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Evaluation of Gen-Probe's *Histoplasma capsulatum* and *Cryptococcus neoformans* AccuProbes

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Gen-Probe's DNA probes were evaluated for use in the identification of clinical isolates of *Histoplasma capsulatum* var. *capsulatum* and *Cryptococcus neoformans*. Ninety-five mould-phase fungi were probed, including 41 isolates of *H. capsulatum* var. *capsulatum*. Similarly, 98 yeasts, including 42 *C. neoformans* isolates, were examined by using the *C. neoformans* DNA probe. In the study, both probes demonstrated 100% specificity and 100% sensitivity. Their use in the clinical laboratory may significantly reduce the time required for definitive identification of fungi.

Fungi associated with systemic disease including *Histoplasma capsulatum* var. *capsulatum* and *Cryptococcus neoformans* are increasingly recovered from an expanding population of immunosuppressed individuals. Selection of effective treatment regimens for these patients is dependent on timely and accurate identification of fungal isolates. Unfortunately, several problems exist in the current laboratory methodology for identification of fungi, particularly *H. capsulatum* var. *capsulatum*.

Colonies of *H. capsulatum* var. *capsulatum* are slow growing; mycelial forms usually mature within 15 to 20 days, but maturation may take up to 8 weeks (20). One problem encountered in the macroscopic identification of *H. capsulatum* var. *capsulatum* is that variations in colony morphotypes exist (2). Wet preparations of the mycelial phase of *H. capsulatum* var. *capsulatum* may reveal distinct tuberculate macroconidia; however, macroconidia may be present as smooth-walled structures in some cultures (2, 17).

Differentiation of *H. capsulatum* var. *capsulatum* from other dimorphic or saprophytic fungi having similar colony morphologies may be accomplished by testing for the production of exoantigens or conversion to the yeast phase (20). Prolonged incubation of cultures for at least 4 weeks may be required for production of sufficient exoantigen (3). Testing for exoantigen production by immunodiffusion is an involved procedure requiring over 36 h and demands technical expertise in test interpretation (9). Conversion of *H. capsulatum* var. *capsulatum* to the yeast phase is often difficult and may take even longer than exoantigen testing (3).

Multiple tests are available for the identification of *C. neoformans*. The encapsulated yeasts may be detected directly in cerebrospinal fluid by using India ink; however, the test sensitivity varies depending on the patient population (12, 18). Another direct detection method, latex agglutination, demonstrates capsular antigen in the cerebrospinal fluid or serum of 90% of patients with cryptococcal meningitis (1). Infrequently, false-positive results are observed (5, 7). When *C. neoformans* is recovered in culture, the yeasts may be presumptively identified within 3 h by demonstrating urease production (21), phenol oxidase production (10), and failure to utilize an inorganic nitrate substrate (8). Several commercial systems reliably identify most *C. neoformans* isolates, although 24 to 72 h is usually required for a definitive identification (4, 6, 15, 19).

Recently developed methods for the identification of fungal isolates such as species-specific DNA probes might decrease the time to identification while maintaining a high degree of accuracy (11, 13). The first commercially available fungal DNA probes were released recently by the Gen-Probe Corporation. The goal of our study was to evaluate the Gen-Probe AccuProbe Culture Identification Reagent Kits (Gen-Probe Corp., San Diego, Calif.) for use in the identification of *H. capsulatum* var. *capsulatum* and *C. neoformans* recovered from clinical specimens at Parkland Memorial Hospital, Dallas, Tex.

Gen-Probe's chemiluminescence-labeled DNA probes are complementary to rRNA sequences. Labeled DNA-RNA hybrids are differentiated from nonhybridized probe and measured in a luminometer (PAL luminometer; Gen-Probe Corp.). A positive result is based on a manufacturer's cutoff value of greater than or equal to 1,500 PAL light units (PLU). Positive and negative controls, as recommended by the manufacturer, include *H. capsulatum* var. *capsulatum* ATCC 38904 and *C. neoformans* ATCC 32045, as well as *Blastomyces dermatitidis* ATCC 60916 and *Candida albicans* ATCC 18804, respectively. Approximately 1.5 to 2 h is required to complete the AccuProbe assays.

At Parkland Memorial Hospital, isolates of *C. albicans* were identified by the germ tube test (18). Yeasts other than *C. albicans* were identified by using either the Vitek Yeast Biochemical Card (Vitek Systems, Hazelwood, Mo.) or the API 20C system (Analytab Products, Inc., Plainview, N.Y.). Mould-phase isolates were identified by their macroscopic and microscopic morphologies. Identification of *H. capsulatum* var. *capsulatum* and other dimorphic fungi was confirmed by testing for the presence of exoantigen (20).

All fungal isolates were grown at room temperature on potato dextrose agar. The isolates were probed in accordance with the manufacturer's guideline concerning age of cultures. The majority of the filamentous colonies were probed within 1 to 2 weeks after the first appearance of growth. Yeasts were usually tested within 2 days. Briefly, 1- to 2-mm^3 samples of yeasts or filamentous growth were suspended in lysis reagent and hybridization buffer (supplied by Gen-Probe). The samples were sonicated for 15 min; this was followed by a 10-min incubation at 95°C. Aliquots of lysed samples were transferred to tubes containing probe and were hybridized for 15 min at 60°C. Gen-Probe's selec-

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TABLE 1. Isolates probed with the *H. capsulatum* AccuProbe

Strain	No. of isolates[a]
Histoplasma capsulatum var. *capsulatum*	41
Absidia sp.	1
Alternaria sp.	1
Aspergillus niger[b]	6
Aspergillus nidulans	1
Aspergillus clavatus	1
Aspergillus flavus	1
Aspergillus terreus	1
Bipolaris spp.	2
Blastomyces dermatitidis[b]	5
Chaetomium sp.	1
Chrysosporium spp.[b]	6
Cladosporium spp.	4
Coccidioides immitis[b]	2
Curvularia sp.	1
Drechslera sp.	1
Fonsecaea pedrosoi	1
Fusarium sp.	1
Microsporum canis	1
Microsporum gypseum	1
Mucor sp.	1
Nigrospora sp.	1
Paecilomyces spp.	2
Penicillium spp.	4
Scopulariopsis sp.	1
Sporothrix schenckii	1
Syncephalastrum spp.	2
Trichophyton rubrum	1
Trichophyton tonsurans	1
Trichophyton schoenleinii	1
Ustilago sp.	1

[a] All isolates were recovered from clinical specimens at Parkland Memorial Hospital, unless otherwise noted.

[b] Five isolates of *A. niger*, five isolates of *Chrysosporium* species, and all isolates of *B. dermatitidis* and *C. immitis* were kindly provided by the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio.

TABLE 2. Isolates probed with the *C. neoformans* AccuProbe

Strain	No. of isolates[a]
Cryptococcus neoformans	42
Cryptococcus albidus	6
Cryptococcus laurentii	2
Cryptococcus luteolus	1
Candida albicans	7
Candida lipolytica	3
Candida parapsilosis	6
Candida tropicalis	5
Candida krusei	5
Candida lusitaniae	2
Candida lambica	1
Candida guilliermondii	1
Rhodotorula rubra	2
Saccharomyces cerevisiae	3
Sporobolomyces salmonicola	1
Torulopsis glabrata	8
Trichosporon beigelii	1
Trichosporon pullulans	1
Torulaspora delbrueckii	1

[a] All isolates were recovered from clinical specimens at Parkland Memorial Hospital.

tion reagent, which allows for differentiation of nonhybridized and hybridized probes by the hydrolysis of a single-stranded probe, was added. The tubes were incubated for 5 min at 60°C. The chemiluminescence of the hybridized probes was read with a PAL luminometer.

Ninety-five mould-phase isolates, 41 *H. capsulatum* var. *capsulatum* and 54 isolates of 23 other genera, were probed with the *H. capsulatum* Culture Identification Reagent Kit (Table 1). Fungal isolates of *Coccidioides immitis* and *B. dermatitidis* were included in the study because dimorphic fungi may be macroscopically indistinguishable from one another (20). Fungi with some degree of microscopic similarity to *H. capsulatum* var. *capsulatum*, such as *Chrysosporium* spp., were also tested. Isolates frequently encountered in the laboratory and possible plate contaminants were included in the study to determine whether false-positive results would be obtained.

Ninety-eight yeast isolates, including 42 strains of *C. neoformans* and 56 isolates of 18 species and 7 other genera, were probed with the AccuProbe *C. neoformans* Culture Identification Reagent Kit (Table 2). Several species of *Cryptococcus* other than *C. neoformans* including *C. albidus*, *C. laurentii*, and *C. luteolus* were examined for possible cross-species hybridization. Other commonly encountered yeast isolates were also included in the study.

All 41 isolates of *H. capsulatum* var. *capsulatum* demonstrated positive results according to the manufacturer's cutoff value of greater than or equal to 1,500 PLU. Negative test results were recorded for the other 54 filamentous fungal isolates. Similar results were obtained with the species-specific probe for *C. neoformans*. All 42 isolates of *C. neoformans* reacted with the probe, whereas RNA from the other 56 yeast isolates did not hybridize with the probe. Therefore, in this study, the specificities and sensitivities of both probes were 100%.

The ranges of values for positive and negative isolates, using the *H. capsulatum* probe, were 2,236 to 9,929 PLU and 21 to 1,276 PLU, respectively. The value of 1,276 PLU, obtained from an isolate of *Chaetomium* species, was strikingly higher than results from other negative isolates, which all had readings below 650 PLU. Use of the *C. neoformans* probe yielded ranges of positive and negative values for isolates of 7,905 to 9,693 PLU and 22 to 391 PLU, respectively. Except for the single high negative result obtained with the *Chaetomium* species, the positive and negative test results were widely separated.

Similar to our results with the commercial probe, Keath and coworkers (11) developed an *H. capsulatum*-specific DNA probe which hybridized with five *H. capsulatum* var. *capsulatum* isolates tested but which failed to hybridize with two strains of *B. dermatitidis* and one isolate of *Chrysosporium* species. Data from an abstract presented by Padhye and coworkers (14) support the utility of the commercial *H. capsulatum* probe for culture identification. A second group of researchers (16) used the *H. capsulatum* Gen-Probe product to test 54 clinical isolates including 13 *H. capsulatum* var. *capsulatum* strains. In their study, a single strain of *Aspergillus niger* probed with the *H. capsulatum* AccuProbe demonstrated a false-positive chemiluminescence result. Six isolates of *A. niger* were tested in the current study; positive results were not observed for any of the isolates. The reason for the discrepancy in results between the two studies is unclear.

A major advantage in using AccuProbe kits is the rapid identification of *C. neoformans* and *H. capsulatum* var. *capsulatum* isolates. The test is technically easy to perform. A small inoculum is required for probing. This is in contrast to the exoantigen test, which requires confluent growth on a

slant or plate. Positive and negative test results are easily differentiated. An additional advantage is the short time period needed to perform the test (approximately 2 h). Disadvantages associated with the use of AccuProbe kits include the requirement for expensive instrumentation and a probe shelf-life of 6 months, which may be insufficient for small laboratories that receive few specimens.

To our knowledge, this is the first study that evaluated both Gen-Probe's *H. capsulatum* and *C. neoformans* AccuProbe Culture Identification Kits. The sensitivities and specificities of both AccuProbe kits tested were found to be 100%; no isolate that we probed yielded a false-positive or a false-negative result. Additionally, the use of nucleic acid hybridization may significantly decrease the time required for identification of fungal isolates, particularly *H. capsulatum* var. *capsulatum*, and may confirm the identity of an isolate with a high degree of accuracy.

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