

Evaluation of a Commercial DNA Probe Assay for the Identification of *Mycobacterium kansasii*

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A commercially available DNA probe (the AccuProbe *Mycobacterium kansasii* culture identification test, Gen-Probe, USA) for the identification of *Mycobacterium kansasii* was tested on a panel of 143 fully characterized mycobacterial strains. The isolates included 70 *Mycobacterium kansasii* and 73 mycobacteria other than *kansasii*. The specificity was 100 % while the sensitivity was 72.8 %. This sensitivity is unusually low in comparison with that of commercial DNA probes for other mycobacteria and confirms a previous study that found genetic heterogeneity within the species *Mycobacterium kansasii*. Strains that do not hybridize with the AccuProbe are particularly prevalent in Italy and perhaps elsewhere in Europe.

Before the beginning of the AIDS era, *Mycobacterium kansasii* was, in various areas of the world, the major cause of mycobacterial pulmonary disease other than tuberculosis (1). In AIDS patients the infections caused by the *Mycobacterium avium* complex are the major clinical problem involving mycobacteria, but *Mycobacterium kansasii* has been reported to be the second most common agent of disseminated nontuberculous mycobacterial disease (2). Conventional identification of *Mycobacterium kansasii* (3) is feasible on the basis of several features: nitrate reduction, Tween hydrolysis, photochromogenicity, catalase (> 45 mm of foam), urease and inhibition by thiazetazone (10 µg/ml); the procedure requires at least three weeks.

Acridinium-ester-labeled DNA probes for the identification of cultures of *Mycobacterium kansasii* (AccuProbe *Mycobacterium kansasii* culture identification test, Gen-Probe, USA),

which became commercially available about three years ago, have dramatically reduced the time and the workload necessary for the identification of this species; so far only one extensive evaluation of this system has been reported (4). The purpose of this study was to assess the sensitivity and specificity of the AccuProbe *Mycobacterium kansasii* culture identification test, as we had previously noticed that it failed to identify a number of strains that behaved as *Mycobacterium kansasii* in conventional tests (5).

Materials and Methods. The hybridization test was performed on a panel of mycobacterial strains that included 70 *Mycobacterium kansasii* and 73 non-*Mycobacterium kansasii*. The panel included reference cultures and clinical isolates detected at three different Italian laboratories (Ancona, Florence, Milan). Duplicate isolates from the same patient were eliminated.

The reference strains were obtained from the Institut Pasteur Collection (1 each of *Mycobacterium kansasii* and *Mycobacterium haemophilum*) and the American Type Culture Collection (ATCC) (1 *Mycobacterium paratuberculosis*). All strains except *Mycobacterium haemophilum* and *Mycobacterium genavense* were grown on Lowenstein-Jensen medium for no more than one month. *Mycobacterium haemophilum* was grown on Middlebrook 7H11 medium supplemented with 0.4 % hemoglobin (BBL, USA). *Mycobacterium genavense* was subcultured from radiometric broth Bactec 12B (Becton Dickinson, USA) on Middlebrook 7H11 medium supplemented with 2 µg/ml of mycobactine J (Rhône-Mérieux, France); this one strain required a longer incubation (7 weeks).

The test was performed according to the manufacturer's instructions. Briefly, a 1 µl loopful of mycobacterial colonies was sonicated for 15 min (Bransonic B1200E4, Branson, USA) in tubes containing glass beads and "lysing" reagent and heated for 10 min at 95 °C. The hybridization with the lyophilized DNA probe was performed at 60 °C for 15 min, and then the "selection" reagent was added. The incubation time of the selection step at 60 °C was increased for the majority of strains (n = 133) from the previously suggested 5 min to the currently recommended time of 8 min, with no effect on the results. Hybridization results were read with a PAL/AccuLDR luminometer (Gen-Probe) after 5 min at room temperature and were expressed as PAL light units (PLU). According to the manufacturer's

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Table 1: Results of tests performed for the conventional identification of 70 *Mycobacterium kansasii* strains.

Test	No. (%) of positive results
Niacin	1 (1.4)
Nitrate reduction	69 (98.6)
Heat-stable catalase	70 (100)
Catalase > 45 mm of foam	70 (100)
Photochromogenicity	70 (100)
β -glucosidase	0 (0)
Tween 80 hydrolysis (10 days)	70 (100)
Slow growth rate	70 (100)
Growth at 25 °C	70 (100)
Growth at 45 °C	0 (0)
MacConkey agar	0 (0)
Tellurite reduction	0 (0)
Arylsulfatase	0 (0)
Rough colonies	69 (98.6)
Urease	70 (100)
Resistance to:	
NaCl (5 %)	0 (0)
p-nitrobenzoate (500 μ g/ml)*	18 (25.7)
Thiophene-2-carboxylic hydrazide (5 μ g/ml)	70 (100)
Thiacetazone (10 μ g/ml)	0 (0)
Hydroxylamine (500 μ g/ml)	4 (5.7)
Isoniazid (1 μ g/ml)	39 (57.7)
Oleate (250 μ g/ml)	0 (0)

*Of 18 strains that tolerated p-nitrobenzoate, 10 were AccuProbe negative and 8 AccuProbe positive.

cutoff value, samples producing signals greater than 899 PLU were considered positive.

Three *Mycobacterium kansasii* isolates with unexpected negative hybridization results were tested twice and two others thrice. Duplicate and triplicate results of these tests were all negative and were not considered further. Conventional identification (3) of the strains was performed using an extended set of tests (Table 1); the results were also assessed using a computer-aided identification program (6).

Results and Discussion. Hybridization results are listed in Table 2. None of the 73 strains of mycobacteria other than *Mycobacterium kansasii* were positive; the PLU value of these strains ranged from 23 to 250, with an average of 81.25 and a standard deviation of 49.56. No significant difference in PLU emission was noticed between the various species. Of the 70 strains conventionally identified as *Mycobacterium kansasii*, 51 hybridized and 19 (27.14 %) failed to hybridize with the specific probe.

The mean hybridization signals were 8,060.35 for positive and 59.68 for negative *Mycobacterium*

kansasii strains. None of the tests performed fell within the manufacturer's repeat range (600–899 PLU). All probe-negative strains exhibited typical characteristics by conventional identification and were confirmed by testing four isolates at a reference laboratory (Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, Paris). The prevalence of probe-negative isolates did not differ appreciably from one contributing center to the other (data not shown).

Tween hydrolysis was the only phenotypic trait that appeared to be associated with the hybridization results: it was weak in AccuProbe-negative strains (mean time 5.00 days) in comparison with positive ones (1.57 days); the difference was statistically significant ($p < 0.01$, Student's t-test). The likelihood of the computer-assisted identification was equally high (ranging from 98.88 to 100 %), both for hybridizing and non-hybridizing isolates.

In our series the specificity of the AccuProbe *Mycobacterium kansasii* culture identification test was 100 %, while its sensitivity was 72.86 %. The complete specificity of the *Mycobacterium kansasii* AccuProbe is consistent with the favorable reports on other commercially available hybridization systems for the *Mycobacterium tuberculosis* complex (7–10), the *Mycobacterium avium* complex (7–12) and *Mycobacterium gordonae* (13). In contrast, such a low sensitivity has not been reported. Working with 105 referenced strains of *Mycobacterium kansasii*, Ross et al. (4) recently reported an overall sensitivity of 93 % for the AccuProbe *Mycobacterium kansasii* test, a value which dropped to 81 % if only the 26 European isolates were considered. In a limited study on nine French isolates of *Mycobacterium kansasii*, five failed hybridization (14). Our results reinforce the idea that, at least in Europe, there is a consistent prevalence of *Mycobacterium kansasii* strains that do not hybridize with the AccuProbe.

No evaluation of *Mycobacterium kansasii* probes with strains isolated in the USA has been reported, apart from the Gen-Probe pre-marketing trial. The reported overall sensitivity, 92.8 %, suggests that the trial panel had a high prevalence of typical strains. All mycobacterial probes manufactured by Gen-Probe aim at rRNA, but their exact sequences are not stated. The recently described variability among 16S rRNA in *Mycobacterium kansasii* (4) might, in principle, account for the inability of some strains to hybridize with commercial probes. Latest studies confirmed the genetic heterogeneity of *Mycobacterium kansasii*

Table 2: Hybridization (expressed in PAL light units) of various mycobacteria with the AccuProbe *Mycobacterium kansasii* test.

Organism ^a	No. of strains	PAL light units ^b		
		Mean	± SD	Range
<i>M. kansasii</i> probe positive ^c	51	8,060.35	± 1,899.53	3,466 – 10,432
<i>M. kansasii</i> probe negative	19	59.68	± 26.47	31 – 148
<i>M. fortuitum</i> complex	20	75.55	± 15.45	50 – 105
<i>M. avium</i> complex	12	119.25	± 52.60	28 – 190
<i>M. xenopi</i>	11	82.45	± 52.58	23 – 170
<i>M. goodii</i>	9	34.56	± 5.01	28 – 46
<i>M. tuberculosis</i>	4	57.00	± 21.79	32 – 92
<i>M. flavescens</i>	3	86.67	± 11.79	70 – 95
<i>M. malmoense</i>	2	67.50	± 7.50	60 – 75
Others ^d	12	95.67	± 73.72	25 – 250

^a Identified by conventional methods.

^b Cutoff between positive and negative reaction = 899.

^c Including the reference strain.

^d One strain each of the following organisms: *M. gastri*, *M. genavense*, *M. haemophilum* (reference strain), *M. marinum*, *M. paratuberculosis* (reference strain), *M. phlei*, *M. scrofulaceum*, *M. shimoidei*, *M. simiae*, *M. smegmatis*, *M. terrae*, unidentified rapid grower.

(15), and changes in the present taxonomy of this species will probably follow.

In conclusion, the AccuProbe *Mycobacterium kansasii* identification test appears to be 100 % specific, with no evidence of cross-reaction with other mycobacterial species, and can thus provide, if positive, a rapid, definitive identification. A negative result should not, however, be considered conclusive; and, for photochromogenic strains, a complete conventional identification continues to be mandatory.

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