JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1456–1458 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1456–1458.2005

False-Positive *Histoplasma capsulatum* Gen-Probe Chemiluminescent Test Result Caused by a *Chrysosporium* Species

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Received 8 September 2004/Returned for modification 21 October 2004/Accepted 26 October 2004

We describe a case in which the *Histoplasma capsulatum* AccuProbe test displayed cross-reactivity with a respiratory isolate thought to be *Histoplasma* but not morphologically consistent with *H. capsulatum*. The isolate was later identified as the *Chrysosporium* anamorph of *Nannizziopsis vriesii* by sequence analysis and phenotypic data.

CASE REPORT

A 40-year-old human immunodeficiency virus-positive male was admitted to the hospital with a diagnosis of hypoxia and pneumonia. His $CD4^+$ T-cell count on admission was 13 cells/μl. Bronchoscopy was performed, and bronchial washings were collected. A white mold was isolated from bronchial washings. The patient was ultimately discharged with a diagnosis of cytomegalovirus infection. The white mold was not thought to have contributed to the primary episode of pneumonia, and no antifungal therapy was given. Because the patient lived in an area of histoplasmosis endemicity, however, the isolate was sent to the University Hygienic Laboratory for confirmation of *Histoplasma capsulatum*. At this laboratory, an AccuProbe test for *H. capsulatum* (GenProbe, San Diego, Calif.) was positive, but the morphology was noted as inconsistent with that of *H. capsulatum*. The isolate was referred to the Fungus Reference Unit, Centers for Disease Control and Prevention, for further investigation.

At the Centers for Disease Control and Prevention, the isolate grew on potato dextrose agar at 25, 37, and 40°C as a white downy colony. Slide culture on oatmeal agar and on Leonian's agar showed abundant cylindrical and pyriform microconidia produced from the sides and termini of hyphae (Fig. 1). No macroconidia or sexual structures (ascomata or asci) were observed on either medium after 4 weeks of incubation. No conversion to yeast form was observed after 4 weeks of incubation on Kelley's medium, Pine's medium, or brain heart infusion agar with blood at 37°C; the growth remained hyphal under these conditions. Tests for urease and hair perforation were positive, as was growth on agar containing cycloheximide at 0.04 g/liter. The AccuProbe test for *H. capsulatum* was positive at 100,277 relative light units (RLU), where a positive control (*H. capsulatum*) generated 588,467 RLU and a negative control (*Coccidioides immitis*) generated 36,856 RLU. A signal greater than or equal to 50,000 RLU is considered positive according to the manufacturer's test definition. DNA was prepared, and the ribosomal internal transcribed spacer (ITS) region was sequenced with ITS-5 and ITS-4 primers (15). The closest match in GenBank was a 94% match to *Nannizziopsis vriesii* CBS 407.71 (sequence AJ131687). No matches were found to any of the approximately 77 *H. capsulatum* ITS sequences in the GenBank database. The isolate was sent to the University of Alberta Microfungus Collection and Herbarium (UAMH), where it was deposited as UAMH 10417. Given that no sexual structures were obtained after additional attempts, the isolate was identified as the *Chrysosporium* anamorph of *Nannizziopsis vriesii* based on morphology and DNA sequence analysis.

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The pathogenic fungus *H. capsulatum* causes histoplasmosis throughout the world. In the United States, this organism resides in soils of the Ohio and Misssissippi River valleys, and the infection is contracted by inhalation of infectious conidia. Most immunocompetent individuals have mild infection that resolves readily. However, in immunosuppressed individuals and the elderly, disseminated extrapulmonary disease may occur, causing serious illness and death.

Historically, definitive identification of *H. capsulatum* has required demonstration of characteristic tuberculate macroconidia and microconidia and conversion from the filamentous mold phase to the yeast phase at 37°C. Because of the biosafety level 3 biohazard level of the mold phase, many laboratories are today reluctant to work extensively with the organism. Special media may be required to obtain sporulation, and macroconidia may resemble conidia produced by fungi belonging to other genera, such as *Sepedonium*. The process of conversion to the yeast stage is lengthy and laborious, requiring multiple subcultures, and is not always successful. A confirma-

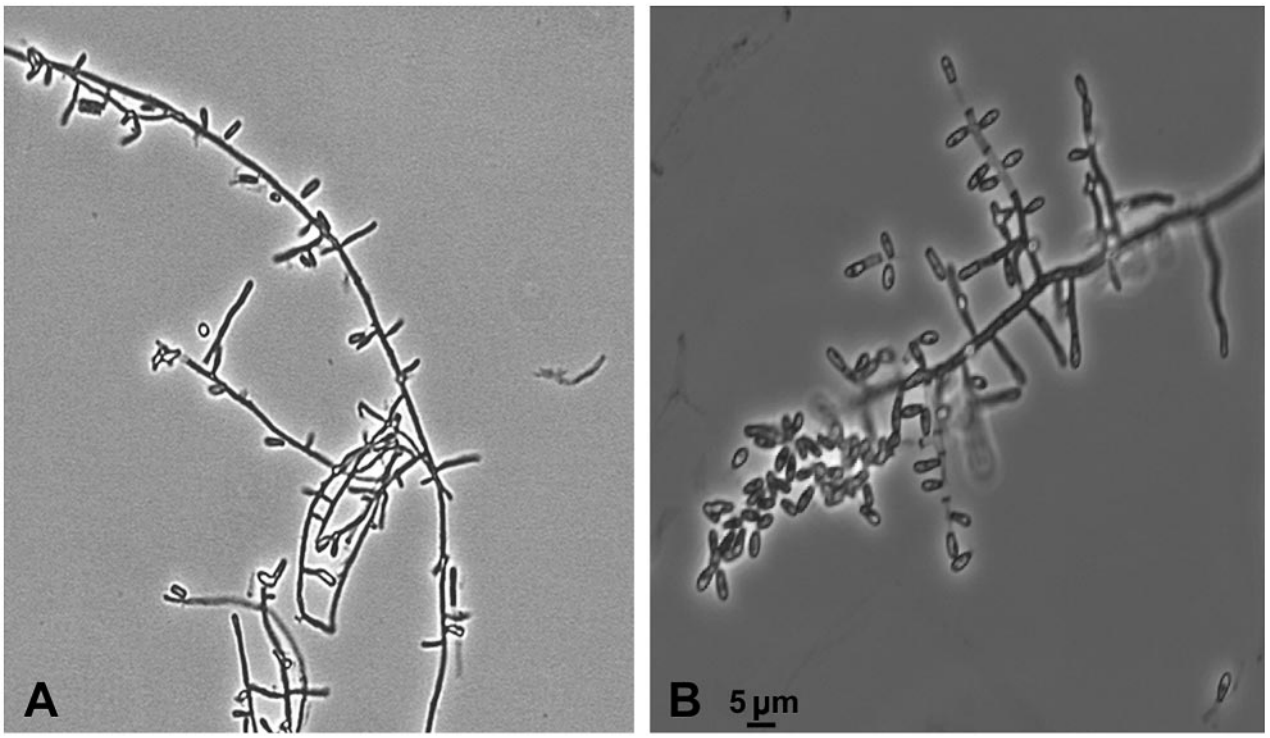

FIG. 1. (A) Slide culture of UAMH 10417 on Leonian's medium showing microconidia on the sides and termini of hyphae. (B) Slide culture of *N. vriesii* CANV 10351 from a reptile.

tory method based on the production of exoantigens has been described, but this method requires technical expertise and lengthy incubation prior to extraction of the exoantigens (5). The DNA probe-based method used in this case study, the AccuProbe (Gen Probe), was introduced some years ago for identification of *H. capsulatum* (6). In this method, a chemiluminescent DNA probe binds to rRNA of the target organism. Unbound probe is chemically degraded, and the light emitted from the labeled DNA-RNA hybrid is measured in a luminometer. Several studies have evaluated the AccuProbe and have found the test generally sensitive and specific (2, 3, 9, 12).

This case is instructive for three reasons. First, it demonstrates that false-positive results may be obtained in the *H. capsulatum* AccuProbe test. This isolate displayed RLU results that were considered positive according to the manufacturer's test definition. However, they were only twofold over the cutoff for positivity of 50,000 RLU instead of displaying the much higher RLU values that are customarily obtained with morphologically typical positive organisms. These results were obtained in two different laboratories, each performing the test according to the manufacturer's instructions with isolates grown on Sabouraud dextrose agar. The AccuProbe test has been reported to be highly specific (2, 9, 12), although these studies tested cross-reactivity with very few *Chrysosporium* species or representatives of the ascomycete order Onygenales (11). A previous report demonstrated a positive *Blastomyces dermatitidis* AccuProbe result with an isolate of the thermotolerant ascomycete *Gymnascella hyalinospora*, which caused pneumonia in a leukemia patient (4). Direct sequencing of the ribosomal ITS region and BLAST searching (1) of our cross-reactive isolate revealed the closest match as *Nannizziopsis vriesii*. The choice of the sequencing locus is important, as identifications based on comparisons of sequence data from highly conserved regions such as the 18S coding region or the 5.8S region may be misleading at the species level. Millar et al. emphasized this point in their report of a *Chrysosporium keratinophilum* isolate that was investigated by sequencing the 18S coding region and the 5.8S ITS-2 region (7). The partial 18S sequence displayed high homology to the dimorphic pathogen *C. immitis*, whereas the ITS-2 region, which is more suitable for investigations of taxonomy at the species level, showed homology to the saprophytic organism *C. keratinophilum*.

Second, the reliability of molecular identifications is dependent on the phylogenetic breadth and taxonomic accuracy of the sequence database being consulted. Both the study by Millar et al. (7) and this one show that gaps in the public databases can lead to misleading results or make results difficult to interpret. For fungi, the current number and diversity of ITS region sequences in GenBank are greater than those of sequences for other gene loci. Missing data contributed to the results of Millar et al. because at that time GenBank contained ITS sequences but no 18S rRNA gene sequences for *C. keratinophilum*. Another important factor in using BLAST searches to identify an unknown isolate is careful interpretation of the results. Queries using an 18S sequence from the target fungus will typically retrieve multiple equally close matches, particularly when only a portion of the 18S coding region, consisting of more than 1,600 bases for ascomycetous fungi, is queried. These types of results, therefore, would suggest only an approximate phylogenetic placement for the fun-

gus. Furthermore, in a BLAST result, the percentage identity between the target and matched sequence is based on the number of bases compared, which may differ from the number of bases queried. In the BLAST result for our isolate, the 94% sequence identity with *N. vriesii* AJ131687 was based mostly on the 5.8S conserved coding region where 291 of 307 bases were identical; in contrast, the ITS-1 and -2 regions were highly divergent and initially suggested only a very poor match to the species. The phylogenetic relationship was confirmed only by comparison with sequences derived from undescribed species of the *N. vriesii* complex, members of which are still under taxonomic investigation (L. Sigler and S. Hambleton, unpublished data).

Third, this report emphasizes the importance of considering both phenotypic and molecular data in identifying fungi of clinical significance. In this case, the morphology of the organism was inconsistent with the positive AccuProbe result. Tuberculate macroconidia of *H. capsulatum* were not observed. Furthermore, the isolate could not be converted to the yeast phase at 37°C but rather remained hyphal at higher temperatures. Finally, this isolate produced strong positive results in the hair perforation test, a sign of keratinophilic properties. These discrepancies prompted further testing, DNA sequencing, and, ultimately, the identification of this isolate as the *Chrysosporium* anamorph of *N. vriesii*. The characteristics of the isolate, including cylindrical and pyriform microconidia, urease production, and hair digestion with perforating bodies, agreed with those described for this species in recent reports of reptile infection (8, 10, 13). However, neither the reptile isolates nor the present human isolate has produced sexual structures typical of *N. vriesii*, introducing some doubt as to their conspecificity with *N. vriesii*. Preliminary molecular analyses have revealed that isolates morphologically resembling *N. vriesii* are members of a complex consisting of several undescribed species (Sigler and Hambleton, unpublished). The 94% sequence similarity between our isolate and *N. vriesii* (AJ131687) provides evidence that these are not the same species but are close relatives. This human isolate differs from most reptile isolates in growing well at 37°C, but thermotolerance and urease activity are variable among members of the *N. vriesii* complex.

The false-positive results with the commercial probes have involved species that are phylogenetically somewhat distant. The dimorphic pathogens *H. capsulatum* and *B. dermatitidis* are now placed within the new family *Ajellomycetaceae* (Onygenales), together with their relatives *Emmonsia* species and *Paracoccidioides brasiliensis* (11, 14). The cross-reacting fungi are classified in separate families of the Onygenales: the *Chrysosporium* anamorph of *N. vriesii* in the *Onygenaceae* and *Gymnascella hyalinospora* in the *Gymnoascaceae*.

It is important that, during their development, molecular assays be screened for cross-reactivity against a range of phylogenetically relevant species, whether or not such species are human pathogens. Furthermore, the results of nucleic acid-based testing should not be followed blindly but considered as one aspect of the body of information that is assessed in identifying an unknown organism. Many organisms formerly considered saprophytes are now recognized as agents of infection in compromised hosts, and organisms mimicking primary pathogens may be recovered from clinical materials.

Individuals competent in classical medical mycology should interpret and reconcile discrepant results, using knowledge of the genetic relatedness as well as the typical phenotypic features of various groups of medically significant fungal organisms.

Nucleotide sequence accession number. The sequence of the isolate examined in this study has been deposited in the GenBank database under accession no. AY744467.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Hall, G., S. K. Pratt-Rippin, and J. A. Washington.** 1992. Evaluation of a chemiluminescent probe assay for identification of *Histoplasma capsulatum* isolates. J. Clin. Microbiol. **30:**3003–3004.
3. **Huffnagle, K. E., and R. M. Gander.** 1993. Evaluation of Gen-Probe's *Histoplasma capsulatum* and *Cryptococcus neoformans* AccuProbes. J. Clin. Microbiol. **31:**419–421.
4. **Iwen, P. C., L. Sigler, S. Tarantolo, D. A. Sutton, M. G. Rinaldi, R. P. Lackner, D. I. McCarthy, and S. H. Hinrichs.** 2000. Pulmonary infection caused by *Gymnascella hyalinospora* in a patient with acute myelogenous leukemia. J. Clin. Microbiol. **38:**375–381.
5. **Kaufman, L., and P. G. Standard.** 1978. Immunoidentification of cultures of fungi pathogenic to man. Curr. Microbiol. **1:**135–140.
6. **Kohne, D. E., A. G. Steigerwalt, and D. J. Brenner.** 1984. Nucleic acid probe specific for members of the genus *Legionella*, p. 107–108. *In* C. Thornsberry et al. (ed.), Legionella. Proceedings of the 2nd International Symposium. American Society for Microbiology, Washington, D.C.
7. **Millar, B. C., X. Jiru, M. J. Walker, J. P. Evans, and J. E. Moore.** 2003. False identification of *Coccidioides immitis*: do molecular methods always get it right? J. Clin. Microbiol. **41:**5778–5780.
8. **Nichols, D. K., R. S. Weyant, E. W. Lamirandel, L. Sigler, and R. T. Mason.** 1999. Fatal mycotic dermatitis in captive brown tree snakes (*Boiga irregularis*). J. Zoo Wildl. Med. **30:**111–118.
9. **Padhye, A. A., G. Smith, D. McLaughlin, P. G. Standard, and L. Kaufman.** 1992. Comparative evaluation of a chemiluminescent DNA probe and an exoantigen test for rapid identification of *Histoplasma capsulatum*. J. Clin. Microbiol. **30:**3108–3111.
10. **Paré, J. A., L. Sigler, D. B. Hunter, R. C. Summerbell, D. A. Smith, and K. L. Machin.** 1997. Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (Apinis) Currah. J. Zoo Wildl. Med. **28:**443–453.
11. **Sigler, L.** 2003. Ascomycetes: the Onygenaceae and other fungi from the order Onygenales, p. 195–236. *In* D. H. Howard (ed.), Pathogenic fungi in humans and animals. Marcel Dekker, New York, N.Y.
12. **Stockman, L., K. A. Clark, J. M. Hunt, and G. D. Roberts.** 1993. Evaluation of commercially available acridinium ester-labeled chemiluminescent DNA probes for culture identification of *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*. J. Clin. Microbiol. **31:**845–850.
13. **Thomas, A. D., L. Sigler, S. Peucker, J. H. Norton, and A. Nielan.** 2002. *Chrysosporium* anamorph of *Nannizziopsis vriesii* associated with fatal cutaneous mycoses in the salt-water crocodile (*Crocodylus porosus*). Med. Mycol. **40:**143–151.
14. **Untereiner, W. A., J. A. Scott, F. A. Naveau, L. Sigler, J. Bachewich, and A. Angus.** 2004. The *Ajellomycetaceae*, a new family of vertebrate-associated Onygenales. Mycologia **96:**811–820.
15. **White, T. J., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, New York, N.Y.