Case Report:
PCR Detection of *Histoplasma capsulatum* var. *capsulatum*
in Whole Blood of a Renal Transplant Patient with Disseminated Histoplasmosis

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**Abstract.** We report the identification of *Histoplasma capsulatum* var. *capsulatum* from whole blood in a renal transplant patient with disseminated histoplasmosis using colorimetric microtiter-plate PCR. This modality demonstrated utility in reaching a definitive diagnosis in a timely manner. Blood fungal cultures in this case remained negative, suggesting that molecular assays may facilitate the laboratory diagnosis of disseminated histoplasmosis.

**Keywords:** *Histoplasma capsulatum*, histoplasmosis, PCR-EIA

**Case Report**

A 35-yr-old woman presented to our emergency department (ED) complaining of fever on 3 separate occasions during the course of a week. At each visit she was found to be afebrile and was sent home. She had received a living related donor renal transplant 5 mo prior for renal failure secondary to bilateral ureteral reflux. The transplant was complicated by an episode of vascular rejection that required splenectomy, anti-thymocyte globulin, and rituximab therapy. Her current medications included prednisone, tacrolimus, mycophenolic acid, doxycycline, valganciclovir, and coumadin.

The patient returned to the ED for the fourth time within that same week complaining of cough, poor appetite, and decreased urine output. Her physical examination was significant for sinus tenderness and nonproductive cough. Initially she was afebrile, but several hr later became acutely febrile to 39.4°C with rigors. Initial laboratory studies revealed a white blood cell count of 1,100/μl (reference range 3,900–10,900/μl) with absolute neutrophil count 420/μl (1,400–7,700/μl), hematocrit 29% (37-44%), platelet count 528,000/μl (135,000–371,000/μl), LDH 1,098 U/L (<226 U/L), SGOT 156 U/L (4–40 U/L), and creatinine 2.18 mg/dl (patient baseline 1.4 mg/dl, reference range 0.7–1.5 mg/dl). Urinalysis was normal, and chest x-ray showed a small right pleural effusion. The clinical differential diagnosis was allograft rejection vs infection. Aerobic and anaerobic blood cultures, urine culture, quantitative CMV and EBV viral loads, hepatitis B, C, and HIV serologic tests, urine histoplasma antigen, and serum cryptoccocal antigen tests were ordered.

A peripheral blood smear on hospital day 4 revealed small, intracellular yeast forms within a monocyte (Fig. 1). An inquiry was made that day to the reference laboratory (MiraVista Diagnostics, Indianapolis, IN) performing the urine histoplasma antigen test, and a positive preliminary result was obtained. A final report confirming this finding was received the next day. In addition, a PCR assay (see below) was performed on hospital day 4 using a leftover whole blood specimen collected on...
hospital day 3: this assay confirmed disseminated *Histoplasma capsulatum*.

The patient immediately received Abelcet (amphotericin B lipid emulsion) at half dosage due to renal insufficiency. On the second day of therapy, she experienced respiratory failure and required pressor support and intubation. A preliminary diagnosis of sepsis due to immune recovery inflammatory syndrome (IRIS) was entertained. She recovered rapidly over the next 48 hr and was discharged 4 days later after switching to oral itraconazole therapy. Over the next month, her marrow function improved and blood counts returned to baseline.

**Methods and Test Results**

Nucleic acid was extracted from 0.2 ml of EDTA whole blood by using the easyMAG system according to the manufacturer’s instruction (bioMerieux, Durham, CA) [1]. Extracted DNA samples were resuspended in 50 μl of water. A colorimetric microtiter-plate PCR assay (PCR-EIA) was performed to detect *H. capsulatum* DNA [2]. In brief, 50 μl of PCR mixture contained the following: 1× buffer, 1.5 mM MgCl₂, 10% glycerol, 200 μM dATP, dCTP, and dGTP, 100 μM dTTP, 90 μM dUTP, 10 μM digoxigenin-11-dUTP (Roche, Indianapolis, IN), 1 μM each primer, 0.01 units/μl uracil N-glycosylase (UNG, Epicentre Technologies, Madison, WI), 0.025 units/μl AmpliTaq gold DNA polymerase (Applied Biosystems), and 5 μl of DNA extract. The reaction mixtures were placed in an ABI 9700 thermal cycler (Applied Biosystems, Foster City, CA) programmed for a 3-step PCR procedure followed by an initial UNG activation. The primer set (Hc447F, 5’- CGC AGT TTT CCG TGC AGA A-3’ and Hc545R, 5’- CCA CAG CAT CAC GGA GGT ATT -3’) was designed to amplify a 99-bp portion of a *H. capsulatum*-specific 100-kDa protein gene [3]. Amplification products were identified by detecting digoxigenin-labeled PCR products with a PCR ELISA kit (Roche). A *H. capsulatum*-specific 5'-biotinylated capture probe (Hc480P, 5’- CCG ATA CAG TTC TCT CCT TCT TGC AAC TC -3’) was used to detect and confirm the amplification product. A positive result was defined as OD₄₅₀-OD₄₉₀ value ≥0.1 [2].

Histoplasma antigen was measured by a second-generation EIA, and results were expressed as units as previously described [4,5]. Testing was performed at Miravista Diagnostics (Indianapolis, IN). A blood culture drawn on hospital day 3 was cultured in BACTEC MYCO/F lytic medium and incubated in the BACTEC 9240 blood culture system (Becton-Dickinson, Sparks, MD) at 35°C for 4 weeks. Additional tracheal cultures were taken during the patient’s brief period of intubation.

PCR-EIA performed on the whole blood specimen collected on hospital day 3 yielded an OD₄₅₀-OD₄₉₀ value of 3.226. A negative control consisting of pooled negative whole blood read as 0.038. A pooled negative whole blood specimen spiked with 5,000 colony forming units/ml of *H. capsulatum var. capsulatum* was used as a positive control and yielded an OD₄₅₀-OD₄₉₀ value of >3.800.

Histoplasma quantitative urine antigen testing yielded a high positive (>39.0 ng/ml) result from two samples taken on the second and third days of hospitalization. The test was performed on day 4 of hospitalization, and final results were available on day 5.

The fungal blood culture failed to turn positive after 4 weeks of incubation. The tracheal fungal cultures grew *Candida tropicalis* only. Multiple bacterial blood and urine cultures were negative.

**Discussion**

Disseminated histoplasmosis is an unusual, but life-threatening, event in the solid organ transplant population. The mortality rate in transplant patients approaches 40% [6]. Serologic testing is useful, but false negatives occur in acute phases of infection, and previous infection can produce antibodies for years [7]. The presence of antigen is indicative of active infection and is the current test modality of choice. Antigen testing is performed with a urine sample, since the antigen is more concentrated in urine than in serum in disseminated histoplasmosis. Major drawbacks to antigen testing are a well-documented cross-reactivity with other closely related fungal species and a prolonged result turnaround time when testing is not available in-house [8,9]. Nucleic acid methods such as PCR are not feasible in urine samples due to physical disruption of the microorganism [2]; blood does...
not suffer this limitation, making it a viable option for testing.

The fortuitous finding of yeast forms in a peripheral blood smear is a rare occurrence and should correlate with a high fungal load given the small volume analyzed. *Histoplasma capsulatum* is a slow-growing microorganism in the laboratory, taking on average 2 to 4 weeks for a blood culture to become positive [7]. Culture remains the gold standard for diagnosis, but such confirmation is not available in a time-frame appropriate for patient care. In our case, we were unable to culture the organism. This brings into question the original fungal load in the bloodstream and demonstrates the ability of a PCR assay to detect fungemia that a blood culture system was unable to detect.

Most reports of PCR-based diagnoses of histoplasmosis have focused on tissue specimens [10-12]. Other specimens, such as ocular and cutaneous specimens, sputum, urine, bronchoalveolar lavage, and blood have also been used in PCR assays [2,13-17]. Recently, PCR assays have been employed to diagnose disseminated histoplasmosis in blood specimens from patients with AIDS [16,18-20]. We report the successful use of PCR-EIA on a whole blood sample for *H. capsulatum* detection for the diagnosis of disseminated histoplasmosis.

As documented in this report, PCR-EIA on a whole blood sample may be used successfully for *H. capsulatum* detection. With an additional signal amplification incorporated in the amplicon detection step, the PCR-EIA assay reaches an excellent analytical sensitivity of 0.2-2 colony-forming units/reaction for detection of *H. capsulatum* [2]. This test, performed in-house, represents a faster diagnostic modality than other testing modalities such as culture and, in the case of our institution, antigen testing. This improved result turnaround time may be particularly useful and cost-effective in a population of immuno-suppressed patients where *Histoplasma capsulatum* is endemic. The PCR-EIA assay has a test turnaround time within 8 hr from the time of specimen collection, which is superior to the roughly 4 day turnaround time for send-out urine antigen testing. Prompt initiation of effective antifungal therapy in this population may improve clinical outcomes. Reliance on culture results as confirmatory evidence of infection can be misleading, and a quicker, more sensitive method is needed. Due to its high sensitivity and specificity as well as rapid test turnaround time, the real-time PCR-based molecular assay is a useful tool that facilitates the diagnosis of disseminated histoplasmosis [14,21].

Further studies are warranted to determine the clinical significance of the more rapid diagnosis of histoplasmosis provided by molecular-based assays. Future studies should also determine the specificity and sensitivity of this and other molecular-based assays using a combination of antigen testing and culture for diagnosis of *Histoplasma capsulatum*. A prospective study of several patients would help to establish these values and might identify factors responsible for negative culture results in clearly infected patients.

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References