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Comparative Evaluation of a Chemiluminescent DNA Probe and an Exoantigen Test for Rapid Identification of *Histoplasma capsulatum*

A. A. PADHYE,* G. SMITH, D. McLAUGHLIN, P. G. STANDARD, AND L. KAUFMAN

Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 29 June 1992/Accepted 8 September 1992

A chemiluminescent DNA probe (Accuprobe) assay developed by Gen Probe, Inc., for the rapid identification of *Histoplasma capsulatum* was evaluated and compared with the exoantigen test by using 162 coded cultures including *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii*, *Histoplasma capsulatum* var. *farciminosum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, and morphologically related saprobic fungi. Each test uses a chemiluminescent, acridinium ester-labeled, single-stranded DNA probe that is complementary to the rRNA of the target organism. Lysates of the test cultures were prepared by sonication with glass beads and heat treated. After the rRNA was released from the target organism, the labeled DNA probe combined with the target *H. capsulatum* rRNA to form a stable DNA-RNA hybrid. A hybridization protection assay was used, and the chemiluminescence of hybrids was measured initially with a Leader 1 luminometer as relative light units and later during the investigation with a probe assay luminometer as probe light units. Of the 162 coded mycelial cultures tested by the Accuprobe assay, 105 were identified as *H. capsulatum*. The test could be performed with an inoculum of a few square millimeters (1 to 2 mm^2) of growth. In the primary evaluation, the Accuprobe identified 103 of the 105 cultures as *H. capsulatum* within 2 h. The remaining two cultures, contaminated with bacteria, had to be purified before the Accuprobe assay identified them correctly as *H. capsulatum*. Since each coded culture was concurrently tested for *H. capsulatum*, *B. dermatitidis*, and *C. immitis* exoantigens, the identification of all three dimorphic pathogens was provided simultaneously. Of the 162 coded cultures tested, 105 were identified by the exoantigen test as *H. capsulatum*, 12 were identified as *B. dermatitidis*, 13 were identified as *C. immitis*, and 32 were negative for *H. capsulatum*, *B. dermatitidis*, and *C. immitis*. The bacterial contamination in two isolates did not interfere with the exoantigen testing. The exoantigen test required 7- to 10-day-old colonies and required 48 to 72 h of incubation before definitive identification was obtained.

The conventional identification of *Histoplasma capsulatum* entails in vitro conversion of its mold anamorph to the yeast anamorph or vice versa in order to establish its dimorphic nature. The conversion is necessary because the morphology of the mold anamorph grossly resembles that of many saprobic fungi belonging to the genera *Chrysosporium*, *Corynascus*, *Renispora*, and *Sepedonium* and of other fungi and is not conclusive for rapid identification. To further confuse matters, many of these saprobic fungi are occasionally isolated from clinical specimens and have even been reported as *H. capsulatum* in the literature (1, 6).

The first successful advance in rapid and accurate identification of dimorphic fungi was made by Standard and Kaufman (11) when they introduced the exoantigen test for immunoidentification of the varieties of *H. capsulatum*. Extensive evaluations (3–5, 7, 8, 10) over the last 15 years have demonstrated that the exoantigen test is a reliable and rapid method for the specific identification of dimorphic and other pathogenic fungi.

More recently, DNA probe (Accuprobe) assays were introduced by Gen Probe, Inc., San Diego, Calif., for the rapid identification of mycelial- and yeast-form isolates of dimorphic fungi (2, 9, 12). Four chemiluminescent, homogeneous DNA probe assays were developed for identifying mycelial- or yeast-form cultures of *Blastomyces dermatitidis*, *Coccidioides immitis*, *H. capsulatum*, and *Cryptococcus neoformans*. We evaluated the Accuprobe for *H. capsulatum* culture identification and compared it with the exoantigen test by using 162 coded cultures. The results of that study are presented.

MATERIALS AND METHODS

Cultures. A total of 162 coded mycelial isolates belonging to *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii*, *Histoplasma capsulatum* var. *farciminosum*, *B. dermatitidis*, *C. immitis*, *Paracoccidioides brasiliensis*, *Chaetomium histoplasmoides*, *Chrysosporium asperatum*, *Chrysosporium* anamorphs of *Arthroderma multifidum*, *Arthroderma tuberculatum*, *Ctenomyces serratus*, a *Chrysosporium* sp., *Corynascus sepedonium*, *Renispora flavissima*, and a *Sepedonium* sp. were selected for the study. Many of the isolates of the three varieties of *H. capsulatum* selected from our culture collection were either typical (producing microconidia and tuberculate macroconidia) or sterile, or they produced only microconidia or smooth-walled macroconidia. All 30 isolates of saprobic fungi selected for the study produced asperulate to spiny conidia in the same size range as those of *H. capsulatum*. The sources of these cultures are summarized in Table 1.

Accuprobe testing. The nucleic acid hybridization test for *H. capsulatum* is based on the ability of complementary nucleic acid strands to specifically align and to form stable

* Corresponding author.

TABLE 1. Sources of coded cultures tested by the Accuprobe assay and the exoantigen test for *Histoplasma capsulatum*

Test organism	No. of isolates	Country of origin	Source
H. capsulatum var. *capsulatum*	74	United States	Humans (13 patients with AIDS, 61 without AIDS)
	5	United States	Soil
	2	Australia	Humans
	4	Argentina	Humans
	2	Guatemala	Humans
	3	Hong Kong	Humans
	4	India	Humans
H. capsulatum var. *duboisii*	5	Nigeria	Humans
H. capsulatum var. *farciminosum*	2	India	Horses
	4	Egypt	Horses
B. dermatitidis	8	United States	Humans
	4	South Africa	Humans
C. immitis	13	United States	Humans
P. brasiliensis	2	Brazil	Humans
Chaetomium histoplasmoides	1	United States	Soil
Chrysosporium asperatum	4	United States	Soil
Chrysosporium anamorph of *Ctenomyces serratus*	5	United States	Soil
Chrysosporium anamorph of *Arthroderma multifidum*	2	Scotland	Soil
Chrysosporium anamorph of *A. tuberculatum*	6	United States	Soil
Chrysosporium sp.	2	India	Soil
Corynascus sepedonium	2	India	Humans
Renispora flavissima	6	United States	Bat droppings
Sepedonium sp.	2	United States	Soil
Total	162		

double-stranded complexes (2). The Accuprobe system uses a chemiluminescent, labeled, single-stranded DNA probe that is complementary to the rRNA of the target culture. Each coded culture was tested by following the instructions provided with each kit. rRNA samples were prepared as follows. (i) A pinhead-size (1 to 2-mm^2) mycelial-growth inoculum from a 7-day-old colony on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) was suspended in a tube with a lysing reagent containing a buffered solution with 0.04% sodium azide and a buffered solution of probe diluent and glass beads. The mixture was vortexed briefly, and the resulting cell lysate was heated to 95°C in a sonicator water bath to inactivate any potentially infectious cells. (ii) One hundred microliters of the lysed specimen from the lysing reagent tube was pipetted into a tube containing the labeled DNA and incubated at 60°C in a water bath for 15 min. This hybridization step allowed the labeled probe to combine with the test organism's rRNA, if present, to form a stable DNA-RNA hybrid. (iii) The probe reagent tube was removed from the water bath, and 300 μl of a selection reagent was pipetted into the tube. The selection reagent preferentially hydrolyzed 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. (iv) The amount of the labeled, hybridized probe was measured initially in a Leader 1 luminometer as relative light units (RLUs) and later during the investigation in a probe assay luminometer as probe light units (PLUs). For each batch of coded cultures that was tested, a positive control culture of *H. capsulatum* ATCC 38904 and a negative control culture of *B. dermatitidis* ATCC 60916 was used. Samples with values of >50,000 RLUs or 1,500 or more PLUs indicated a positive reading for *H. capsulatum*. The identities of samples showing readings between 40,000 and 49,999 RLUs or between 1,200 and 1,499 PLUs were equivocal and necessitated reexamination of the cultures and repetition of the procedure. Samples showing values of <40,000 RLUs or of <1,200 PLUs were considered negative for *H. capsulatum*. The coded cultures were tested in batches, each batch containing 10 to 15 cultures. The final readings with the Accuprobe for each batch of cultures required a period of at least 2 h.

Exoantigen test. A 7- to 10-day-old duplicate colony of each coded culture on Sabouraud dextrose agar was simultaneously tested by the exoantigen tests for *H. capsulatum*, *B. dermatitidis*, and *C. immitis* according to the procedures of Kaufman and Standard (8). The results were read after 48 to 72 h.

RESULTS

Accuprobe identification. Of 162 coded mycelial cultures tested by the Accuprobe, 105 were identified as *H. capsulatum*. In the primary evaluation, the Accuprobe identified 103 of the 105 cultures as *H. capsulatum*. The remaining two isolates were identified as presumptive *P. brasiliensis* because the mycelial isolates were nonconidiating and the patients were from South and Central American countries. The histologic examination of the lung tissue in both patients showed budding yeast cells suggestive of *H. capsulatum*. Both cultures gave readings between 42,000 and 46,000 RLUs (Table 2). According to kit instructions, the aforementioned isolates were examined microscopically and were found to be contaminated with bacteria. After their purification, both cultures were retested. Both gave values between 250,000 and 300,000 RLUs and were identified as *H. capsulatum*. The remaining 57 heterologous mycelial fungal cultures gave low luminometer RLU and PLU readings (Table 3), indicating that they were negative for *H. capsulatum*. None of the other dimorphic pathogens or morphologically related fungi that were tested with the Accuprobe showed

TABLE 2. Accuprobe RLU and PLU value determinations for 105 mycelial isolates of the three varieties of *H. capsulatum*

Test organism[a]	Value range (no. of isolates)	
	RLUs	PLUs
H. capsulatum var. *capsulatum*	62,000–100,000 (20)	6,000–7,000 (5)
	101,000–150,000 (4)	7,100–8,000 (21)
	151,000–200,000 (3)	8,100–9,000 (8)
	201,000–250,000 (7)	9,100–10,000 (6)
	251,000–300,000 (2)	10,100–11,000 (4)
	301,000–350,000 (12)	
	42,000–46,000 (2)[b]	
H. capsulatum var. *duboisii*	160,000–280,000 (2)	
	350,000–390,000 (3)	
H. capsulatum var. *farciminosum*		4,000–7,000 (4)
		7,100–11,000 (2)
H. capsulatum var. *capsulatum* (+ control)	280,476–394,481	8,200–9,610
B. dermatitidis (− control)	3,600–17,661	97–250

[a] + control, positive control; − control, negative control.
[b] Two isolates of *H. capsulatum* contaminated with bacteria gave equivocal readings. When the isolates were purified, readings were between 250,000 and 300,000 RLUs.

any cross-reactivity with the *H. capsulatum* probe, thus showing that the probe was specific for *H. capsulatum*.

Exoantigen tests. Each coded culture was concurrently tested for *H. capsulatum*, *B. dermatitidis*, and *C. immitis* exoantigens, and thus the identifications of all three dimorphic pathogens were provided simultaneously. Of the 162 coded cultures tested, 105 were identified by the exoantigen test as *H. capsulatum*, 12 were identified as *B. dermatitidis*, 13 were identified as *C. immitis*, and 32 were negative for *H. capsulatum*, *B. dermatitidis*, and *C. immitis*. The bacterial contamination in two isolates of *H. capsulatum* did not interfere with the exoantigen test, and both were identified as *H. capsulatum*. The specific identities of the 32 isolates were determined by conventional methods. Two of the 32 were identified as *P. brasiliensis*, and 30 were identified as saprobic fungi producing asperulate to spiny conidia which grossly resembled the tuberculate conidia of *H. capsulatum*.

TABLE 3. Accuprobe RLU and PLU values for 57 isolates of heterologous fungi tested for *H. capsulatum* Accuprobe specificity

Test organism	Value range (no. of isolates)	
	RLUs	PLUs
B. dermatitidis	2,108–3,160 (2)	79–197 (10)
C. immitis	5,390–6,299 (2)	69–250 (11)
P. brasiliensis		210–231(2)
Chaetomium histoplasmoides		76 (1)
Chrysosporium asperatum	4,800–6,630 (4)	
Chrysosporium anamorph of *Ctenomyces serratus*	3,500–4,600 (5)	
Chrysosporium anamorph of *A. multifidum*		80–125 (2)
Chrysosporium anamorph of *A. tuberculatum*	2,309–7,584 (6)	
Chrysosporium sp.		120–160 (2)
Corynascus sepedonium		80–168 (2)
Renispora flavissima		80–190 (6)
Sepedonium sp.	4,615–4,800 (2)	

DISCUSSION

When a dimorphic pathogen such as *H. capsulatum* is identified by the Accuprobe, the step-by-step procedure to be followed according to the instructions involves a minimum time of 45 to 50 min, irrespective of the number of cultures. The chemiluminescent DNA probe correctly identified 103 of the 105 test cultures of the three varieties of *H. capsulatum* to the species level within 2 h and successfully differentiated among other dimorphic pathogens as well as morphologically similar saprobes from *H. capsulatum*. Two isolates contaminated with bacteria gave equivocal readings when tested by the Accuprobe. When both cultures were purified and retested, both were identified as *H. capsulatum*. The Accuprobe required 1 to 2 mm^2 of early growth free from bacterial contamination in order to provide correct identifications of *H. capsulatum*. On the other hand, the exoantigen test identified all of the 105 test cultures correctly as *H. capsulatum* within 72 h. However, as stated earlier, a colony at least 25 mm in diameter (a 7- to 10-day-old colony) was required for the exoantigen test in order to extract a sufficient quantity of the antigens from the slant culture. DiSalvo et al. (4) had reported that saprobic fungal contamination did not interfere with the exoantigen test identification of *H. capsulatum* isolates. However, none of their tested cultures were contaminated with bacteria. In the present study, of 162 cultures that we tested by the Accuprobe and the exoantigen test, only two cultures were contaminated with bacteria. None were contaminated with saprobic fungi. Both systems do not depend upon recognition of characteristic morphologic features or conversion of *H. capsulatum* for definitive identification. Relatively early mycelial or yeastlike growth of *H. capsulatum* from a primary isolation medium can be correctly identified by the Accuprobe assay provided that the growth is free of contamination. The concurrent testing of each unknown isolate with the Accuprobe for *B. dermatitidis*, *C. immitis*, and *H. capsulatum* may not be cost-effective. To minimize costs, workers should be able to anticipate the identity of the possible etiologic agent on the basis of available cultural, histologic, serologic, and clinical data so that an appropriate probe(s) can be selected to rapidly identify the fungal agent isolated in culture.

Since we concurrently tested each coded culture by the exoantigen test for *H. capsulatum*, *B. dermatitidis*, and *C. immitis*, the identifications of all three of these dimorphic pathogens were provided simultaneously at a substantially lower cost. The exoantigen test is based on the interaction between antigens released by fungi in culture and homologous antibodies that are specifically generated to precipitate them. The precipitates formed are readily checked for fusion with preselected reference precipitate systems in immunodiffusion tests to establish the identity of the fungus producing the antigen(s). In the present study, the exoantigen test had a sensitivity of 100%. Mature (7- to 10-day-old) colony growth is necessary in order to extract a sufficient quantity of antigens from the test slant culture.

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