Title: False Positive Culture Results from Patient Tissue Specimens Due to Contamination of RPMI Medium with *Cryptococcus albidus*

Running title: False Positive Cultures Due to *C. albidus* Contamination

Authors: Lisa G. Winston, MD*, Marguerite Roemer, Carol Goodman, Barbara Haller, MD, PhD

Institutional address: San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, California 94110

Affiliations: University of California, San Francisco / San Francisco General Hospital

*Corresponding author:

Mailing address: San Francisco General Hospital, 1001 Potrero Avenue, Room 5H22, San Francisco, California 94110

Phone: 415-206-8703

Fax: 415-206-8965

Email: lisa.winston@ucsf.edu
Abstract: Cryptococcus albidus, a rare opportunist, was isolated from biopsy specimens from three patients over four days. Investigation showed the specimens had been contaminated by placement in RPMI medium. The importance of rapid communication between Microbiology, Infectious Diseases/Infection Control, and other involved parties in the event of unusual occurrences is highlighted.
Cryptococcus albidus is a fungus that can be isolated from the environment. It is a rare pathogen, with fewer than thirty cases of human infection reported. The Microbiology laboratory at our institution isolated C. albidus from biopsy specimens obtained from three different patients over a four-day period in August 2006. This highly unusual occurrence prompted an investigation into possible sources of contamination of the specimens.

Patient #1 was a 63 year-old man with hemoptysis for several months. Chest computed tomography scanning revealed an upper lobe mass concerning for primary bronchogenic carcinoma. On 8/22/06, Interventional Radiology performed a computed tomography guided aspiration of the mass, and specimens were sent both to Pathology for examination and to the Microbiology laboratory for fungal and mycobacterial cultures. A Gram stain of the specimen was not performed since a bacterial culture was not ordered. Acid fast staining of the tissue performed by the Microbiology laboratory showed no organisms, and cultures were subsequently negative for mycobacterial growth. However, a fungal culture grew C. albidus, which was definitively identified by the Microbiology laboratory on 8/31/06. The C. albidus was germ tube negative and there were no hyphal/pseudohyphal elements seen on cornmeal agar with trypan blue (Remel, Lenexa, KS). The negative reaction on birdseed agar indicated that the yeast was not C. neoformans. The C. albidus was definitively identified using the API 20 C AUX identification strip (bioMérieux, Marcy-l’Etoile, France). Cytology, reported 8/25/06 by the Pathology Department, revealed squamous cell carcinoma and necrosis without evidence of infection. The patient was not treated for cryptococcal infection.
Patient #2 was a 25 year-old man with a history of intravenous drug use. He had been diagnosed with pan-sensitive tuberculosis of the thoracic spine. He was admitted with fevers despite ongoing treatment with appropriate antituberculous drugs. The patient refused testing for human immunodeficiency virus (HIV). On 8/19/06, computed tomography of the abdomen and pelvis was performed due to abdominal pain and showed rim-enhancing lesions in the liver and spleen, a lytic lesion in the T12 vertebral body, and enhancing paraspinal soft tissue. On 8/24/06, Interventional Radiology performed percutaneous needle biopsies of the liver and paraspinal lesions. Specimens were sent to Pathology and to the Microbiology laboratory. Gram stains of the liver and paraspinal specimens sent to Microbiology showed many round and oval budding yeast. Staining for acid fast bacilli in Microbiology was negative. Cultures from both specimens grew C. albidus, which was reported 9/05/06. Culture and identification methods were the same as those described for Patient #1. Pathological examination of both the liver and paraspinal specimens showed organisms consistent with mycobacteria using Fite staining. Given the clinical presentation and possible risk factors (injection drug use and the question of immunocompromise), this patient was initially treated with amphotericin B deoxycholate until an investigation indicated contamination of the specimens during collection.

Patient #3 was a 31 year-old man with HIV infection. He presented to clinic and was noted to have a large axillary lymph node. A fine needle aspirate (FNA) was performed in clinic on 8/25/06 by Cytopathology. Tissue was sent to the Microbiology laboratory and to Pathology. In Microbiology, acid fast staining revealed few acid fast bacilli. Culture for mycobacteria subsequently grew Mycobacterium avium complex.
Gram staining of the tissue revealed few oval budding yeast. Culture grew *C. albidus* reported 9/5/06. Again, culture and organism identification were performed as described for Patient #1. Cytological examination performed by Pathology showed acute necrotizing inflammation and numerous organisms consistent with mycobacteria by Fite staining. The patient was not treated with antifungal therapy.

On 9/6/06, Infectious Diseases and Infection Control became aware that three patients had grown *C. albidus* from specimens taken within a four-day period. An investigation had been undertaken in Microbiology to look for possible mechanisms of contamination after the specimens reached the laboratory. This investigation included a check of the labeling on the specimen tubes and testing to demonstrate sterility of media used in Microbiology for specimen processing and culture inoculation. No evidence of contamination within the Microbiology laboratory was uncovered. On 9/7/06, review of the original laboratory requisitions revealed that one cytopathologist had collected the specimens obtained by Interventional Radiology for the first two patients and had collected the specimen from the third patient in clinic. On 9/8/06, representatives from the Microbiology laboratory and Infectious Diseases/Infection Control met with the cytopathologist to examine the pathology slides and to review the procedures used when specimens were collected and transported to Pathology and Microbiology.

All the specimens had been obtained by needle aspiration using a fresh sterile needle and syringe. None of the specimens submitted to Pathology showed the presence of yeast. Special fungal stains were not performed on the lung biopsy specimen from Patient #1; however, both Pathology specimens from Patient #2 (liver and paraspinal lesions) had Gomori methenamine silver (GMS) staining performed. Yeast forms were
not identified in the Pathology specimens. The lymph node biopsy specimen from Patient #3 was also negative for yeast by GMS staining.

Specimens submitted to the Microbiology laboratory were collected with a needle and syringe and then they were rinsed into tubes of RPMI cell culture medium (named for Roswell Park Memorial Institute) before being transported to the laboratory. RPMI medium was not used for any of the specimens submitted to Pathology. The original bottle of RPMI medium was stored at 4°C in the Pathology laboratory. Aliquots of RPMI were periodically poured into sterile capped tubes which were stored at room temperature in the biopsy tray carried by the cytopathologist to the site of specimen collection.

The Microbiology laboratory obtained a capped tube of RPMI medium from the FNA tray that had been poured from the refrigerated bottle. Gram stain of this medium on 9/8/06 revealed many round budding yeast consistent with Cryptococcus spp. Clinicians caring for all three patients were notified of this finding on 9/8/06, as this provided evidence that the patient specimens were contaminated by use of contaminated RPMI medium. Subsequent culture of both the capped tube of RPMI and the original, refrigerated bottle grew C. albidus, which was identified by API 20 C AUX 9/14/06.

The Microbiology laboratory also obtained an unopened bottle of RPMI of the same lot number from Pathology and cultured it. The RPMI medium from the sealed bottle was sterile with no yeast or bacteria recovered. The contaminated RPMI medium was discarded. Policies and procedures have been created which eliminate use of RPMI medium for collection of specimens for Microbiology cultures and institute use of single-use sterile saline tubes for collection of these specimens.
This report describes three clinical cases in which *C. albidus* was isolated from patient specimens as a result of contamination of RPMI medium. *Cryptococcus neoformans* has been reported previously as a laboratory solution contaminant in a pseudoutbreak of cryptococcal meningitis (3). However, we did not find any previous cases in which *C. albidus* was found in patient specimens as a result of laboratory contamination. True infections with *C. albidus* are extremely rare. Systemic infections have been reported largely from immunocompromised patients (4-6, 8, 10). Ocular infections (1, 2) and cutaneous infections (7, 9) have also been reported in patients with underlying risk factors for opportunistic infections.

Due to the rarity of finding *C. albidus* in clinical specimens and the proximity of the cases in time, contamination was quickly suspected. However, it was not immediately apparent where the contamination had occurred or whether one of the patients could have true disease with the ‘real’ specimen contaminating the laboratory environment. Patient #2 was the only patient clinically suspected of having disease due to an environmental cryptococcal species after culture results were reported. It was thought that he might have become infected through frequent injection drug use. The finding of large numbers of yeast on Gram stain also suggested the possibility of true infection to his clinicians. However, the presence of both *C. albidus* cultured by Microbiology and acid fast bacilli seen on staining by Pathology from the same specimens was puzzling.

Discussion with the cytopathologist who obtained the specimens pointed to a likely mechanism of contamination, which then was rapidly confirmed. RPMI solution is known to sustain growth of bacteria and fungi, and it is not needed for transport of tissue.
specimens to the Microbiology laboratory. In addition, diluting the specimens in a fairly large and non-standardized volume of fluid may lead to decreased culture yield. In order to decrease the risk of specimen contamination and to decrease needlestick exposures from re-capping needles, our institution now uses single-use sterile saline tubes for specimens collected for Microbiological culture with needle and syringe.

The false positive cultures described in this report demonstrate how important it is that unusual occurrences in the Microbiology laboratory are immediately reported to hospital Infectious Diseases/Infection Control and investigated thoroughly so corrective actions can be addressed and inappropriate therapy can be avoided.
References


