Human fungal infections cause a range of diseases, from common, mild superficial infections to serious life-threatening diseases. Healthy individuals are not normally infected by environmental fungi, but for immunocompromised patients such infections can be severe. Serious infection due to the yeast *Candida* is becoming increasingly prevalent. A range of isolating media and detection methods for diagnosing yeasts and moulds in clinical samples are described in this article. Differential diagnosis of these micro-organisms is important because they exhibit such varied susceptibility to antifungal agents.

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<th>Description</th>
<th>Species</th>
<th>Clinical Manifestation</th>
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<td>Yeasts</td>
<td><em>Candida albicans</em>&lt;br&gt; <em>Candida spp.</em></td>
<td>Infections of the skin and mucosa in immuno-compromised patients can cause pneumonia, septicaemia and endocarditis&lt;br&gt; Meningitis and pulmonary disease</td>
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<tr>
<td>Superficial Mycoses</td>
<td><em>Dematophytes</em> (Keratinophilic fungi)&lt;br&gt; <em>Trichophyton spp.</em>&lt;br&gt; <em>Microsporum spp.</em>&lt;br&gt; <em>Epidermophyton spp.</em></td>
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<td>Filamentous Fungi</td>
<td><em>Soil-borne fungi</em>&lt;br&gt; <em>Zygomycosis spp.</em>&lt;br&gt; <em>Aspergillosis spp.</em></td>
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<td>Chronic pulmonary and skin infections&lt;br&gt; Systemic infections&lt;br&gt; Acute pulmonary infections&lt;br&gt; Infections of skin, mucosa and pulmonary tract&lt;br&gt; Chronic cutaneous and subcutaneous infections including ‘Rose Gowers Disease’</td>
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Table 1. Summary of the different types of fungal infection [2].

Unlike the toxigenic fungi, which cause problems in foodborne illness, pathogenic fungi do not produce toxins, but they do show physiological modifications during an infection (e.g. increased metabolic rate, modified metabolic pathways and modified cell wall structure). They are also capable of withstanding many host defences.

The establishment of a mycotic infection usually depends on the size of the inoculum and the resistance of the host. The severity of the infection appears to mostly depend on the immunological state of the host. For example, the demonstration of fungi in blood drawn from an intravenous catheter can be due to colonisation of the catheter, to transient fungaemia (i.e. fungi in the blood stream) or to a true infection.

**Yeasts**

Yeasts are by far the most common fungi isolated from patients. In general it is recommended that yeasts should be identified if they come from normally sterile body fluids, including cerebrospinal fluid, blood, urine and peritoneal fluid, or from seriously ill or immuno-compromised patients or patients where a mycotic infection is suspected. Yeasts recovered in large amounts from any clinical source should also be identified, as should yeasts recovered from several successive specimens, with the exception of respiratory secretions. Yeasts isolated from respiratory secretions do not need to be routinely identified; however, they should be screened for the presence of *Cryptococcus neoformans*. *Candida* species are responsible for the most frequently encountered opportunistic fungal infections, with *C. albicans* the most frequent aetiological agent.

Hospital-acquired (nosocomial) infections are increasingly caused by *Candida* species. These opportunistic pathogens are often isolated from critically ill patients on intensive care units (ICUs), e.g. patients receiving broad-spectrum antimicrobial therapy. This increase started in the late 1980s and was associated with the increase in immuno-compromised or severely ill individuals as the result of the spread of Human Immunodeficiency Virus (HIV), the increased use of immunosuppressive agents in association with organ transplants, chemotherapy, and improved life-saving medical techniques necessitating indwelling catheters [3].

However, advances made during the 1990s led to the introduction of new antifungal agents, with 15 different drugs marketed worldwide. Azoles are currently the most widely used of these [3]. Azole antifungal agents prevent the synthesis of ergosterol, a major component of fungal plasma membranes. This disrupts both the structure of the membrane and several of its functions such as nutrient transport and chain synthesis. The net effect is to inhibit fungal growth. The azole antifungal agents in clinical use are classified as imidazoles (e.g. ketoconazole, miconazole, Figure 1. Isolation and identification of *Aspergillus* species.)
Fungal Infections

A recent review of 1,591 cases of candidal infections found the non-albicans Candida species to be causative agents in 46% of systemic infections [4].

- **C. tropicalis** 25%
- **C. glabrata** 8%
- **C. parapsilosis** 7%
- **C. krusei** 4%

Patients with leukaemia are most likely to be infected with either *C. albicans* or *C. tropicalis*, while bone marrow transplant recipients are more likely to be infected with yeasts that are resistant to antifungal agents, i.e., *C. krusei*, *C. lusitaniae* and *C. glabrata* [4]. Rapid identification of the Candida species causing the infection is therefore critical for determining the correct antifungal agent.

Macroscopic and microscopic detection of yeasts and moulds in clinical samples

The appropriate examination of a clinical specimen is essential prior to proper processing of the material. Additionally, examination will often aid the technician and physician in a preliminary identification, either ruling in or out certain pathogenic yeasts and fungi. Certain staining methods are universal to the preliminary observation of fungi in a specimen e.g. Gram stain, calcifluor and the use of 30% potassium hydroxide.

However, others such as India ink preparation for the demonstration of a capsule, are used for yeasts and yeast-like fungi only. This preparation is carried out with cultures, and on specimens of urine, cerebrospinal fluid etc., that have been centrifuged.

During microscopic examination of a specimen for yeasts, a range of morphological features that aid in identification should be considered. These include the size and shape of the organism, the mode of attachment of any buds, the presence or absence of a capsule, the thickness of the cell wall and whether pseudohyphae, true hyphae or arthroconidia are present. It is also important to consider the purity of the culture, and any unusual structures should also be noted.

Isolation media for Candida species

Rapid identification of micro-organisms in general has been shown to have a major impact on the morbidity and mortality of patients, and the duration of their hospitalisation. For Candida species involved in bloodstream infections on ICUs, it was shown by Ibrahim et al. [5] that initial therapy was inadequate in 95% of cases because no antifungal agent was administered. Due to the inadequacy of the initial treatment, a mortality rate of about 60% was observed in the patient group with *Candida* infections. Hence early recognition of a *Candida* infection can aid in the selection of appropriate treatment.

Combined with rapid identification of the causative organism, this treatment could be optimised to include a non-azole group anti-fungal agent, if required, at an early stage of the infection. The direct microscopic examination of clinical specimens containing *Candida* will reveal budding yeast cells (blastoconidia) 2 to 4 μm in diameter and/or pseudohyphae showing regular points of constriction, or true septate hyphae. The blastoconidia, hyphae, and pseudohyphae are strongly Gram-positive.

**BIGG AGAR (Bismuth sulphate glucose glycerine yeast agar)**

This medium is based on the formulation developed by Nickerson [6] and may be used for the isolation and presumptive identification of *Candida* species, particularly *C. albicans* and *C. tropicalis* from sputum and vaginal smears. The medium differentiates *Candida* species based on their ability to reduce a bismuthyl hydroxy polysulphite to bismuth sulphide, which results in black colonies. However, strong reducing ability is confined to *C. tropicalis*. Variations in results from other species are caused by the medium (the nature of which are incorporated in their enzyme patterns).

![Table 2. Typical enzyme patterns and colour reactions on oxoid chromogenic Candida agar.](image)

**Oxoid chromogenic Candida medium**

Oxoid Chromogenic *Candida* agar (OCCA) is a selective differential medium for the rapid identification of several clinically important *Candida* species. The medium incorporates two chromogenes that indicate the presence of the target enzyme.

- X-NAG (5-bromo-4-chloro-3-indolyl-N-acetyl-D-glucosaminide) which detects the activity of neuramidinase.
- BCP (5-bromo-6-chloro-3-indolyl phosphate-p-sulfonic acid salt) which detects alkaline phosphatase activity.

The typical enzyme patterns of *Candida* species are shown in Table 2. An opaque agent is also incorporated into the formulation to improve the colour definition on the agar. This modification of Sabouraud agar is suitable for the cultivation and differentiation of *Candida* species. The medium is often used with added antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

**Sabouraud maltose agar fungal isolation media**

This medium differs from Sabouraud dextrose agar, only in that carbohydrate is incorporated. Sabouraud maltose agar may be used with or without added antibiotics, where a maltose-based medium is preferred.

**Dermao agar fungal isolation media**

This is a selective medium for dermatophyte fungi recommended for the examination of hair, skin scrapings, nails, etc. Emmons [8] suggested that media for growth of dermatophytes should have a pH of 6.8-7.0, as a near neutral pH is better for the growth of some fungi. The pH 7.0 used in other media to suppress bacterial contaminants can be replaced by cycloheximide and chloramphenicol, which inhibit the growth of saprophytic fungi, yeasts and bacterial skin flora [9].

**References**

1. PHL Fungal Infections Homepage

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