Cost-Effective Method for Identification of Dimorphic Fungi

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Traditional methods to identify dimorphic fungi dictate that the mold be converted to the yeast phase at 35 to 37°C. We present a time- and cost-saving method of confirming the identification of a dimorph by using special stains to demonstrate the yeast phase directly growing in the original clinical specimens.

Our patient was a 45-year-old male with a history of severe alcoholism. He presented with an episode of near syncope, abdominal pain, nausea and vomiting, and melanoctic stool for 4 days. Chest X ray revealed interstitial and air space opacities bilaterally. The patient developed fever and progressive obtundation requiring intubation and mechanical ventilation. Multiple blood and sputum specimens were submitted to pathology.

Three sets of sputum cultures, a lung biopsy specimen, and four blood cultures were collected from this patient. Three primary plates at 30°C were set up from the sputum specimens as follows: Emmons Sabouraud dextrose agar (SDA), Emmons SDA with antibiotics (Mycosel), and brain heart infusion with sheep blood, chloramphenical, and gentamicin. Potassium hydroxide (KOH) preparations and permanent stains (methenamine silver and periodic acid-Schiff) were also performed.

The KOH and permanent stains revealed only bacteria and Candida-like yeast. After 2 days of incubation at 30°C, scant amounts of Candida grew on all the primary plates. After 23 days, the SDA and Mycosel plates all grew a white mold. The brain heart infusion plates grew a tan, dry, wrinkly colony. Lactophenol cotton blue stains of the white mold growing on SDA revealed delicate, true, septate hyphae, teardrop microconidia, and doubled-walled spherical and pear-shaped macroconidia. A few of these macroconidia were tuberculated. These findings are all suggestive of Histoplasma capsulatum.

The blood cultures collected during the same period were as follows: two sets of aerobic and anaerobic Bactec bottles, with a 6-day incubation protocol at 35°C; and two myco F lytic Bactec bottles, specifically for fungus, with a 28-day incubation protocol at 35°C. The aerobic and anaerobic sets were finalized as negative at 6 days. The Bactec 9240 flagged one myco F lytic bottle as positive on day 23 of incubation. Gram stains revealed no organisms, but the bottle was subcultured to two sets of primary media: one set at 35°C and the other at 30°C.

This was done because the respiratory cultures were growing a possible Histoplasma isolate. At 4 days, a dull, white haze of growth appeared on both 30 and 35°C sets of plates. By day 7, white mycelial growth had developed. A lactophenol cotton blue tape preparation (2) showed the same mold as was visible on the respiratory cultures.

In order to identify the fungus growing on SDA as Histoplasma, the yeast phase of this dimorphic fungus had to be demonstrated (3). Traditional methods dictate that the mold be converted to the yeast phase at 35 to 37°C. This requires time and expense, including special media, weeks of incubation to make the conversion, additional exposure to the organism, and more technologist time to perform the subcultures and additional tape preparations.

We propose an alternate method of confirming the identification of the dimorph by using special stains on direct specimens, instead of culture conversion. The standard stain used to demonstrate the tiny (3 to 5 μm) Histoplasma yeast form in clinical specimens is Giemsa (4). The standard Giemsa stain has an affinity for staining the tiny yeast of Histoplasma, which is found in the macrophages of the reticuloendothelial system. However, this permanent stain has a drying and staining time of several hours. The calcofluor white stain (1) is usually employed to examine clinical specimens as they are initially set up for fungal culture and smear. We decided to try the quicker wet stain of KOH with calcofluor white.

The positive myco F lytic blood culture was the only remaining specimen to work with. It had been incubating at 35°C continuously for 30 days. White mold, consistent with Histoplasma, grew from every subculture at 30 and 35°C. We used the calcofluor stain to examine the growth from the blood culture. Several drops of the 30-day-old myco F lytic blood was mixed with the KOH with calcofluor white and examined under the fluorescent microscope. Small, single budding yeast cells (3 to 4 μm) compatible with H. capsulatum were clearly evident. These yeast cells grew as a mold on the fungal media at 30°C. The dimorphic nature of the fungus in our specimen was confirmed. This was proof of the yeast and mold phases of Histoplasma from the same specimen.

As many laboratories face budgetary constraints, hiring freezes and loss of personnel, we propose a simple and cost-effective method to identify a dimorphic fungus. This method conserves media and decreases the time needed for diagnosis.

The tissue phase of each of the four respiratory dimorphic fungi, when seen in either direct wet mounts of clinical material or observed in 35°C cultures, is easily identifiable based on size, morphology, and relationship within tissue (intracellular or extracellular). Their subsequent growth as a mold in culture at 30°C is positive proof that the fungal species is dimorphic.
For example, the morphology of the yeast of *Blastomyces dermatitidis* is pathognomonic in tissue. Its large, broad-base, nonencapsulated, single buds are positive identification. *Paracoccidioides brasiliensis* is the only dimorph with multiple, non-encapsulated budding yeast forms. *Coccidioides immitis* forms spherules in tissue, not budding yeast: therefore, its identification is not based on its conversion in the laboratory. The yeast phase of *Penicillium marneffei* is similar in size to *H. capsulatum*, but the mold phase has a unique red pigment. *Candida glabrata* is the only other yeast similar in size to *H. capsulatum*, but it is a monomorph. Both *P. marneffei* and *C. glabrata* have much faster growth rates (2 to 3 days) than *H. capsulatum*, which has a growth rate of 3 weeks.

Positive identification of a dimorphic fungus requires demonstrating the yeast and mold phases of the organism. The presumed need to convert *H. capsulatum* to the yeast phase is based on the occasional isolation of the saprophytic monomorph *Sepedonium* sp., which produces tuberculate macroconidia similar to *H. capsulatum*. It does not produce microconidia, nor does it grow at 35°C. The laboratory’s often-futile attempt to convert the mold-phase to the yeast-phase to prove the identification of the isolate as *H. capsulatum* is time-consuming. Therefore, when yeast are seen in tissue or grown in culture at 35°C and converted to a mold phase at 30°C, a dimorphic identification can be assigned. The mold phase can be demonstrated by standard culture technique, and the yeast phase can be demonstrated directly from the specimen with calcofluor white. Tech time and media are conserved, exposure to pathogenic fungi is lessened, and diagnosis is expedited.

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REFERENCES