriate initial empiric antifungal-therapy choices. The ability to grow the mold in vitro for definitive identification and drug susceptibility, however, remains critical for assigning treatment and prognosis.
Culture

Growth in culture remains essential for the optimal diagnosis of infections due to filamentous fungi, especially when one of multiple potential pathogens may be present. In these cases, culture offers a broader approach than pathogen-specific nucleic acid-detection technologies. In addition, drug-susceptibility determination still primarily relies on phenotypic techniques that require a growing isolate.

Molds can be grown on a variety of artificial media routinely used within the clinical laboratory. For optimal recovery of fungal pathogens, a combination of both selective and nonselective media should be used (10, 151). Brain heart infusion (BHI) and Sabouraud dextrose with BHI (SABHI), both of which can be supplemented with antibiotics, or Inhibitory Mold Agar (IMA) contain antibiotics to inhibit the growth of contaminating bacteria. This is especially critical when culturing isolates from non-sterile sites such as the respiratory tract. Cycloheximide suppresses the growth of saprophytes while allowing the growth of dimorphic fungi or dermatophytes (151). It is important to remember that cycloheximide may inhibit certain opportunistic pathogens such as Aspergillus, Fusarium, Scopulariopsis, Pseudallescheria, Mucorales, and some dematiaceous fungi, as well as yeasts, such as Cryptococcus neoformans and some Candida spp.

Once an isolate is recovered from fungal culture, identification is made using a variety of methods. Macroscopic and microscopic morphology is one of the most basic and traditional methods used. There are multiple references that describe these phenotypic characteristics in great detail (7, 8). Differential growth on specialized media, temperature-dependent growth studies, as well as some biochemical assays, also contribute to the fungal-identification process. Not all laboratories, however, are equipped with trained personnel who are able to appropriately perform and interpret the information gained from these classical techniques. Even when performed by experienced mycologists, morphological similarities across species and genera may still lead to misidentification. Therefore, molecular methods such as Sanger sequencing of the internal-transcribed spacer (ITS) and/or D1/D2 regions have been used successfully for the identification of many fungal pathogens. MALDI-TOF analysis (described later in this section) is also being investigated for fungal-identification purposes.

Despite its obvious utility in making a diagnosis of IFD, culture also has significant limitations which include poor sensitivity (directed lung sampling with BAL is at best 50% sensitive (152)), long turnaround times for slow-growing organisms, and misidentifications. Furthermore, differentiating environmental contamination or colonization from true IFD is difficult. Clues to the clinical significance of a mold isolated in culture include visualization of compatible fungal elements on direct stain, the same organism isolated from multiple specimens, multiple colonies of the same organism growing from a single specimen, and the anatomic source of the specimen placed in the context of the host and clinical symptoms (e.g., Mucorales isolated from the sinus of an HSCT recipient).

Antibody-detection assays

While multiple methods for the detection of antifungal antibodies in both serum and cerebrospinal fluid (CSF) samples exist, assays of the humoral response to pathogens other than Coccidioides and Histoplasma have a limited role in the diagnosis of filamentous fungi (153, 154). As a ubiquitous part of the environment, molds are inhaled routinely. Therefore, antibody detection does not differentiate prior exposure from current infection. Furthermore, the ability of the host to mount a humoral response may be limited by immunosuppression. For recent HSCT recipients and those requiring multiple blood transfusions, the measured humoral response is more relevant to the donor than the patient being evaluated. For these reasons, serologic assays rarely provide diagnostic information in the immunocompromised host.

Antigen-detection assays

The detection of the fungal cell-wall biomarkers galactomannan and (1,3)-β-D-glucan have become an integral part of the management of severely immunocompromised patients. These tests, which are described below, are optimally used in conjunction with other diagnostic procedures and should not be interpreted as stand-alone tests for the diagnosis of IFD.

Galactomannan

Galactomannan (GM) is a cell-wall polysaccharide that is released during in vivo fungal growth and can be detected in serum, often before the onset of symptoms (155). It is present in and released by Aspergillus, as well as other fungi, such as Penicillium, Paecilomyces, and Histoplasma. Assays for GM detection have been optimized for target organisms (i.e., Aspergillus or Histoplasma), but cross-reactivity due to other invasive fungi is possible.

There is currently only one Food and Drug Administration (FDA)-approved assay for the detection
of *Aspergillus* GM (Plateia *Aspergillus* EIA, Bio-Rad; Marnes La Coquette, France). It has been approved for testing of serum and BAL specimens, but studies have shown that it may also be effective using additional specimens such as CSF (156, 157). The test is an immunoenzymatic-sandwich assay which utilizes monoclonal antibodies directed against *Aspergillus* GM. In the presence of GM antigen, a monoclonal antibody – GM–monoclonal antibody/peroxidase complex is formed. Upon addition of a substrate, a colorimetric reaction occurs that can be detected by spectrophotometry. The presence or absence of GM antigen in a test sample is determined by calculating an index using the optical density (OD) value of the specimen divided by the mean OD of a cut-off control. An index of <0.5 is considered negative for GM antigen, whereas an index of ≥0.5 is considered positive (158). Several recent BAL studies, however, suggest that increasing the OD threshold to 1.0 or 1.5 may increase specificity without affecting sensitivity (159, 160).

The manufacturer recommends repeat testing for both positive and negative serum results. A negative *Aspergillus* GM result does not rule out the diagnosis of IA and repeat testing is recommended if clinical suspicion for disease remains high. Additionally, repeat testing of a new aliquot of the same sample, as well as collection of a new sample from the patient for follow-up testing, are also recommended for confirming a positive test result (158). The utility of a single GM determination is of limited diagnostic value. Surveillance strategies for at-risk patients using *Aspergillus* GM generally use serially collected specimens to maximize the clinical utility of this test. While a range of testing frequencies have been studied (161, 162), most experts agree that testing two or three times per week is appropriate to balance the need for both rapid detection of IFD and cost containment (163). Twice-weekly testing for patients at risk for IA is also recommended by the manufacturers (158).

Serum *Aspergillus* GM-test characteristics vary by patient population and disease prevalence (164, 165). In the adult and pediatric HM/HSCT setting, pooled sensitivity and specificity estimates range from 70% to 89% and 85% to 92%, respectively (164). In contrast, serum *Aspergillus* GM in SOT recipients has very poor sensitivity (164) and is not routinely recommended. It is likely that neutropenic patients have significantly higher concentrations of circulating GM antigen than do non-neutropenic SOT recipients. The specificity and positive-predictive value of *Aspergillus* GM are limited by false-positive tests that result from coadministration of certain antibiotics derived from fungi (e.g., beta-lactam drugs such as piperacillin), certain food stuffs (e.g., milk formulas or popsicles), and the translocation of *Bifidobacterium* from the guts of neonates (166, 167).

*Aspergillus* GM testing has also been validated for BAL specimens. Sensitivity appears to be increased for BAL specimens relative to serum in patients with IPA. This observation holds true for both HM/HSCT and SOT patients (157, 168). Meta-analyses have reported a pooled sensitivity and specificity of 82% to 87% and 89% to 97% in BAL, respectively (169, 170). The detection of GM and/or fungal DNA in BAL, however, does not differentiate colonization from invasive disease. Additionally, combining GM results with polymerase-chain reaction (PCR) testing may enhance early detection of IPA over GM alone, both for serum and BAL testing (171, 172).

(1, 3)-β-D-glucan

(1, 3)-β-D-glucan (BDG) is another polysaccharide component of the fungal cell wall. BDG is produced at detectable levels in a variety of medically important fungi including *Candida* spp., *Pneumocystis jiroveci*, and multiple filamentous fungi such as *Aspergillus* spp., *Fusarium* spp., and *Acremonium* spp.; notable exceptions are *Cryptococcus* spp., the *Mucorales*, and the yeast phase of *B. dermatitidis* (173). There is currently only one FDA-approved assay for the detection of BDG in clinical samples (Fungitell Assay, Associates of Cape Cod Inc.; Falmouth, MA) that is approved for use on serum specimens. The assay is based on the principles of the *Limulus*-lysate reaction, which is a biological coagulation cascade found in horseshoe-crab amebocytes. The cascade has been modified so that the presence of BDG leads to activation of a clotting enzyme that cleaves a chromogenic substrate. The OD of the reaction well is recorded and results from test samples are compared to a standard calibration curve, allowing quantitation of BDG levels. Concentrations of BDG ranging from 10 to 40 pg/ml are considered normal background, possibly due to the presence of commensal yeast in the alimentary and gastrointestinal tract, whereas values above 80 pg/ml in at-risk patients are considered positive (173).

Overall, the sensitivity of serum BDG testing appears to be similar to GM for detection of *Aspergillus* spp., but BDG testing is expectedly less specific (174). BDG-test characteristics vary by organism, with the greatest sensitivity (96%) and specificity (84%) reported for *Pneumocystis* pneumonia (175). This assay has also been applied to CSF to assist in the diagnosis of fungal meningitis due to *Exserohilum rostratum* (176, 177) and for detecting infections due to dimorphic fungi (178, 179).
Similar to GM-antigen testing, repeat testing is recommended to improve assay sensitivity and specificity (173).

A variety of factors have been linked to false-positive BDG tests. These include the use of certain hemodialysis membranes (180), intravenous immunoglobulin (181), and surgical sponges and gauze products (182), as well as following lung transplantation, possibly as a result of underlying airway colonization specifically in this patient population (183). False positivity due to antibiotics may be less common than sometimes thought (184).

Lastly, BDG testing has not been well validated in children and different cut-offs to define a positive result may be required for optimal performance in pediatrics (185).

Antigen-based assays for dimorphic fungi
Asymptomatic colonization with dimorphic fungi rarely, if ever, occurs; therefore, antigen assays for these pathogens have high clinical specificity. There are different commercially available assays for the detection of *Histoplasma* antigens, but only one has been approved by the FDA for in vitro diagnostic (IVD) use (Alpha Histoplasma Antigen EIA; IMMY, Norman, OK) (186). Depending on the assay, these tests use either monoclonal or polyclonal antibodies for the detection of *Histoplasma* GM or other, proprietary, antigens. Studies suggest that use of monoclonal antibodies improves the analytical performance of these assays (187). While the IVD assay has only been approved for urine specimens, antigen can be detected in other specimens, including serum, CSF, and BAL fluid, using this or other *Histoplasma* antigen-detection methods (188–190). In one study investigating diagnostic techniques for both pulmonary and disseminated histoplasmosis in SOT patients, antigen detection proved to be the most sensitive methodology (antigenuria sensitivity of 93% and antigenemia sensitivity of 86%) compared to fungal culture, antibody detection, or demonstration of organism by cytology or pathology (191). *Histoplasma* antigen detection in urine can be used not only to support the diagnosis of histoplasmosis, but also as a means of monitoring patient response to antifungal therapy. Antigen levels generally decline following treatment, but the rate of this reduction varies depending on assay used (187).

Antigenic similarities between *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis* cause cross reactivity with *Histoplasma* antigen assays (192, 193). Specific assays for the detection of these organisms have also been developed (194, 195). Similar to *Histoplasma* antigen assay, they are subject to cross-reactivity with other dimorphic fungi and must be interpreted in the clinical context of the patient.

Nucleic acid-detection techniques
Molecular techniques currently serve as important adjuncts to, rather than replacements of, traditional fungal diagnostic method, such as culture. Positive molecular results can be used to guide antifungal therapy, but must be interpreted in the context of the host, specimen type, and local epidemiology due to the risk of contamination by environmental fungi. Currently, only one assay has received FDA approval for the direct detection of fungal nucleic acid in a patient specimen (T2Candida T2 Biosystems, Inc.; Lexington, MA). The assay employs PCR and T2 magnetic resonance (T2MR) for the detection of the five most common *Candida* species directly from blood specimens with an overall specificity of 99.4% (95% CI, 99.1% to 99.6%) and overall sensitivity of 91.1% (95% CI, 86.9% to 94.2%) compared to blood culture (196). Currently, there are no FDA-approved tests for the direct detection of filamentous fungi. Instead, currently available assays consist of different laboratory-developed tests that vary based on type and volume of specimen tested, as well as the method of cell-wall disruption, DNA extraction, and gene-target amplification and detection (197). This lack of standardization limits assay comparisons between laboratories as well as across clinical studies.

Specimen type is important for both determining the performance of the assay and interpreting of the results. Studies have demonstrated that fungal nucleic acid can be detected from a wide range of specimens including the various fractions of blood, CSF, tissue, and BAL fluid (67, 198, 199). The preferred specimen type used for a clinical diagnosis depends largely on the suspected site of infection and host characteristics. Detection of fungal nucleic acid from a normally sterile site can provide more rapid results compared to lengthier and potentially less-sensitive culture methodologies. Differentiation of airway colonization from invasive disease is not possible with current methods. However, patients with invasive lung disease tend to have higher fungal burdens and lower PCR-crossing thresholds than do those with simple airway colonization (200). Targeting genes involved in fungal growth and tissue invasion has also been discussed as a potentially more-specific predictor of infection (201), but, to date, no such assay has been developed. Specimens can also become contaminated with ubiquitous environmental fungi during collection and processing. Studies have reported contaminating fungal nucleic acid in phlebotomy tubes (202) and reagents used during the nucleic acid-extraction procedure (203), such that assay design should account for low-level background fungal-DNA contamination.
Direct detection of fungal nucleic acids in tissue can be especially beneficial in situations where culture was not ordered at the time of specimen collection or when fungi are seen microscopically but the cultures remain negative. Remaining tissue samples may already be fixed in formalin for pathological exam, thus making them unacceptable for culture. Detection and identification of nucleic acid directly from these specimens can improve the diagnostic yield. In fact, recent studies have demonstrated that broad-range fungal PCR followed by sequencing can be accomplished using formalin-fixed, paraffin-embedded tissue with 86% to 94% sensitivity in culture-proven cases and 64% to 89% in cases with fungal elements seen by histopathology (204–207). Factors influencing assay sensitivity in these studies included gene target and extraction method used.

Fungal nucleic acids can be detected or amplified by targeting a variety of sequences. The choice of target is the key factor in determining the specificity of the assay for fungal organisms. Targets can either be pan-fungal, which allow for a broader range of organisms detected with the same method, or they can be genus- or species-specific. In each case, multi-copy genes are desirable targets as they improve the sensitivity of the assay by providing a larger volume of nucleic-acid template per specimen. Some examples of potential targets include the ribosomal RNA (rRNA) gene cluster, which contains both highly conserved (18S and 28S) and variable (ITS and D1/D2) regions, as well as genus-specific targets such as the Aspergillus mitochondrial and alkaline-proteinase genes. A more detailed discussion of Aspergillus-specific direct-detection techniques is included later in this section of the chapter.

Once the target has been detected or amplified, identification can be accomplished using a variety of methods, of which sequencing is the most commonly used. Combining amplification of nucleic acids with mass-spectrometry detection of amplified material is also possible with electro-spray ionization (208). When using pan-fungal approaches, sequence reads must then be compared to a database of known organisms to determine genus- and species-level identifications. Unfortunately, publicly available databases available for fungal organisms often contain erroneous entries. Therefore, when performing a sequence analysis, appropriate and thorough adjudication of references is required before identification can be confirmed.

Aspergillus-specific molecular-detection techniques
While laboratory-developed assays have been developed for endemic mycoses (209) and Mucorales (210), molecular methods targeting an individual genus have been most widely studied for Aspergillus infections. In a meta-analysis completed by Mengoli et al., 16 studies completed between the years of 2000 and 2008, including greater than 10,000 samples from 1,618 at-risk patients, were reviewed (197). Overall, performing a single PCR targeted to Aspergillus had a sensitivity of 88% and specificity of 75% for proven/probable IA; specificity was increased to 87% when two consecutive PCR tests were performed. While this is encouraging, this meta-analysis emphasized the lack of standardization between studies, with varying assay characteristics. Therefore, much work is needed before such molecular methods are optimized and more universally utilized by clinical laboratories.

Contributing to this goal, the performance of molecular techniques using different fractions of blood has been compared to determine the optimal specimen type for IA surveillance testing by PCR. Using whole blood and plasma specimens spiked with Aspergillus conidia, Loeffler et al. found that both sources resulted in identical limits of detection (10 CFU/ml and 100 fg DNA) (198). Using samples from patients with IA, however, the authors found that approximately half of the positive samples were only detected using whole-blood, not plasma, samples (198). Similarly, Springer et al. demonstrated an increased sensitivity for identifying cases of IA using detection of Aspergillus nucleic acid in whole-blood versus serum specimens. Furthermore, because specimens were tested serially as part of a surveillance program, the authors also reported a more rapid detection of fungal nucleic acid in whole blood (15 days) versus serum (36 days). Specificity for a diagnosis of IA, however, was decreased using whole-blood specimens (211). The European Aspergillus PCR Initiative (EAPCRI) also performed a multicenter comparison of existing PCR protocols used to detect Aspergillus nucleic acid in whole-blood specimens (212). From this review, researchers found that the volume of specimen tested (≥3 ml), the use of bead-beating for mechanical disruption of fungal cell walls, and the inclusion of an internal control were all positively associated with assay sensitivity. In contrast, there was a negative association with elution volumes greater than 100 μl (212). As a result of this research, the EAPCRI has proposed a standardized whole-blood fungal DNA-extraction protocol (213).

Proteomic methods
Mass spectrometry (MS) is an increasingly powerful tool for the identification of fungi. MALDI-TOF involves laser ionization of proteins directly from culture
growth with MS detection of the ionized particle. Identification of fungi in this manner is rapid and potentially economical relative to genomic sequence-based technologies, after purchase of an instrument. Current commercial MALDI-TOF MS reference databases, however, contain a limited number of filamentous fungal spectra. Significant augmentation of the spectral library is required for routine use in the clinical laboratory (214) and is particularly important for reaching a strong consensus between proteomic and sequence-based identifications for filamentous fungi. Without the construction of a highly stringent supplemental database, MALDI-TOF MS analysis is often unable to achieve adequate resolution for species, and sometimes even genus, level identification compared to that of multilocus sequencing (214). More recently, gas chromatography-MS has been used for the detection of volatile organic compounds from the breath of patients with suspected IPA (215). How these rapidly evolving technologies will integrate into current practice remains to be seen.

**Radiologic Technologies**

Imaging techniques including computed tomography (CT) and magnetic-resonance imaging (MRI) are important tools for the early diagnosis of IFD. CT is more sensitive for the detection of lung nodules than plain radiographs (216). No radiologic feature is pathognomonic for fungal infection and imaging is neither genus- nor species-specific. However, certain findings are suggestive of invasive mycoses. A dense nodule surrounded by ground-glass opacity characterizes the classic “halo sign.” A series of 235 patients with probable or confirmed IPA demonstrated the presence of a nodule ≥1 cm in 95% of cases, a halo sign in 61%, an inacrt-shaped nodule in 27%, and a cavity in 20%. Because radiographic changes may be detectable prior to onset of symptoms, pulmonary nodules suggestive of mold infection may be found on studies done for screening or discovered incidentally during cancer staging. Initiating antifungal therapy based on presence of a typical nodule rather than waiting for microbiologic confirmation has demonstrated mortality benefit (217). Although the halo sign is the earliest radiological manifestation of IPA, it is transitory and may be missed by symptom-driven testing (218). As a result, many centers integrate protocol-driven chest CTs into their fungal-surveillance strategies; examples of a prompt for use of chest CT include an abnormal chest X-ray, elevated fungal biomarkers, symptom of cough, or fever despite antibiotic therapy.

**ANTIFUNGAL AGENTS AND SUSCEPTIBILITY TESTING**

Antifungal-susceptibility testing for filamentous fungi has accrued substantial interest as the incidence of IFDs and the number of available antifungal agents have increased. A general knowledge of the available antifungal agents and their spectrum of activity is important for the clinical microbiologist.

**Mold-Active Antifungal Agents**

There are currently three classes of antifungal drugs with activity against the filamentous fungi discussed in this chapter; these include the polyenes, the triazoles, and the echinocandins. Their mechanisms of action and spectra of activity are described briefly in this section.

**Polyenes**

The era of systemic antifungal therapy began in 1958 with the introduction of amphotericin B deoxycholate (AmB). The polyene class of drugs now also includes three lipid-associated formulations (amphotericin B lipid complex [ABLC], liposomal amphotericin B [L-AmB], and amphotericin B colloidal dispersion [ABCD]). The lipid preparations were developed in an attempt to improve the therapeutic index of AmB and became available between the years of 1995 to 1999. Polyenes work by targeting the sterol component (ergosterol) of fungal plasma membranes, which differs from cholesterol found in mammalian plasma membranes. It has been hypothesized that 8–10 molecules of drug bind to form a pore within the fungal lipid bilayer, thus promoting spillage of potassium ions and disruption of the cellular proton gradient. In addition to the cell-membrane effects, polyenes are also thought to induce oxidative damage in fungal cells (219).

The amphotericin formulations have a broad-spectrum of fungicidal activity (Table 3). However, there are also a variety of opportunistic fungi with intrinsically elevated amphotericin minimal-inhibitory concentrations (MICs) (220). The clinical laboratory should alert clinicians to the possibility of amphotericin non-susceptibility when reporting significant isolates of S. prolificans and S. apiospermum (teleomorph P. boydii), P. lilacinum, Fusarium spp. (especially F. solani), A. terreus, Acremonium spp., and some of the dematiaceous fungi (221).

**Triazoles**

The triazole class of antifungal drugs acts by interrupting sterol biosynthesis, a multistep process involved in the conversion of lanosterol to ergosterol. Specifically, azoles inhibit lanosterol 14α-demethylase (P45014dm),
a cytchrome P450-dependent enzyme containing a heme moiety in its active site. Azole compounds bind to the iron atom within the P450_{450} heme group through an unhindered nitrogen in the azole ring. The azole-heme complex then prevents demethylation of lanosterol, which is required for ergosterol formation. The resultant ergosterol depletion in conjunction with accumulation of lanosterol and other methylated sterol precursors interferes with fungal-membrane structure and function.

The newer azole compounds (voriconazole [2002], posaconazole [2006], and isavuconazole [2014]) have an extended spectrum of antifungal activity including activity against many filamentous fungi (Table 3). These drugs are generally fungistatic against yeast, but may be fungicidal against certain mold strains (222, 223). Voriconazole has activity against Aspergillus, some Fusarium and Scedosporium isolates, as well as the dimorphic fungi (224). In addition to these organisms, posaconazole and isavuconazole also have activity against many of the Mucorales (225).

**Echinocandins**

The current generation of echinocandins includes caspofungin (2001), micafungin (2005), and anidulafungin (2006). These compounds are non-competitive inhibitors of (1, 3)-β-D-glucan synthase, an enzyme involved in the production of glucan polymers in the fungal cell wall. The degree of (1, 3)-β-D-glucan polymerization in the fungal cell wall and the expression of the glucan-synthase enzyme target largely define the spectrum of this antifungal class. The echinocandins are generally considered to have fungistatic activity against Aspergillus spp., but are fungicidal against Candida; they do not have activity against Mucorales or the endemic fungi (Table 3).

**In Vitro Susceptibility Testing**

The internationally recognized reference method for mold antifungal-susceptibility testing is broth dilution (BD), a technique that involves serial two-fold dilutions of an antifungal drug in a liquid medium that is inoculated with a standardized number of conidia and incubated for a prescribed period of time. Standardized BD methods for mold have been established by the Clinical and Laboratory Standards Institute (CLSI) (221) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (226). The two approaches are similar in that both use RPMI 1640 broth as the base medium, have similar incubation durations, and a prominent inhibition endpoint. Results are reported as a MIC or a minimum effective concentration (MEC) for the echinocandins only. The MEC is defined as the lowest drug concentration that results in macroscopic changes in vitro and the glucose content of the medium.

Currently, there are no data to correlate mold MICs with treatment outcome; as a result, clinical interpretive breakpoints (CBPs) for in vitro mold-susceptibility testing have not been established. MIC determinations may still be clinically useful, especially when interpreted in the context of an individual patient and the pharmacologic properties of a given drug.

In the absence of CBPs, epidemiological-cutoff values (ECVs) can be used for differentiating organisms with decreased antimicrobial susceptibility (227). ECVs may be useful for identifying isolates that are less likely to respond to treatment due to acquired-resistance mechanisms as well as for following in vitro-susceptibility trends over time. The ECV is defined as an MIC cut-off

![Table 3](https://example.com/table3.png)

**Table 3** Summary of possible in vitro antifungal susceptibility profiles for selected opportunistic filamentous fungi

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>ITRA</th>
<th>VORI</th>
<th>POSA</th>
<th>ISA</th>
<th>AMB</th>
<th>Echinocandins</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acremonium</em> spp.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>ND</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td><em>Fusarium</em> spp.</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Gibberella fujikuroi</em></td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td><em>Purpureocillium lilacinum</em></td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td><em>Paecilomyces</em> spp.</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. variotii</em></td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Scedosporium apiospermum</em></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td><em>S. prolificans</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td><em>Scopulariopsis brevicaulis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

*Refers to the species complex; ITRA, itraconazole; VORI, voriconazole; POSA, posaconazole; ISA, isavuconazole; AMB, amphotericin formulations; ND, not determined.*
value that discriminates wild-type (WT) isolates from non-WT strains (i.e., organisms harboring mutational- or acquired-resistance mechanisms) (228). The ECV takes into account the WT MIC distribution, modal MIC, and the inherent variability of the susceptibility-test method (±1 doubling dilution). The MIC distribution for WT organisms typically covers three to five doubling dilutions surrounding the modal MIC (229, 230). For most MIC distributions, the ECV occurs at a threshold of approximately two dilutions above the modal MIC and encompasses ≥95% of the WT MIC distribution (230).

ECVs and WT MIC distributions have been established for the most clinically relevant Aspergillus spp. and Mucorales. Ninety-five percent ECVs expressed in μg/ml with the percentage of isolates for which MIC values were equal to or greater than the ECV are summarized in Table 4. Although the ECVs do not necessarily predict treatment outcome, they may aid in the detection of drug resistance.

Alternative susceptibility methods
Alternative methods for predicting antifungal susceptibilities are being investigated. MALDI-TOF MS is able to detect changes in protein expression in the presence of antifungal agents and may lead to more rapid interpretation of phenotypic-based techniques (231). Furthermore, as the molecular basis for drug resistance is becoming better understood for fungal organisms, nucleic acid-amplification techniques (NAAT) may provide more rapid susceptibility information compared to traditional phenotypic methods. For example, azole resistance for Aspergillus spp. is often caused by mutations in Cyp51a leading to a mutated enzyme involved in ergosterol synthesis (232). NAAT assays designed to detect this mutation have been developed, but are not widely available.

Prevention and Management of Fungal Disease in Immunocompromised Hosts

An understanding of the epidemiology of IFDs affecting immunocompromised patients, risk factors for the development of infection, and approaches to diagnosis greatly facilitate early initiation of pathogen-specific therapy. Prompt diagnosis of IFD is essential because treatment delays are associated with increased morbidity, mortality, and hospital costs. In the highest-risk patient groups, prevention of IFD with the use of antifungal prophylaxis or serial-biomarker surveillance as a guide for preemptive therapy may be preferred. Selection of the optimal management strategy should be based on the patient population, local microbiology, and availability of laboratory-test results in conjunction with the pharmacologic properties (e.g., spectrum of activity, pharmacokinetics/dynamics, safety, and efficacy) of currently available antifungal agents.

Primary Antifungal Prophylaxis
Primary prophylaxis involves administration of an antifungal drug to an at-risk patient during a defined period of risk (e.g., during chemotherapy-induced neutropenia or periods of treatment for GvHD). Mold-active antifungal prophylaxis is typically reserved for the highest-risk HM and HSCT patients, lung-transplant recipients, and/or individuals colonized with filamentous fungi headed into immunosuppressive procedures. Posaconazole and micafungin have FDA-approved indications for prophylaxis in selected immunocompromised populations (233, 234), but other echinocandins or voriconazole may be used off-label on an institution-specific basis. From a microbiologic perspective, breakthrough IFDs that occur in patients receiving antifungal prophylaxis tend to be with organisms that are either intrinsically resistant to the prophylactic agent or, more rarely, have developed secondary resistance as a result of prolonged drug exposure.

Preemptive Antifungal Therapy
Preemptive approaches involve serial surveillance of at-risk patients using one or multiple of the biomarker tests described in Laboratory Tests, this chapter. Treatment is initiated when patients develop a positive test. For preemptive surveillance to be of benefit, the disease should not be so common that most patients eventually develop a positive test, in which case prophylaxis might be preferred, the disease prevalence should not be so low that testing is a wasted expense for patients unlikely to develop IFD, and the results of testing must be available to clinicians in a meaningful time frame (i.e., 48–72-hour turnaround time).

Preemptive approaches have established clinical utility in the HM and HSCT settings. Maertens et al. were the first to show that serial serum-GM testing combined with protocol-driven CT scans lead to a 78% reduction (from 35% to 8%) in the use of antifungals among 41 neutropenic patients who would otherwise have qualified for empirical antifungal treatment based on persistent or recurrent fever, without compromising outcomes (161). A second study of 188 allo-HSCT recipients compared PCR-based preemptive therapy to empiric therapy
based on refractory febrile neutropenia (235). Survival curves showed better 30-day survival when close PCR monitoring was performed (mortality 1.5% vs 6.3%; \( P = 0.015 \)), but there was no mortality difference at post-transplant day 100.

Recent clinical trials also suggest that combining GM with Aspergillus DNA detection in blood improves the diagnostic accuracy of surveillance testing by increasing sensitivity without affecting specificity (211, 236). An open-label, randomized study involving 240 adult

### TABLE 4 Epidemiologic cut-off values determined by the CLSI M38-A2 broth microdilution method for Aspergillus and Mucorales species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal</th>
<th>MIC or MEC μg/ml</th>
<th>Range</th>
<th>Mode</th>
<th>ECV (% non-WT isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Itraconazole</td>
<td>≤0.03–2</td>
<td>0.5</td>
<td>1 (2.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.03–≤4</td>
<td>0.25</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>≤0.015–4</td>
<td>0.06</td>
<td>0.5 (2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.06–8</td>
<td>0.5</td>
<td>1 (5.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.016–32</td>
<td>0.25</td>
<td>0.5 (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>3.03–16</td>
<td>0.5</td>
<td>2 (0.3)</td>
<td></td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>Itraconazole</td>
<td>0.03–2</td>
<td>0.5</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.06–4</td>
<td>0.5</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>≤0.03–2</td>
<td>0.06</td>
<td>0.25 (5.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.06–2</td>
<td>0.5</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.016–≥32</td>
<td>0.06</td>
<td>0.25 (2.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.032–8</td>
<td>1</td>
<td>2 (2.6)</td>
<td></td>
</tr>
<tr>
<td><em>A. nidulans</em></td>
<td>Itraconazole</td>
<td>0.03–2</td>
<td>1</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.03–≤4</td>
<td>2</td>
<td>2 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>≤0.03–2</td>
<td>0.5</td>
<td>1 (2.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.06–1</td>
<td>0.12</td>
<td>0.25 (27.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.032–16</td>
<td>0.15</td>
<td>0.5 (14.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.06–32</td>
<td>1</td>
<td>4 (1.1)</td>
<td></td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Itraconazole</td>
<td>0.03–2</td>
<td>1</td>
<td>2 (8.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>≤0.03–4</td>
<td>0.5</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>≤0.03–2</td>
<td>0.5</td>
<td>0.5 (5.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.06–8</td>
<td>1</td>
<td>4 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.016–2</td>
<td>0.06</td>
<td>0.25 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.03–2</td>
<td>0.5</td>
<td>2 (0)</td>
<td></td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>Itraconazole</td>
<td>0.03–1</td>
<td>0.25</td>
<td>1 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.03–≤4</td>
<td>0.5</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>≤0.03–2</td>
<td>0.25</td>
<td>0.5 (2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.06–2</td>
<td>0.25</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.016–2</td>
<td>0.06</td>
<td>0.25 (8.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.12–32</td>
<td>2</td>
<td>4 (3.9)</td>
<td></td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>Itraconazole</td>
<td>0.03–2</td>
<td>2</td>
<td>2 (5.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.03–≤4</td>
<td>2</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>0.03–4</td>
<td>1</td>
<td>1 (13.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isavuconazole</td>
<td>0.05–≤8</td>
<td>0.25</td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.032–2</td>
<td>0.12</td>
<td>0.25 (6.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.032–8</td>
<td>1</td>
<td>2 (8.1)</td>
<td></td>
</tr>
<tr>
<td><em>Lichtheimia corymbifera</em></td>
<td>Itraconazole</td>
<td>0.06–8</td>
<td>0.25</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>0.06–4</td>
<td>0.5</td>
<td>1 (1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.06–16</td>
<td>0.5</td>
<td>1 (2.9)</td>
<td></td>
</tr>
<tr>
<td><em>Mucor circinelloides</em></td>
<td>Itraconazole</td>
<td>0.25–16</td>
<td>4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>0.06–16</td>
<td>1</td>
<td>4 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.03–4</td>
<td>0.25</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus arrhizus</em></td>
<td>Itraconazole</td>
<td>0.06–16</td>
<td>0.5</td>
<td>2 (5.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>0.03–32</td>
<td>0.5</td>
<td>2 (10.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.03–4</td>
<td>1</td>
<td>2 (1.2)</td>
<td></td>
</tr>
<tr>
<td><em>R. microsporus</em></td>
<td>Itraconazole</td>
<td>0.25–32</td>
<td>1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posaconazole</td>
<td>0.06–16</td>
<td>0.5</td>
<td>1 (5.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin</td>
<td>0.06–4</td>
<td>0.5</td>
<td>2 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

1Adapted from Espinel-Ingroff et al. (263–267).

2MIC, minimum inhibitory concentration; MEC, minimum effective concentration; ECV, epidemiologic cutoff values; WT, wild-type; ND, not determined.

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neutropenic HM and HSCT patients compared a biomarker-based preemptive strategy (GM plus PCR) to empiric antifungal therapy driven by clinical symptoms, culture, and histology (237). Overall, the biomarker group received less antifungal treatment than the empiric-therapy group (difference 17%, 95% confidence interval [CI] 4–26; \( P = 0.002 \)), with no differences in the incidence of histologically proven IA, all-cause mortality, or *Aspergillus*-attributable mortality between groups. The greatest benefit was observed in patients receiving antifungal prophylaxis targeting yeast only or no antifungal prophylaxis. A second randomized study compared GM surveillance alone to combination GM-PCR testing. Combined testing was associated with an earlier diagnosis and a lower incidence of IA in high-risk hematological patients receiving fluconazole prophylaxis (172).

Taken together, these studies suggest that preemptive therapy is an acceptable alternative to empiric treatment of IFD, especially for patients already receiving antifungal prophylaxis. A second randomized study compared GM surveillance alone to combination GM-PCR testing. Combined testing was associated with an earlier diagnosis and a lower incidence of IA in high-risk hematological patients receiving fluconazole prophylaxis (172).

**Empiric Antifungal Therapy**

The risk of death from IFD in immunocompromised patients remains unacceptably high, largely because of difficulties and delays in making a definitive diagnosis by culture and histology (59, 238–244). As a result, antifungal therapy is routinely administered to neutropenic patients suspected of having an IFD based on persistent fevers despite treatment with broad-spectrum antibacterial drugs for three to five days (245). This approach, however, has never been definitively shown to confer a survival benefit, likely because fever is an insensitive and nonspecific surrogate marker for IFD (246). Furthermore, approximately 22% to 34% of neutropenic patients with cancer will receive an antifungal drug by these criteria, yet only 4% have a demonstrated invasive-fungal infection (243). The drawbacks of empiric therapy therefore include overtreatment of many patients without an IFD with expensive antifungals, the potential for drug-drug interactions, and the emergence of antifungal resistance. There is an ongoing need for additional nonculture-based fungal diagnostics, especially in the setting of febrile neutropenia.

**Targeted Therapy**

Making a genus or, ideally, species-level diagnosis is essential for guiding targeted-antifungal therapy. Although CBPs have not been established for any of the medically important filamentous fungi, susceptibility testing may be useful when the infecting organism has an unpredictable susceptibility profile, when the patient history includes prolonged prior exposure to antifungal drugs, and/or when the infection is refractory to standard therapy. In general, voriconazole is the treatment of choice for IA (247) and may be useful for treating fusariosis (248) as well as scedosporiosis (249). Amphotericin formulations remain the treatment of choice for mucormycosis, often in combination with surgical debridement and reductions in immunosuppression when possible (250). Isavuconazole was recently approved by the FDA in September 2014 for the treatment of IA and mucormycosis, but at the time of this review, the drug is not yet clinically available. Posaconazole has primarily been studied as salvage treatment for IA and mucormycosis in patients who are refractory or intolerant of other agents (251–253). Lastly, Infectious Diseases Society of America guidelines exist for the treatment of the various endemic mycoses (19, 254, 255).

**Hospital Environmental Monitoring**

In addition to recreational, occupational, and environmental exposures, IFDs may also be nosocomially acquired. A large review concluded that most *Aspergillus* outbreaks resulted from air contamination due to ongoing hospital construction (256). The lack of high-efficiency particulate-air (HEPA) filters in patient rooms (257) and potted plants and shower heads have also been linked to cases of IA that develop in the hospital (258, 259). Rammert et al. reviewed published cases of health-care-associated mucormycosis (260). A total of 169 cases were identified between 1970 and 2008. Prolonged steroid therapy, SOT, DM, and severe prematurity were the most common comorbidities identified and skin was the most common site of infection (57%). Outbreaks and clusters were related to adhesive bandages, wooden tongue depressors, ostomy bags, water-circuitry damage, and adjacent building construction. Therefore, while routine fungal cultures of hospital-environmental samples are not currently recommended, clinical laboratories should be prepared to partner with infection preventionists in order to identify and limit potential outbreaks under conditions of higher risk, such as building construction or renovation (261).

**SUMMARY**

Fungi are ubiquitous in the environment, and exposure through contact with air, soil, and water is unavoidable.
The host immune response plays an essential role in determining the outcome of human exposure to potential fungal pathogens. Depending on the effectiveness of immune mechanisms, fungi can either be cleared or infection can progress to a potentially fatal invasive disease. Patient populations with impaired granulocyte and T-cell function are at higher risk for invasive disease. Laboratory-diagnostic methods including culture, nucleic acid and antigen detection, and some antibody-based tests provide vital information to the clinicians that helps determine the optimal-management strategies. Despite these resources and tools, however, the morbidity and mortality associated with IFD remains unacceptably high. Continued development of more-rapid diagnostic methods, with improved negative- and positive-predictive values, is essential to improve outcomes related to invasive fungal infections in the immunocompromised host.

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