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Evaluation of Commercially Available Acridinium Ester-Labeled Chemiluminescent DNA Probes for Culture Identification of *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*

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Four commercially available acridinium ester-labeled DNA probes directed against rRNA were evaluated for their ability to identify *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans* in culture. rRNA was extracted by sonication of 1- to 2-mm^2 portions of cultures of fungi in two chaotropic reagents with glass beads. Following a heat inactivation step, the extracts were hybridized in solution with probes specific for each pathogen. The acridinium ester reporter moiety of nonhybridized probe was selectively hydrolyzed, and chemiluminescence of specific DNA:RNA hybrids was quantitated in relative light units with a luminometer. A positive identification required a relative light unit value of ≥50,000. Sensitivity and specificity of the probes were determined by probing cultures of the respective pathogenic fungi (target) and nontarget fungi. Both mycelial and yeast forms of the dimorphic fungi (*B. dermatitidis* and *H. capsulatum*) were tested. For *B. dermatitidis*, sensitivity and specificity were 87.8 and 100%, respectively (74 target and 219 nontarget fungi tested). For *C. immitis*, sensitivity and specificity were 99.2 and 100%, respectively (122 target and 164 nontarget fungi tested). For *H. capsulatum*, sensitivity and specificity were 100 and 100%, respectively (86 target and 154 nontarget fungi tested). For *C. neoformans*, sensitivity and specificity were 97 and 100%, respectively (100 target and 230 nontarget fungi tested). For *B. dermatitidis*, *C. immitis*, and *C. neoformans*, repeat testing increased the respective sensitivities to 97.3, 100, and 100%. The high sensitivities and specificities of the probes, the relatively short time (less than 1 h) required to perform the assay, and the availability of standardized reagent kits make the acridinium ester-labeled DNA probes well suited to laboratories in need of a rapid method to identify these fungal pathogens. Further, use of the probes to identify pathogenic fungi as soon as colonies appear on primary recovery media significantly shortens the time to reporting.

Rapid molecular diagnostic methods for the detection and/or identification of prokaryotic and eukaryotic microorganisms based on unique rRNA sequences are being developed in rapidly increasing numbers (4, 6). Such probe-based methods are especially important for the dimorphic fungal pathogens, which often grow slowly and require an extended time for identification (10, 11). This report presents an evaluation of four nucleic acid probes designed by Gen-Probe, Inc. (San Diego, Calif.) for the culture identification of *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*.

(A portion of the information in this report was presented at the 90th Annual and 91st General Meetings of the American Society for Microbiology [17, 18].)

MATERIALS AND METHODS

Commercially available AccuProbe culture identification reagent kits (Gen-Probe) designed for identification of four fungal pathogens, *B. dermatitidis*, *C. immitis*, *H. capsulatum*, and *C. neoformans*, were evaluated against target as well as nontarget organisms. These comprised clinical and reference isolates of fungi in the Mayo Clinic culture collection which were identified by conventional methods (5). Cultures varied in age at the time of testing, but all were less than 30 days old. Fungi were grown at 30°C on a variety of selective and nonselective media commonly used in a clinical microbiology laboratory, and the dimorphic fungi were converted to their yeast form at 35°C. Spherules of *C. immitis* were produced in Converse culture medium (2). Media included brain heart infusion agar containing 10% sheep blood, 16 μg of chloramphenicol per ml, and 5 μg of gentamicin per ml, with and without 0.5 mg of cycloheximide per ml; inhibitory mold agar; cottonseed agar; lactrimel agar; Middlebrook 7H10 agar; mycobiotic agar; Sabouraud dextrose agar (Emmon's modification); yeast extract agar; and yeast nitrogen base agar (5, 20).

Methods for sample preparation and hybridization provided by the manufacturer were followed. Briefly, rRNA was extracted by sonication of 1- to 2-mm^2 samples of fungal cultures in chaotropic reagents with glass beads for 15 min at 25°C. Following a heat inactivation step at 95°C for 15 min, the RNA extracts were hybridized for 15 min at 60°C in solution with probes specific for *B. dermatitidis*, *C. immitis*, *H. capsulatum*, or *C. neoformans*. The acridinium ester reporter moiety of nonhybridized probe was hydrolyzed in

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TABLE 1. Summary of AccuProbe results used for culture identification

Characteristic	Result for probe:			
	C. immitis	*C. neoformans*	*H. capsulatum*	*B. dermatitidis*
No. of target organisms (>50,000 RLU)	121	97	86	65
No. of target organisms (<50,000 RLU)	1	3	0	9
Mean RLU (positive)	494,231	441,005	341,111	200,943
RLU range (positive)	55,955–672,500	91,063–503,185	82,841–454,333	56,632–510,363
No. of nontarget[a] organisms (<50,000 RLU)	164	230	154	219
Mean RLU (negative)	2,640	2,863	3,994	5,704
RLU range (negative)	346–15,510	703–22,457	703–37,675	963–32,071
Sensitivity (%)	99.2	97.0	100	87.8
Specificity (%)	100	100	100	100
Sensitivity after repeat testing (%)	100	100	100	97.3

[a] See Table 2 for nontarget fungi and other organisms tested with each probe.

selection reagent for 5 min at 60°C, and formation of specific DNA-RNA hybrids was quantitated in relative light units (RLU) with a luminometer (Leader 1; Gen-Probe). A positive identification required an RLU value of 50,000 or greater, as specified by the manufacturer. An equivocal range of 40,000 to 50,000 RLU indicated retesting. Both filamentous and yeast forms of the dimorphic fungi were tested. All organisms with discrepant RLU results were retested with the appropriate probe and reidentified by conventional methods.

RESULTS

A total of 1,149 fungi were tested in this assay format. All target organisms tested (n = 382) yielded an $\bar{x}$ RLU of 369,322, and all nontarget (n = 767) organisms gave an $\bar{x}$ RLU of 3,800. These results are shown in Table 1.

A total of 74 cultures of *B. dermatitidis* (62 mold and 12 yeast forms) were tested with the *B. dermatitidis* AccuProbe. Sixty-five target cultures initially yielded positive results, with an $\bar{x}$ RLU value of 200,943. Of the nine target cultures that initially yielded negative results ($\bar{x}$ RLU of 25,114), seven were positive when subcultures were retested ($\bar{x}$ RLU of 175,177). For the remaining two false-negative cultures, the initial probe results were 35,980 and 39,118 RLU, which on retesting yielded RLU values of 5,538 and 36,587, respectively. A total of 219 nontarget fungi (Table 1) were tested with the probe and yielded negative results ($\bar{x}$ RLU values = 5,704).

A total of 122 cultures of *C. immitis* (119 mold and 3 spherule forms) were tested with the *C. immitis* AccuProbe, and 121 yielded positive results ($\bar{x}$ RLU = 494,231). The culture initially yielding a negative result (RLU = 47,578) was retested and gave a positive result of 647,241 RLU. A total of 164 nontarget fungi (Table 1) were tested, and all were negative ($\bar{x}$ RLU = 2,640).

A total of 86 cultures of *H. capsulatum* (80 mold and 6 yeast forms) were tested with the *H. capsulatum* AccuProbe. All yielded positive results ($\bar{x}$ RLU = 341,111). A total of 154 nontarget fungi (Table 1) were tested, and all yielded negative results ($\bar{x}$ RLU = 3,994).

A total of 100 isolates of *C. neoformans* were tested with the *C. neoformans* AccuProbe. Ninety-seven initially yielded positive results ($\bar{x}$ RLU = 441,005). The three organisms having negative results were retested and gave positive results. Initial and subsequent RLU values were 1,229, 3,169, and 8,621 and 480,210, 428,653, and 489,359 RLU, respectively. A total of 230 nontarget fungi (Table 1) were tested, and all yielded negative results ($\bar{x}$ RLU = 2,863).

DISCUSSION

For ease of comparison, the sensitivity and specificity for each AccuProbe are presented in Table 1. Culture identification was used as the standard for identifying cultures tested with the probes. The sensitivity of the probes is high, ranging from 87.8 to 100% on initial testing, and increased to 97.3 to 100% after retesting of false negatives. The 100% specificity of all four probes indicates that no false-positive results were seen among all the nontarget fungi tested.

The AccuProbes are now in routine use in the clinical microbiology laboratory at the Mayo Clinic and have virtually replaced exoantigen testing, which was used for many years to confirm the identification of dimorphic pathogens. Exoantigen testing for definitive identification of the dimorphic pathogens requires subculture of the organism and preparation and filtering of merthiolate water extracts of the subculture, followed by concentration of the extract and agar immunodiffusion with reference antisera for up to 48 h. The readily available and highly specific AccuProbes significantly shorten the time to reporting results. One group has reported use of the AccuProbe to identify cultures of *B. dermatitidis* which were exoantigen negative (15). A very significant advantage of the AccuProbes is their utility for testing isolated colonies of suspected pathogenic fungi growing on primary recovery media in the midst of other saprophytic fungi, enabling the clinical laboratory to shorten time to result reporting by days to weeks.

All four probes were used to test three aerobic actinomycetes and one culture of *Prototheca wickerhamii*. All were negative (data not shown) with the exception of the *H. capsulatum* probe, which gave 62,494 RLU when tested with *Nocardia brasiliensis*. The culture was grown on medium containing 10% sheep blood. Blood components can yield false-positive probe results because of nonspecific chemiluminescence. Testing from blood-containing media does not always present a problem but should be considered when evaluating discrepant positive results. If blood-containing media are used, the medium alone should be used as the negative control to rule out potential false-positive results. In practice, by the time a clinical laboratory was ready to probe, it would be known the culture was not a *Nocardia* sp. on the basis of colony morphology and microscopic exam.

TABLE 2. Fungi tested with probes

Organism	No. of organisms tested with each probe:			
	Coccidioides immitis	*Cryptococcus neoformans*	*Histoplasma capsulatum*	*Blastomyces dermatitidis*
Dimorphic pathogens				
Blastomyces dermatitidis	10	2	17	74
Coccidioides immitis	122	1	6	5
Histoplasma capsulatum	20	1	86	86
Sporothrix schenckii	3	3	3	4
Dermatophytes				
Epidermophyton floccosum	1	1	1	1
Microsporum audouinii	1	0	0	0
Microsporum canis	1	1	1	1
Microsporum cookei	1	1	1	1
Microsporum distortum	1	1	0	0
Microsporum gypseum	1	1	1	1
Microsporum nanum	1	1	1	1
Microsporum persicolor	1	1	1	1
Microsporum racemosum	1	1	1	1
Microsporum vanbreuseghemii	1	1	1	1
Trichophyton ajelloi	0	1	0	0
Trichophyton concentricum	1	1	1	1
Trichophyton equinum	1	1	1	1
Trichophyton megninii	0	1	1	0
Trichophyton mentagrophytes	3	2	4	4
Trichophyton rubrum	4	2	5	5
Trichophyton schoenleinii	1	0	1	1
Trichophyton terrestre	2	2	2	2
Trichophyton tonsurans	1	1	0	0
Trichophyton verrucosum	0	1	2	1
Trichophyton violaceum	1	1	1	1
Zygomycetes				
Absidia sp.	1	1	1	1
Circinella sp.	1	1	1	1
Cunninghamella sp.	1	1	1	1
Mucor sp.	1	1	1	1
Rhizopus sp.	1	1	1	1
Syncephalastrum sp.	1	1	1	1
Hyaline hyphomycetes				
Acremonium sp.	2	2	2	2
Aspergillus clavatus	1	1	1	1
Aspergillus flavus	2	2	2	2
Aspergillus fumigatus	2	2	2	2
Aspergillus glaucus	1	1	0	0
Aspergillus nidulans	1	1	1	1
Aspergillus niger	2	2	2	2
Aspergillus sp.	2	0	1	1
Aspergillus terreus	1	1	1	1
Aspergillus uniguis	1	1	1	1
Aspergillus versicolor	1	1	1	1
Auxarthron sp.	2	1	2	2
Beauveria sp.	1	1	1	1
Chaetomium sp.	1	1	1	1
Chrysosporium sp.	2	1	2	2
Fusarium sp.	1	1	1	1
Fusarium solani	1	1	1	1
Geotrichum sp.	4	3	2	2
Gliocladium sp.	1	1	1	1
Gliomastix sp.	1	1	1	1
Graphium sp.	2	1	1	1
Malbranchea sp.	3	2	2	2
Metarrhizum sp.	1	1	1	1
Oedocephalum sp.	1	1	1	1
Paecilomyces sp.	1	1	1	1
Penicillium marneffei	1	1	1	1
Penicillium sp.	1	1	1	1
Phoma sp.	2	2	2	2
Pseudallescheria boydii	0	1	1	1
Scedosporium inflatum	1	1	1	1
Scopulariopsis brumptii	0	1	0	0

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TABLE 2—*Continued*

Organism	No. of organisms tested with each probe:			
	Coccidioides immitis	*Cryptococcus neoformans*	*Histoplasma capsulatum*	*Blastomyces dermatitidis*
Scopulariopsis sp.	2	1	2	2
Sepedonium sp.	1	2	1	1
Sporothrix cyanescens	1	1	1	1
Sporotrichum sp.	1	0	0	0
Trichoderma sp.	1	1	1	1
Trichothecium sp.	0	0	1	1
Tritirachium roseum	1	1	1	1
Verticillium sp.	1	1	1	1
Dematiaceous hyphomycetes				
Alternaria sp.	1	1	1	1
Aureobasidium sp.	1	1	1	1
Beltrania sp.	1	1	0	0
Bipolaris sp.	2	2	2	2
Bispora sp.	1	1	1	1
Cephalotrichum sp.	1	1	1	1
Cladosporium sp.	2	2	2	2
Curvularia sp.	1	1	1	1
Drechslera sp.	1	1	1	0
Epicoccum sp.	0	1	0	0
Exophiala jeanselmei	1	1	1	1
Exserohilum sp.	3	2	2	2
Fonsecaea compacta	1	1	1	1
Fonsecaea pedrosoi	1	1	1	1
Helminthosporium sp.	1	1	1	1
Hendersonula toruloidea	1	1	1	1
Leptosphaerulina sp.	0	1	1	1
Madurella mycetomatis	1	1	1	1
Nigrospora sp.	1	1	1	1
Nodulosporium sp.	0	1	0	0
Papularia sp.	0	1	1	1
Phaeoannellomyces werneckii	1	1	1	1
Phaeococcomyces sp.	1	2	1	1
Phialophora richardsii	1	1	1	1
Phialophora verrucosa	1	0	1	1
Puciola spinosa	1	1	1	1
Rhinocladiella sp.	1	1	1	1
Stachybotrys sp.	0	1	0	0
Stemphylium sp.	1	1	1	1
Ulocladium sp.	2	1	1	1
Wangiella dermatitidis	1	1	1	1
Xylohypha bantiana	0	1	1	1
Yeasts and yeastlike organisms				
Blastoschizomyces capitatus	0	2	0	0
Candida albicans	1	6	1	1
Candida famata	0	2	0	0
Candida (*Torulopsis*) *glabrata*	1	6	1	1
Candida guilliermondii	1	4	1	1
Candida kefyr	1	3	1	1
Candida krusei	1	4	1	1
Candida lambica	0	2	0	0
Candida lipolytica	0	2	0	0
Candida lusitaniae	0	2	0	0
Candida parapsilosis	1	8	1	1
Candida rugosa	0	1	0	0
Candida stellatoidea	0	1	0	0
Candida tropicalis	2	8	2	2
Cryptococcus albidus	1	18	0	0
Cryptococcus laurentii	0	9	0	0
Cryptococcus luteolus	0	1	0	0
Cryptococcus neoformans	1	100	2	1
Cryptococcus sp.	0	2	0	0
Cryptococcus uniguttulatus	0	3	0	0
Hanseniaspora sp.	1	1	0	0
Hansenula sp.	1	2	1	1
Kluyveromyces lactis	1	2	1	1
Malassezia furfur	0	0	1	1
Pichia farcinosa	1	1	1	1

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TABLE 2—*Continued*

Organism	No. of organisms tested with each probe:			
	Coccidioides immitis	*Cryptococcus neoformans*	*Histoplasma capsulatum*	*Blastomyces dermatitidis*
Rhodotorula sp.	1	15	1	1
Saccharomyces cerevisiae	1	3	1	1
Sporobolomyces sp.	1	3	1	1
Trichosporon beigelii	1	3	1	1
Ustilago sp.	1	1	1	1

Table 2 summarizes the nontarget fungi tested with each of the probes and emphasizes that the fungi commonly and less commonly encountered in the clinical laboratory do not give false-positive results. Although none of the target fungi are dematiaceous hyphomycetes, this group of fungi was included since they might have yielded false positives because of pigment chemiluminescence (3). These fungi also occur commonly and may not appear as dematiaceous when cultures are young.

The fungi possibly confused with *B. dermatitidis* include *Trichophyton rubrum*, which commonly produces singly borne microconidia or sterile hyphae. Likewise, the morphologically similar hyaline hyphomycetes *Pseudallescheria boydii* and *Chrysosporium* sp. must be distinguished from *B. dermatitidis*. The broad-based yeast form of *B. dermatitidis* is relatively unique, though small forms in tissue which might pose identification problems have been described (13). No isolates of *Paracoccidioides brasiliensis* were tested in this study, but previous reports have shown that both mold and yeast forms of this fungus yielded positive results with the *B. dermatitidis* probe (1). The false-negative results we obtained on initial testing with 9 and on repeat testing with 2 of 74 isolates of *B. dermatitidis* indicate that conversion to yeast form and/or exoantigen testing is important for the positive identification of this pathogen. Detection by direct examination of the yeast form, with subsequent growth of the mold form, or in vitro conversion of the hyaline mold to the yeast form, should be included in the identification protocol. It is known that certain rRNA regions are highly conserved (21) and may be responsible for the positive results with *P. brasiliensis* with the *B. dermatitidis* probe. The microscopic morphologic features of yeast forms of these two dimorphic fungi should readily allow differentiation between these species (8).

The characteristic alternating arthroconidia of *C. immitis* generally provide an early presumptive identification of this rapidly growing dimorphic pathogen. Other hyaline molds which produce arthroconidia that may be confused with *C. immitis* include *Geotrichum* spp., *Trichosporon* spp., *Malbranchea* spp., and *Auxarthron* spp. All of these were tested and yielded negative results. Although the zygomycetes usually produce aseptate hyphae, they grow rapidly and young cultures could potentially be confused with *C. immitis*. All morphologically similar nontarget fungi tested yielded negative results. Cultured spherules of *C. immitis* were tested and gave positive results, suggesting that it may eventually be possible to probe tissue spherules.

Fungi effectively distinguished from *H. capsulatum* with the *H. capsulatum* probe included those with morphologically similar microscopic features, namely, dermatophytes, notably *T. rubrum*, and other hyaline hyphomycetes, in addition to *B. dermatitidis*, which produce singly borne microconidia (Table 2). The yeast form of *H. capsulatum* might be confused with *C. neoformans*, *Candida glabrata*, and endospores of *C. immitis* (5), since they are in the same size range (2 to 5 μm).

The high species-specificity of the *C. neoformans* probe is underscored by the results for nontarget yeasts tested, including other potentially pathogenic (7, 9) species of *Cryptococcus* (Table 2). Alternative identification methods such as rapid urease testing (14), carbohydrate utilization, antigen detection in body fluids, and phenol oxidase indicator media are available for the diagnosis of cases of suspected cryptococcosis (5, 20). The *C. neoformans* probe yields rapid, specific results and can reduce time to diagnosis.

The wide variety of nontarget fungi tested and the high specificity for all four probes indicate that the design of probes based on unique rRNA is a sound approach to utilizing molecular biological identification methods in the clinical laboratory. Similarly high specificities for the Accu-Probes have been reported by other laboratories (3, 12, 16, 19). These methods will see increasing use, particularly in view of the simplified format of the probe detection assay. These probes have proved extremely useful because of the ease and rapidity with which they identify suspected dimorphic pathogens from primary recovery media. Nucleic acid probes can save days to weeks for the definitive identification of the previously mentioned dimorphic fungi and the yeast *C. neoformans*.

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