Histoplasmosis, Blastomycosis, Coccidioidomycosis, and Cryptococcosis in the Lung

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Case Presentation

A 62-year-old man presents to clinic with a 4-month history of persistent malaise, shortness of breath, and nonproductive cough. He has a past medical history of psoriatic arthritis for which he is taking a monoclonal antibody immunosuppressive medication. He states that he has felt this way since an extended trip that included visits to Arizona, Mississippi, and Texas. A CT scan shows a 2.2 cm peripheral lung nodule in the right upper lung that has been slowly increasing in size over the past 3 months (Fig. 52.1). Serologic tests are equivocal, and he agrees to a biopsy of the pulmonary nodule. Histologic sections show a nodule consisting predominantly of caseating necrosis with rare scattered poorly staining spherules and degenerated forms that are highlighted by a GMS stain (Fig. 52.2). These forms appear to range markedly in size from <10 μm to approximately 100 μm. Rare spherules contain many small endospores, while smaller spherules do not contain these structures (Fig. 52.2). A histologic diagnosis of coccidioidomycosis is made, and the patient is prescribed a 3-month course of fluconazole.

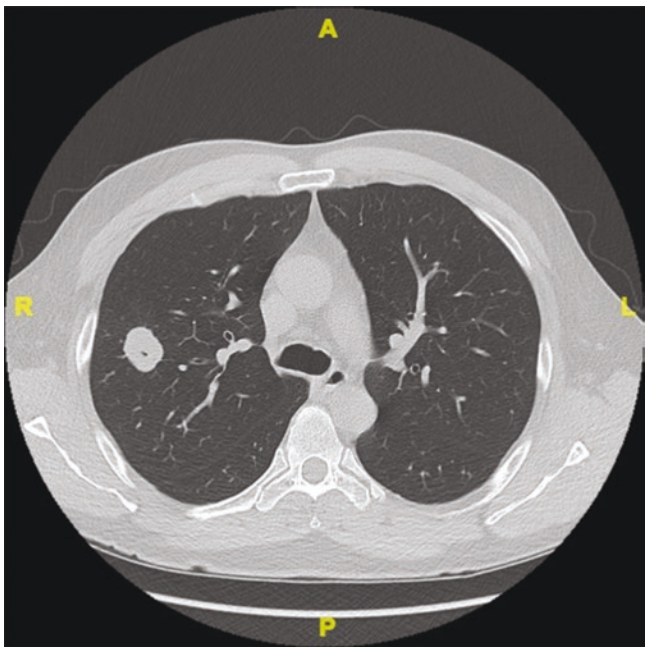

Fig. 52.1 2.2 cm lung nodule with 0.3 cm of cavitation and bronchiectasis in the right upper lobe. Focal hilar lymphadenopathy was also identified; no other masses or consolidations were noted

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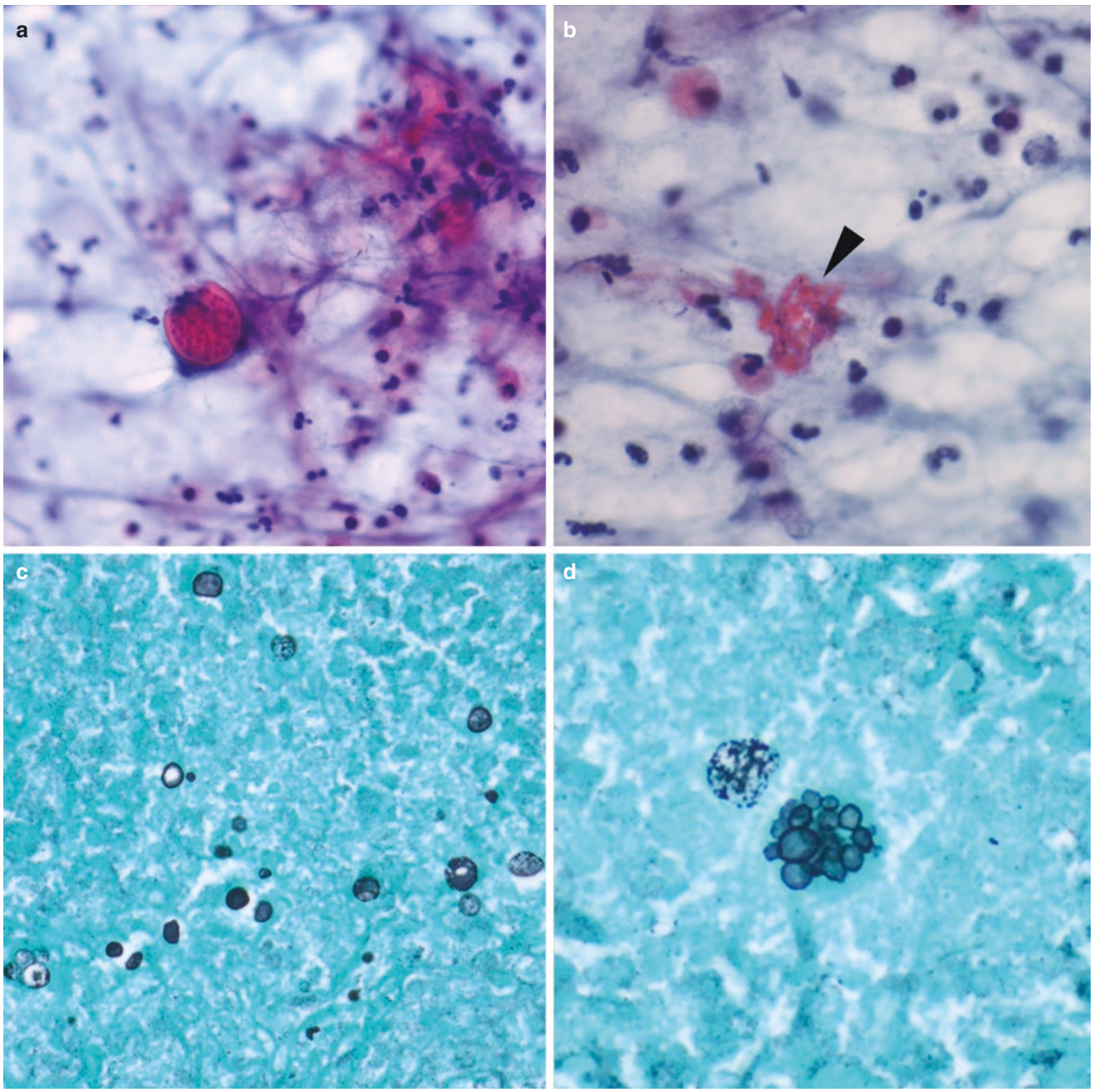

Fig. 52.2 Morphologic variation in coccidioidomycosis. (**a**) Mature spherules of *Coccidioides* spp. containing visible endospores from a Papanicolaou-stained FNA aspirate (400×). (**b**) Free endospores (arrowhead) remain after spherule rupture (Papanicolaou-stained, 400×). (**c**) Immature *Coccidioides* spp. spherules stained with GMS have variable size and shape; endospores are not observed (200×). (**d**) Maturing *Coccidioides* spp. spherules stained by GMS with a degrading spherule in the background (400×)

Final Pathologic Diagnosis: Coccidioidomycosis of the Lung

Clinical Considerations

Histoplasma capsulatum, *Blastomyces dermatitidis*, and *Coccidioides immitis/posadasii* are frequently encountered pulmonary pathogens in immunocompetent hosts. *Cryptococcus neoformans* is more frequently encountered in immunocompromised patients such as those with HIV/AIDS, organ transplant, and prolonged courses of immunosuppressive medications. However, *C. gattii* has been shown to cause clinically severe infections in immunocompetent hosts [1, 2]. While all are environmental soil fungi, the geographic distribution varies. *Histoplasma* is most frequently found in the distribution of the Ohio and Mississippi rivers [3]; *Blastomyces* shares these river valleys but also extends throughout the Great Lakes, St. Lawrence River, and parts of Canada [4]. *Coccidioides* is an endemic pathogen in the southwestern United States, Mexico, parts of Central and South America [5], and arid regions of the northwestern United States [6]. *Cryptococcus neoformans* has a global distribution [7], while *C. gattii* has a more limited distribution with outbreak- [1] and non-outbreak-related infections occurring in the Western and Northwestern regions of North America [2], as well as tropical and subtropical regions [8].

These mycoses all have a wide range of associated clinical severity. Histoplasmosis is most frequently asymptomatic and self-limiting [9] but can produce an acute presentation, a chronic infection akin to mycobacterial disease, or disseminated disease. A similar range of severity and time course exists for blastomycosis, which can have a varied clinical presentation and frequently presents with concurrent skin involvement [10, 11]. *Coccidioides* most frequently presents as a flu-like illness but, like the others, can present with more chronic, fibrocavitary, or disseminated disease [12]. Unlike *Blastomyces* which produces disseminated disease in 20–50% of patients [13, 14], dissemination occurs in ~1% of cases of coccidioidomycosis, and patients of African and Pacific Islander descent are at increased risk [15, 16]. *Cryptococcus* most frequently has a subacute or chronic clinical presentation but has been shown to asymptomatically colonize the respiratory tract in hosts with prior pulmonary disease [17] and can present as disseminated disease in profoundly immunocompromised patients. The most common and feared clinical complication of cryptococcosis is cerebromeningitis. In all of these mycoses, the manner of clinical presentation alone is generally insufficient to rule out any given fungus. However, an appropriate travel history, immunocompetency status, and certain symptoms (e.g., neurological involvement for *Cryptococcus* and skin involvement for *Blastomycosis*) can markedly influence the differential diagnosis.

Serologic testing for *Coccidioides* usually begins with enzyme immunoassays and are confirmed by immunodiffusion and complement fixation, titers of which track with disease states [18]. Antigenic testing for *Cryptococcus* is available in serum and CSF and is an important adjuvant test, particularly to evaluate for cryptococcal cerebromeningitis [19]. Antigenic tests are available for *Coccidioides*, *Blastomyces*, and *Histoplasma*, but antigens are cross-reactive across these and other yeasts [20, 21].

Radiologic Features

Because these different mycoses can have varied clinical presentations and distributions, radiographic findings can vary and overlap. However, there are still characteristic findings in typical presentations of disease. In acute histoplasmosis, radiographs most often show patchy pneumonia in one or more lobes with frequently noted hilar or mediastinal lymph nodes [22]. Severe disease can show diffuse pulmonary reticulonodular pulmonary infiltrates. In blastomycosis, acute presentations are more likely to present with airspace consolidation, and chronic presentations are more likely to present with mass-forming lesions [23]. In coccidioidomycosis, many patients have generally unremarkable radiographic findings; less than half show patchy areas of consolidation, and a minority show hilar lymph node enlargement [12]. In an immunocompetent patient, *Cryptococcus* can show multiple small well-defined nodules or focal areas of consolidation with an upper lobe predominance [24, 25].

Histologic and Immunophenotypic Features

In invasive disease, the shared morphology of these four mycoses is that of yeast or yeast-like forms; it is this feature that groups them as a common differential diagnosis. Upon the detection of candidate yeast forms in a biopsy or surgical specimen, delineation between the different mycoses can be made by several features, including size, shape (oval, round, or pleomorphic), pattern of budding (narrow, broad, or varied), and certain characteristic morphologic or staining features. A summary table of morphologic features is presented in Table 52.1.

Table 52.1 Morphologic differential of endemic mycoses

	Histoplasma	*Blastomycosis*	*Coccidioides*	*Cryptococcus*
Size	2–5 μm	8–20 μm	20–100 μm	5–15 μm
Shape	Round	Varied	Round	Varied
Budding	Narrow-based	Broad-based	None	Narrow-based
Other features	Intracellular pathogen within histiocytes; may be seen extracellularly	Double-contoured and refractile wall	Mature spherules with endospores (2–5 μm), occasionally hyphae	Mucicarmine-staining capsule, acapsular forms exist

Histoplasma spp. infects alveolar macrophages and is predominantly seen as an intracellular pathogen within phagocytic cells [26] with small, ovoid yeast forms (2–5 μm) that are the smallest of the yeasts forms discussed in this chapter (Fig. 52.3a). These typically stain poorly in routine preparations but can be observed as phagocytosed forms in the cytoplasm of macrophages. While other yeasts such as *Cryptococcus* can overlap in size with *Histoplasma*, the overall population of other yeasts is typically pleomorphic with larger forms present. *Histoplasma* shows narrow-based budding that contrasts with *Blastomyces*' broad-based budding.

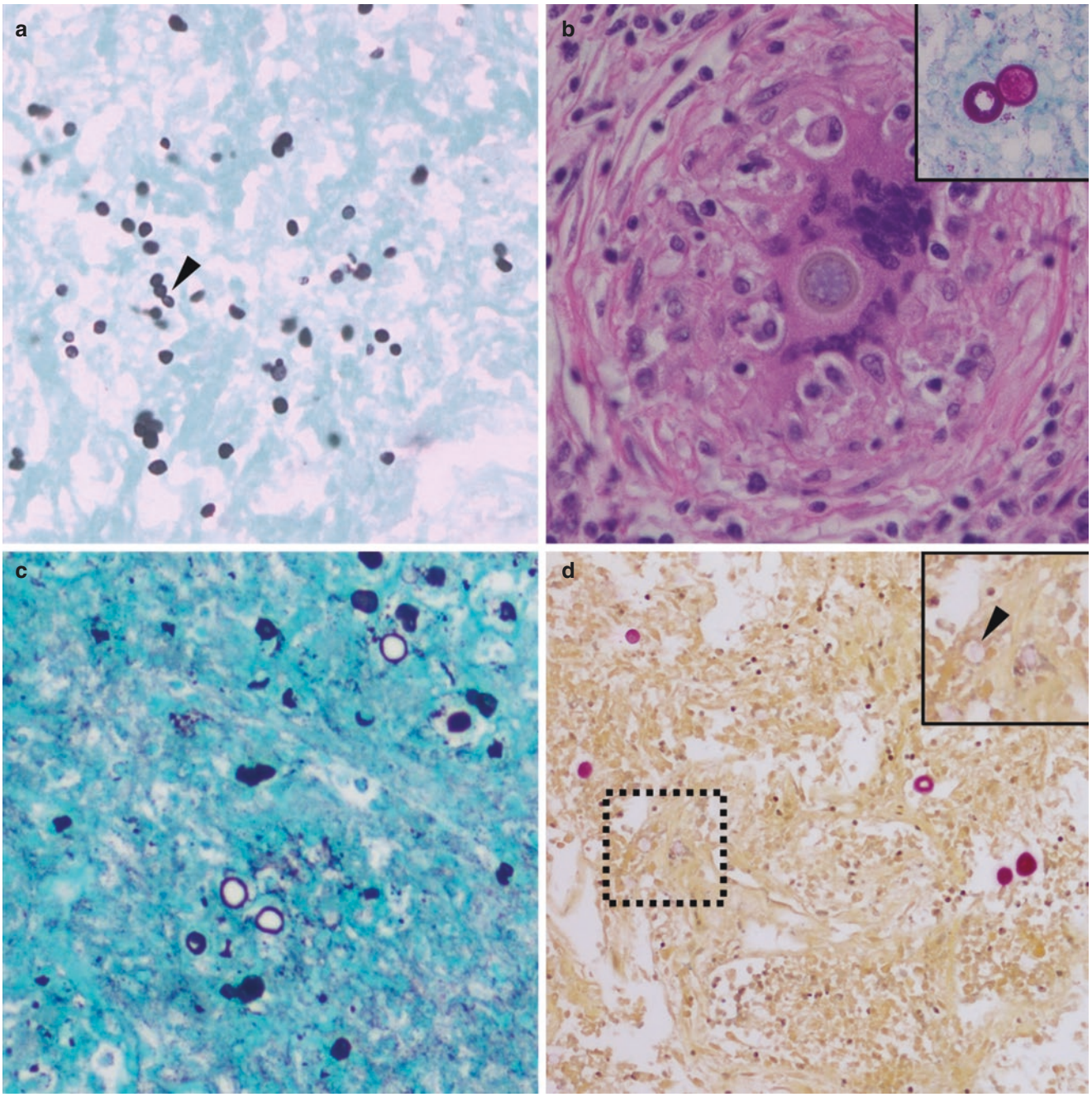

Fig. 52.3 Representative morphology of *Histoplasma*, *Blastomyces*, and *Cryptococcus*. (**a**) Histoplasmosis with small, ovoid yeast forms with narrow-based budding. (**b**) Blastomycosis with a range of intermediate-sized (12–23 μm) spherical yeasts. These are larger than *Histoplasma* and have broad-based budding with double-contoured cell walls (inset: PAS stain). (**c**) GMS stain of *Cryptococcus* with variably shaped and sized spherules without budding. (**d**) Mucicarmine stain highlighting mucoid capsule of *Cryptococcus*. Occasional possible capsule-deficient forms are present (inset)

Blastomyces are double-contoured, refractile, and typically 8–15 μm in size, creating morphologic overlap with either *Cryptococcus* (smaller forms) or *Coccidioides* (larger forms). Occasionally, very small forms mimicking *Histoplasma* in size can occur, but, as with *Cryptococcus*, these will typically exist in a continuum of size, including forms with more classic morphology [27]. The broad-based attachment of blastoconidia to their parent cells is the most reliable feature to distinguish *Blastomyces* from similar sized mimics (Fig. 52.3b, inset). *Blastomyces* (Fig 52.3b) will also be larger than *Histoplasma* (Fig 52.3a) if measurements with a calibrated micrometer can be made. The double cell wall is also a helpful finding (Fig. 52.3b).

The most characteristic finding of *Coccidioides* is that of a large, mature spherule greater than 30 μm containing multiple endospores (Fig. 52.2a). Smaller, immature spherules can be seen, which are generally ovoid and varied in size (Fig. 52.2c, d); these spherules can be confused with the yeast forms of other mycoses. Endospores are small (2–5 μm) (Fig. 52.2b) and may be difficult to identify in the absence of spherules [28]. Unlike other invasive yeasts, occasional hyphae and arthroconidia can occasionally be seen. When mature endospore-laden spherules are present, a confident diagnosis can be made, but if only immature spherules or degenerated forms are present, then a diagnosis is less certain and should be correlated with serology, immunofluorescence, or fungal culture.

Cryptococcus has intermediate-sized (most typically 4–7 μm) yeast forms with narrow budding (Fig. 52.3c), surrounded by a mucoid capsule that characteristically stains with a Mucicarmine stain (Fig. 52.3d). These yeasts may be pleomorphic, ranging up to 15 μm. Capsule-deficient forms can be present. Particularly in caseous or necrotic nodules, cryptococcal cells may be small, nonviable, distorted, and acapsular, becoming potentially confused with *Histoplasma*. Cryptococcal forms are typically not easily seen in an H&E stain, especially in the case of degenerated cells or resolving/resolved infections. In the case of unsuccessful fungal culture, immunofluorescence or molecular studies may be undertaken in order to make a histologic diagnosis.

Molecular Testing

Fungal culture should be performed in all clinical scenarios where invasive fungal disease is suspected. However, invasive fungal disease is often an unexpected finding, and fungal culture can sometimes produce isolates that are of uncertain clinical significance. Histologic evaluation, including assessment of host response, adds valuable information in these cases [28]. There are, however, cases where morphologic findings are not entirely specific for an etiology, and fungal culture has either failed or was not performed. In these cases, PCR-based methods performed on the formalin-fixed, paraffin-embedded tissue are becoming increasingly common and useful diagnostic tools [29, 30]. While molecular-based methods are largely more sensitive than histologic ones, a negative result does not definitively rule out invasive fungal disease in cases with strong histologic evidence or clinical suspicion. Degradation of DNA in nonviable organisms or after formalin fixation can cause a sample's total fungal DNA content to be below the threshold of detection for that assay, and caution in such cases is warranted. For cultured isolates of *Coccidioides*, DNA hybridization probes are available.

Key Points for Differentiating Coccidioides, Cryptococcus, Blastomyces, and Histoplasma

Each of These Pathogens Has Yeast or Yeast-Like Forms in Tissue: What Morphologic Features Distinguish Them?

Size is an important feature distinguishing these organisms (see Table 52.1), but other key morphologic features are often visible. A unique feature of *Coccidioides* distinct from *Histoplasma*, *Blastomyces*, and *Cryptococcus* is the formation of spherules, generally greater than 30 μm diameter; mature spherules are laden with endospores (2–5 μm). *Cryptococcus* spp. usually have a capsule that stains with mucicarmine. *Blastomyces* has a thick, double-contoured cell wall and divides by broad-based budding, whereas *Histoplasma* lacks the double-contoured wall and divides by narrow-based budding.

What Are Important Pitfalls in the Identification of These Organisms?

The pathologist should be aware of capsule-deficient *Cryptococcus*, small-variant *Blastomyces*, and the presence of free *Coccidioides* endospores in the absence of spherules. Free endospores are small and may hide in the background. Small-variant *Blastomyces* typically has a range of sizes, helping to distinguish from *Histoplasma capsulatum*. Serologic tests such as cryptococcal antigen may help diagnose capsule-deficient *Cryptococcus*. Other yeasts, such as *Candida* spp. and *Sporothrix* spp. may appear similar in histologic section.

What Additional Diagnostic Testing Is Important/Should Be Recommended?

Fungal culture and molecular microbiologic testing are far more specific than histologic assessment for species identification. Culture should be taken in lesions with a high suspicion, and the laboratory should be alerted to concern for *Coccidioides* or *Histoplasma* due to the significant risk of occupational exposure. Fungal PCR can be performed on the paraffin block in cases without a microbiologic diagnosis. Serologic testing for either antibodies or antigens is often helpful, but cross-reactivity among these and other yeasts is an important concern.

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