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Limitation of the AccuProbe *Coccidioides immitis* Culture Identification Test: False-Negative Results with Formaldehyde-Killed Cultures

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The AccuProbe *Coccidioides immitis* culture identification test (CI test) yielded false-negative results with formaldehyde-killed *C. immitis* submitted to a reference laboratory. Further evaluation with pure or mixed cultures or stored, heat-killed cultures revealed the CI test to be highly sensitive and specific for *C. immitis* except when the cultures were pretreated with formaldehyde.

Coccidioides immitis is a dimorphic fungal pathogen with distribution in the United States restricted to the Southwest. This pathogen is being increasingly recognized in parts of the world where it is not endemic in people who have a history of travel to the zone of endemicity. The safe laboratory handling of this highly infectious fungus requires a biosafety level III facility, and interstate transport of the fungus is restricted under the provisions of the Anti-Terrorism and Effective Death Penalty Act of 1996 (1). The AccuProbe *C. immitis* culture identification test (CI test) provides rapid, sensitive, and specific confirmation of the pathogen (5, 7). It is expected that a great majority of clinical laboratories in areas where the fungus is not endemic do not have access to the CI test because of infrequent demand. Instead, reference laboratories are more often called upon to provide confirmatory identification of suspected *C. immitis* cultures. We recently observed that formalin-killed *C. immitis* cultures referred from other laboratories or received as part of a proficiency test consistently tested negative with the CI test (9). This report summarizes further evaluation of the CI test with pretreated cultures of *C. immitis*.

The AccuProbe *C. immitis* CI test was purchased from and used as recommended by the manufacturer except for the use of an *Arthrographis* sp. as a negative control in place of the dimorphic pathogen *Blastomyces dermatitidis* (Gen-Probe Inc., San Diego, Calif.). All patient *C. immitis* isolates were previously characterized in our laboratory. The cultures were grown on modified Sabouraud dextrose agar for 14 days at 30°C. A 1- to 2-mm portion of mycelium without agar was processed for identification. A similar portion was also pretreated for 60 min with commercially supplied 37.5% formaldehyde or a diluted 10.0% solution (Sigma Chemical Co., St. Louis, Mo.). In a second pretreatment, 10.0% formaldehyde-treated cultures were rinsed in a microcentrifuge tube with three changes of deionized water. In a third pretreatment, cultures were kept in a boiling water bath for 20 min, followed by storage at 6°C for 48 h. Finally, cultures were mixed with either *Aspergillus fumigatus* or *Candida albicans*. A 48-h-old mold or yeast culture was used as a source of conidia and hyphal fragments or yeast, which were harvested from culture slants in sterile saline with repeated shaking. The suspension was adjusted to 10^7 cells/ml by counting with a hemacytometer. A 100-μl aliquot of this suspension (10^6 cells) was added to the reagent tube containing a 1- to 2-mm portion of the *C. immitis* culture. Finally, an aliquot of each pretreated *C. immitis* culture was transferred to a modified Sabouraud dextrose agar slant for a viability check. Both formaldehyde- and heat-killed cultures tested nonviable. Control cultures and all pretreated cultures were further processed according to the prescribed protocol involving sonication and lysis at 95°C, hybridization of extracted rRNA with a chemiluminescence-labeled DNA probe, and detection in a luminometer.

Pretreatment of *C. immitis* cultures with formaldehyde led to false-negative results due to very low relative luminescence units (RLUs), which improved only marginally upon subsequent rinses with deionized water (Table 1). This was quite unexpected; there was only one previous report in the literature on AccuProbe test inhibition. That report described how high salt in the enriched media interfered with lysis of *Listeria monocytogenes* and subsequent hybridization of the probe with cellular RNA (6). Moreover, the product literature did not list chemicals contraindicated for use with the CI test. The negative results suggested an inhibitory effect(s) of formaldehyde either on one or more of the test reagents or on the target rRNA in *C. immitis*. The concentrations of formaldehyde used in pretreatment reflected routine practice in the diagnostic laboratories for killing fungi and for preservation of histopathological specimens (3). Formaldehyde was previously reported to markedly enhance thermally induced DNA denaturation (8). Furthermore, formaldehyde in cytological fixatives was reported to cause extensive DNA damage in plants and fungi, leading to complete inhibition of PCR (2). It can only be speculated whether similar physical damage of target rRNA led to its nonhybridization with the labeled DNA probe or if sufficient traces of formaldehyde were carried over to denature the DNA probe even after repeated rinses with water. Further studies are also needed to determine if formaldehyde use is contraindicated in AccuProbe tests for other pathogenic fungi (4, 5, 7).

The sensitivity and specificity of the test were not affected when heat-killed cultures stored for 48 h or mixed cultures were used in our experiments (Table 1). The stability of target RNA for 48 h in heat-killed, refrigerated cultures indicated that this pretreatment is preferable when submitting suspect cultures to the reference laboratories to ensure safety without

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TABLE 1. Effect of pretreatment of *C. immitis* cultures on accuracy of AccuProbe test

C. immitis strain no.	Avg RLU[a]						
	AccuProbe protocol[b]	Boiled cultures[c]	Mixed cultures[d]		37.5% Formaldehyde[e]	10% Formaldehyde	
			A. fumigatus	*C. albicans*		Unwashed[e]	3 Washes[f]
NYS 104-94	699,787 (+)	745,639 (+)	62,191 (+)	741,536 (+)	2,869 (−)	6,887 (−)	18,499 (−)
NYS 498-97	717,437 (+)	525,529 (+)	619,501 (+)	277,748 (+)	6,685 (−)	3,942 (−)	52,317 (±)
NYS 56-98	484,562 (+)	398,424 (+)	314,104 (+)	624,013 (+)	3,014 (−)	1,662 (−)	47,769 (±)
NYS 89-99	702,192 (+)	203,661 (+)	811,376 (+)	709,925 (+)	2,178 (−)	4,895 (−)	15,200 (−)
NYS 371-99	750,192 (+)	203,661 (+)	455,968 (+)	803,618 (+)	2,178 (−)	4,895 (−)	186,675 (+)

[a] From two experiments. Interpretations of results are in parentheses; ±, borderline positive.
[b] Includes lysis at 95°C for 5 min. The positive control was *C. immitis* NYS 1442-98 (RLU = 768,904 ± 110,916); the negative control was *Arthrographis* sp. strain NYS 721-95 (RLU = 3,882 ± 1,075). Values are from 10 independent determinations.
[c] Twenty minutes in boiling water bath, storage at 6°C for 48 h, and processing as per AccuProbe protocol.
[d] Mixed with 10^6 conidia or yeast cells and processed as per AccuProbe protocol.
[e] Treated with formaldehyde for 60 min and processed as per AccuProbe protocol.
[f] Formaldehyde treatment followed by three rinses with deionized water and processing as per AccuProbe protocol.

adversely affecting the outcome of the CI test. These results were also in conformity with an earlier report that described the use of heat-killed frozen cultures as positive controls in the CI test over a 10-month period (10).

The presence of either *A. fumigatus* or *C. albicans*, which are likely to be recovered from respiratory tract specimens along with *C. immitis*, did not interfere with either the recovery of target rRNA or accurate identification. This observation was consistent with the results described in the product literature when rRNA mixes equivalent to 5×10^4 cells of *Histoplasma capsulatum* or 5×10^6 cells of *C. albicans* were used along with *C. immitis*. The present study reaffirmed the reported high sensitivity and specificity of CI test for pure or mixed cultures of *C. immitis* except when the cultures were pretreated with formaldehyde.

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