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Comparative Evaluation of Chemiluminescent DNA Probe Assays and Exoantigen Tests for Rapid Identification of *Blastomyces dermatitidis* and *Coccidioides immitis*

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Chemiluminescent DNA probe (Accuprobe) assays developed by Gen-Probe, Inc. (San Diego, Calif.), for the rapid identification of *Blastomyces dermatitidis* and *Coccidioides immitis* were evaluated and compared with the exoantigen test by using 74 mycelial cultures of *B. dermatitidis* and 72 mycelial cultures of *C. immitis*. Seventeen isolates of the dimorphic pathogen *Paracoccidioides brasiliensis* were included because of their gross morphologic and antigenic relatedness to *B. dermatitidis*. The heterologous fungi, namely, species of *Chrysosporium*, which are often confused with *B. dermatitidis*, and species of *Malbranchea*, which morphologically resemble *C. immitis*, were tested. All 74 of the *B. dermatitidis* mycelial isolates were correctly identified by the Accuprobe assay for *B. dermatitidis* within 2 h. However, the *B. dermatitidis* probe cross-hybridized with rRNA extracts of 10 of the 17 *P. brasiliensis* isolates, misidentifying them as *B. dermatitidis*. All 72 of the *C. immitis* isolates were identified correctly with the *C. immitis* probe. None of the other heterologous fungi belonging to *Chrysosporium* spp., *Malbranchea* spp., *Onychocola canadensis*, and *Geotrichum* sp. were cross-reactive with the *B. dermatitidis* and *C. immitis* probes. The exoantigen tests specifically identified 74 *B. dermatitidis*, 72 *C. immitis*, and 17 *P. brasiliensis* isolates within 48 to 72 h and differentiated the related heterologous fungi from the three dimorphic fungal pathogens.

In our comparative evaluation of commercially available, chemiluminescent DNA probes (Accuprobes) and the exoantigen test for the rapid identification of dimorphic fungi, we first found that the DNA probe for culture identification of *Histoplasma capsulatum* provides accurate identification of both conidiating and nonconidiating isolates within 2 h (12). In the present study, DNA probes for *Blastomyces dermatitidis* and *Coccidioides immitis*, made available through the courtesy of Gen-Probe, Inc., San Diego, Calif., were evaluated and compared with the aforementioned exoantigen tests for the two dimorphic fungal pathogens. Two recent evaluations assessed the value of the *B. dermatitidis* and of the *B. dermatitidis* and *C. immitis* probes, respectively (15, 20). However, neither evaluation included any African isolates of *B. dermatitidis* or isolates of *Paracoccidioides brasiliensis*. Earlier studies (8, 21) have shown that the majority of the African *B. dermatitidis* isolates are morphologically and antigenically distinct from North American isolates. In addition, the African isolates are well known for their resistance to in vitro conversion from the mycelial to yeast form at 37°C on commonly used conversion media (11). Most are incompatible when paired with the tester strains of *Ajellomyces dermatitidis* (9). Even though the areas of endemicity of *P. brasiliensis* do not overlap with those of *B. dermatitidis*, imported and long, latent cases of infection caused by *P. brasiliensis* among immigrant populations have been recorded in the United States and Europe (1), and the etiologic agents in such cases may not be correctly identified with the *B. dermatitidis* probe alone (20). Even though both of the previous evaluations (15, 20) included some *Chrysosporium* spp. among the heterologous fungi tested with the *B. dermatitidis* probe, neither study included isolates of *P. brasiliensis* or *Chrysosporium parvum*, the two taxa that morphologically and antigenically resemble *B. dermatitidis* (7, 10). Similarly, atypical variants of *C. immitis* were not tested with the *C. immitis* probe. In light of the aforementioned data, the present study was undertaken to further determine the specificities of the *B. dermatitidis* and *C. immitis* probes.

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MATERIALS AND METHODS

Cultures. Seventy-four mycelial isolates of *B. dermatitidis*, including 51 from North America, 20 from Africa, 1 from the Middle East, and 2 from Asia, were studied. Sixty-seven of the 74 isolates were from humans, 5 were from animals (2 cats, 1 dog, 1 polar bear, and 1 bat), and 2 were isolated from soil containing organic debris (Table 1). The heterologous fungi selected for testing with the *B. dermatitidis* probe were those clinical isolates of *Chrysosporium* spp. which we received as *B. dermatitidis*. These isolates were misidentified as mycelial anamorphs of *B. dermatitidis* on the basis of their gross morphologic similarity with *B. dermatitidis* or results of exoantigen tests with commercial reagents without appropriate references. The selection of *P. brasiliensis* was based on its gross morphologic and antigenic similarities with *B. dermatitidis* (7, 10). The details for the heterologous fungi tested are summarized in Table 1.

Among the 72 isolates of *C. immitis*, 10 of the 12 isolates described by Huppert et al. (4) in 1967 were tested because of their atypical colonial morphologies. Those investigators noted the occurrence of powdery, granular, velvety, cottony to woolly isolates that developed pigmented colonies with hues of pink, a very pale lavender, buff, cinnamon, yellow, and brown pigments. Doubts about the identities of some these isolates were raised by Sigler and Carmichael (17). One of these 12 isolates (isolate C-318 [ATCC 38148] of Huppert et al. [4]) was shown by Kaufman and Standard (6) not to be *C. immitis* by the exoantigen test. Huppert et al. (5) later confirmed Kauf-

TABLE 1. Sources of cultures tested by the Gen-Probe Accuprobe assay and the exoantigen test for *Blastomyces dermatitidis* and *Coccidioides immitis*

Test organism	No. of isolates	Country of origin	Source (no. of isolates)
Blastomyces dermatitidis	45	United States	Humans
Blastomyces dermatitidis	4	United States	Cats (2), dog (1), polar bear (1)
Blastomyces dermatitidis	2	United States	Soil with organic debris
Blastomyces dermatitidis	20	Algeria, Angola, Morocco, Rwanda, South Africa, Tunisia, Uganda, Zaire	Humans
Blastomyces dermatitidis	1	Israel	Human
Blastomyces dermatitidis	2	India	Human (1), bat (1)
Paracoccidioides brasiliensis	17	Colombia, Brazil	Humans
Chrysosporium asperatum	2	United States	Humans
Chrysosporium parvum	4	Canada, United States	Rodents (3), human (1)
Chrysosporium tropicum	2	United States	Humans
Chrysosporium spp.	3	United States	Humans
Aphanoascus fulvescens	2	India	Humans
Coccidioides immitis	39	United States	Humans
Coccidioides immitis	10	United States	Humans (pigmented isolates)
Coccidioides immitis	2	Argentina	Humans
Coccidioides immitis	3	Guatemala	Humans
Coccidioides immitis	3	Mexico	Humans
Coccidioides immitis	3	Venezuela	Humans
Coccidioides immitis	12	United States	Soil
Malbranchea albolutea	1	Argentina	Soil
Malbranchea dendritica	2	United States	Soil
Malbranchea filamentosa	2	Argentina	Soil
Malbranchea gypsea	1	United States	Soil
Malbranchea pulchella	1	United States	Human
Malbranchea spp.	6	United States	Human
Auxarthron zuffianum	2	Argentina	Soil
Auxarthron zuffianum	2	United States	Soil
Auxarthron zuffianum	2	United States	Humans
Uncinocarpus reesii	2	Canada	Mating types
Uncinocarpus reesii	2	United States	Humans
Geotrichum spp.	2	United States	Humans
Onychocola canadensis	1	Canada	Human

man and Standard's (6) results. Further studies of this isolate by Sigler (16a) showed that it was a mixture of *Malbranchea dendritica* and *Uncinocarpus reesii* (6). This isolate was included in the present study as one of the two *M. dendritica* isolates listed in Table 1. Eleven of these 12 atypical isolates described by Huppert et al. (including isolate C-318) were deposited by S. H. Sun, Audie L. Murphy Memorial Veterans' Administration Hospital, San Antonio, Tex., in the American Type Culture Collection, Rockville, Md. We received these isolates through the courtesy of M. Huppert, S. H. Sun, as well as S. C. Jong, American Type Culture Collection. Similarly, five of six soil isolates from Argentina originally described as *C. immitis* by Borghi et al. (3) were included in the study because these isolates were later identified by Sigler et al. (18) as *Malbranchea albolutea*, *Malbranchea filamentosa*, and *Auxarthron zuffianum*. Of the 72 *C. immitis* isolates, 60 (including the 10 isolates described by Huppert et al. [4]) were recovered from patients and 12 were isolated from soil in the United States. Of the 60 isolates from humans, 2 were from Argentina, 3 were from Guatemala, 3 were from Mexico, 3 were from Venezuela, and 49 were from the United States (Table 1). The heterologous fungi tested with the *C. immitis* probe were selected on the basis of either their micromorphologic similarity to *C. immitis* or misidentifications as *C. immitis* by using commercial exoantigen test kits. The culture of *Onychocola canadensis* was included in the study because when tested by a commercially available exoantigen kit, it cross-reacted with the *C. immitis* reference system and was identified by the sender's laboratory as *C. immitis*. The culture was sent to us to verify the exoantigen test results, and confirmation was by the Gen-Probe Accuprobe assay.

Methods. All cultures were grown on Sabouraud dextrose agar (Emmons' modification) (Difco Laboratories, Detroit, Mich.) for 7 to 10 days at 25°C; *P. brasiliensis* cultures were incubated for 21 days at 25°C. We tested only the mycelial anamorphs of the test fungi with the probes and by the exoantigen tests.

Accuprobe testing. A pinhead-size (1- to 2-mm^2) mycelial growth inoculum from a 7- to 10-day-old colony of each isolate was suspended in a tube containing lysing reagent and a buffered solution of probe diluent and glass beads to extract the rRNA. The mixture was vortexed briefly, and the cell lysate was sonicated for 15 min and heated to 95°C in a water bath to inactivate any potentially infectious cells. One hundred microliters of the lysed rRNA extracts from the lysing reagent tubes was pipetted into tubes containing labeled *B. dermatitidis* and *C. immitis* DNAs, respectively, and were allowed to hybridize at 60°C for 15 min. After removing the probe reagent tube from the water bath, 300 μl of a selection reagent was added to preferentially hydrolyze the label on any nonhybridized single-stranded probe, allowing the differentiation of nonhybridized and hybridized probes by retaining the chemiluminescence of the probe hybridized with the rRNA target. The amount of the labeled, hybridized probe was measured in the probe assay luminometer as probe light units (PLUs). For each batch of cultures tested, a positive control culture of *B. dermatitidis*

TABLE 2. PLU determinations by Accuprobe assay of 74 isolates of *Blastomyces dermatitidis*, 17 isolates of *Paracoccidioides brasiliensis*, and 13 isolates of heterologous fungi tested with the *Blastomyces dermatitidis* DNA probe

Test organism	PLU range (no. of isolates)
Blastomyces dermatitidis	2,900–6,000 (9)
Blastomyces dermatitidis	6,100–9,000 (40)
Blastomyces dermatitidis	9,100–12,000 (25)
Blastomyces dermatitidis (positive control)	11,000–30,000
Histoplasma capsulatum (negative control)	120–250
Paracoccidioides brasiliensis	300–1,200 (7)
Paracoccidioides brasiliensis	1,500–3,400 (6)
Paracoccidioides brasiliensis	3,500–4,000 (1)
Paracoccidioides brasiliensis	4,100–5,000 (1)
Paracoccidioides brasiliensis	5,100–6,000 (2)
Chrysosporium asperatum	90–200 (2)
Chrysosporium parvum	90–200 (4)
Chrysosporium tropicum	80–168 (2)
Chrysosporium spp.	80–220 (3)
Aphanoascus fulvescens	90–160 (2)

TABLE 3. PLU determinations by Accuprobe assay of 72 *Coccidioides immitis* isolates and 26 heterologous fungi tested with the *Coccidioides immitis* probe

Test organism	PLU range (no. of isolates)
Coccidioides immitis	3,000–5,000 (2)
Coccidioides immitis	5,100–7,000 (1)
Coccidioides immitis	7,100–9,000 (4)
Coccidioides immitis	9,100–11,000 (2)
Coccidioides immitis	11,200–14,000 (63)
Coccidioides immitis (positive control)	11,186
Blastomyces dermatitidis (negative control)	90–180
Malbranchea albolutea	60–300 (1)
Malbranchea dendritica	150–300 (2)
Malbranchea filamentosa	60–300 (2)
Malbranchea gypsea	60–300 (1)
Malbranchea pulchella	60–300 (1)
Malbranchea spp.	60–300 (6)
Malbranchea anamorph of *Auxarthron zuffianum*	60–300 (6)
Malbranchea anamorph of *Uncinocarpus reesii*	601–900 (4)
Geotrichum sp.	60–300 (2)
Onychocola canadensis	60–300 (1)

ATCC 60916 and a negative control culture of *H. capsulatum* ATCC 38904 were used. For *C. immitis* isolates, *C. immitis* ATCC 38904 (Silveira strain) was used as the positive control culture and *B. dermatitidis* ATCC 60916 was used as the negative control culture. Samples with values of 1,500 PLUs or more indicated positive readings for *B. dermatitidis* and *C. immitis*. Samples with readings of between 1,200 and 1,499 PLUs were equivocal, necessitating reexamination of the cultures and repetition of the procedure. Samples with values of <1,200 PLUs were considered negative for *B. dermatitidis* and *C. immitis*.

Exoantigen test. A 7- to-10-day-old duplicate colony of each isolate on Sabouraud dextrose agar was simultaneously tested by the exoantigen tests for *B. dermatitidis*, *C. immitis*, and *P. brasiliensis* by the procedures of Kaufman and Standard (7). The results were read after 48 to 72 h.

RESULTS

Identification by the Accuprobe assay. Of the 74 mycelial cultures of *B. dermatitidis* tested, all were correctly identified by the Accuprobe assay within 2 h. The values ranged from 2,900 to 30,000 PLUs. The positive control culture of *B. dermatitidis* ATCC 60916 gave a value of 8,500 PLUs. However, rRNA extracts from 10 of the 17 *P. brasiliensis* isolates hybridized with the *B. dermatitidis* probe, giving positive readings of 1,500 to 6,000 PLUs on three separate testings, thus falsely identifying them as *B. dermatitidis*. The remaining seven *P. brasiliensis* isolates were negative with the *B. dermatitidis* probe, with values of <1,400 PLUs. None of the rRNA extracts from the heterologous *Chrysosporium* spp. tested hybridized with the *B. dermatitidis* probe (Table 2).

Surprisingly, none of the 11 isolates of Huppert et al. (4) studied by us showed colony morphology, as was originally shown in their Fig. 1 (4). After 8 weeks of incubation at 25°C on Sabouraud dextrose agar, the colonies were cottony, velvety, often moist, smooth, wrinkled, or with radial striations and very much resembled the colonial morphology of commonly encountered, typical *C. immitis* isolates. The colors of the colonies varied from white to dirty white, with orange-yellow to light tan on the reverse of the colonies. Many colonies developed hyphal tufts in the central areas. Isolate ATCC 38148 (isolate C-318) shown earlier not to be *C. immitis* by Kaufman and Standard (6) and Huppert et al. (5) by the exoantigen test was negative for *C. immitis* when tested with the *C. immitis* probe. The remaining 10 isolates of Huppert et al. (4) had readings ranging from 3,000 to 14,000 PLUs, thus confirming their original identification of the isolates as *C. immitis*. Six Argentinian soil isolates were negative when tested by exoantigen tests and with the *C. immitis* probe (range, 60 to 300 PLUs). These isolates have been shown to belong to the species *Malbranchea arcuata*, *M. albolutea*, *M. filamentosa*, and *A. zuffianum* (18). The 72 *C. immitis* isolates were identified correctly with the *C. immitis* probe (range, 3,000 to 14,000 PLUs). The positive control culture of *C. immitis* ATCC 38904 gave a value of 11,186 PLUs. None of the heterologous fungi tested was positive (range, 60 to 900 PLUs) with the *C. immitis* probe (Table 3).

Exoantigen tests. Each culture was concurrently tested for *B. dermatitidis*, *C. immitis*, and *P. brasiliensis* exoantigens, enabling the identification of all three dimorphic pathogens simultaneously. All 74 *B. dermatitidis*, 72 *C. immitis*, and 17 *P. brasiliensis* isolates were correctly identified by the exoantigen tests after 48 to 72 hours by using 10-day-old colonies (21-day-old colonies for *P. brasiliensis*). The 39 morphologically or antigenically related heterologous fungi were negative for *B. dermatitidis*, *C. immitis*, or *P. brasiliensis*.

DISCUSSION

Results of our studies indicated that the sensitivity of the *B. dermatitidis* probe is 100%. On the other hand, the sensitivity and specificity of the *C. immitis* probe were 100%. The specificity of the *B. dermatitidis* probe, however, dropped to 59% when the probe was tested against *P. brasiliensis*. None of the other heterologous fungi belonging to *Chrysosporium* spp. showed any cross-reactivity with the *B. dermatitidis* probe. Although cases of paracoccidioidomycosis are not routinely encountered in diagnostic laboratories in the United States, imported and latent cases have been diagnosed (1). The mycelial anamorph of *P. brasiliensis* grows much more slowly than that of *B. dermatitidis* does, even though colonies may resemble those of *B. dermatitidis*. Most isolates of *P. brasiliensis* grow for long periods of time without any conidial production (13). A nonconidiating, slow-growing colony may provide the first suspicion that the fungus to be tested by the Gen-Probe

Accuprobe assay may not be *B. dermatitidis*. In vitro conversion of *P. brasiliensis* to the yeast form at 37°C is also quite slow. The exoantigen test provides a rapid and accurate means of identifying such slow-growing isolates of *P. brasiliensis* (19).

Because some clinical isolates of *B. dermatitidis* may not produce diagnostically significant quantities of A or K antigens in slant cultures (2, 14), this method may not be sufficiently sensitive for identifying all *B. dermatitidis* isolates. If one suspects *B. dermatitidis* and the results of testing by a slant extraction method are negative, shake culture filtrates should be tested. Three- to 6-day-old growth extracts of *B. dermatitidis* isolates grown at 37°C in brain heart infusion broth shake cultures should be used for testing against anti-*B. dermatitidis* rabbit serum with control *B. dermatitidis*-specific A and K antigens. This modified procedure has proved to be helpful in accurately identifying troublesome isolates that do not produce A or K antigen in sufficient quantities (16) in slant cultures.

An analysis of the costs and time requirements done by Sandin et al. (14) showed that the costs required to perform the exoantigen test are one-half those of the DNA probe test. However, they found that variations among commercially available exoantigen test reagents may give false-negative results for some *B. dermatitidis* isolates. In order to identify such isolates correctly, exoantigen tests may have to be repeated several times, thus increasing the costs of and labor required to perform the exoantigen tests.

The DNA probes require special equipment such as a sonicator, a heating block, and a luminometer. Identification by the Gen-Probe Accuprobe assay is achieved within 2 h. Without cultural, histologic, serologic, and clinical background data, laboratory workers may not be able to anticipate the identity of the possible etiologic agent. As a result, concurrent testing of the unknown isolate with the three DNA probes for *B. dermatitidis*, *C. immitis*, and *H. capsulatum* with appropriate positive and negative controls for each probe would then be required, thus adding to the costs of the tests. For the exoantigen test, mature (7- to 10-day-old) colony growth is needed in order to extract a sufficient quantity of antigens from the test slant culture. For some *B. dermatitidis* isolates, 3- to-6-day-old growth from shake flask broth cultures grown at 37°C may be necessary. However, each unknown culture extract can be tested concurrently for *B. dermatitidis*, *C. immitis*, *H. capsulatum*, and *P. brasiliensis* antigens, so that tests for all four dimorphic pathogens can be done simultaneously at a substantially lower cost.

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