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Direct Identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* from Amplified Primary Cultures in BACTEC Media Using DNA Probes

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DNA probes (Gen-Probe, San Diego, Calif.) directed at the *Mycobacterium tuberculosis* complex and *Mycobacterium avium-M. intracellulare* complex were used to identify acid-fast bacilli directly from specimens grown in BACTEC 12B bottles (Becton Dickinson and Co., Towson, Md.). Clinical specimens were inoculated directly or after decontamination into a BACTEC 12B bottle, Middlebrook 7H11 agar, and Lowenstein-Jensen medium. Conventional media were incubated at 37°C in 5% CO_2 and examined weekly for 6 weeks. Identification of isolates grown on conventional media by standard biochemicals, morphology, and growth characteristics served as the reference method for identification. BACTEC bottles were incubated at 37°C, and a growth index was taken twice a week. When a growth index of ≥100 was reached, 1 ml of BACTEC 12B medium was put into each of three microfuge tubes which were centrifuged for 15 min at 15,000 × *g*. Pellets were used in hybridization reactions with an *M. tuberculosis* complex probe, an *M. avium* probe, and an *M. intracellulare* probe. The results of the hybridizations of the three probes with the same sample were compared, and the highest percent hybridization was divided by the average of the two lower hybridization values. If this value, the derived patient ratio (DPR), was ≥3, then the specimen was considered positive for the organism giving the highest percent hybridization. Of the 1,988 specimens cultured, the results of conventional tests for the 190 conventional culture-positive specimens were 64 *M. tuberculosis*, 61 *M. avium*, 14 *M. intracellulare*, 30 other *Mycobacterium* spp., and 25 non-acid-fast bacilli. There were four cultures that each contained two different *Mycobacterium* spp. Directly probing the BACTEC 12B sediment, at a DPR of ≥3 the *M. tuberculosis* probe identified 83% (53 of 64) of *M. tuberculosis* isolates, the *M. avium* probe identified 92% (56 of 61) *M. avium* isolates, and the *M. intracellulare* probe identified 86% (12 of 14) of *M. intracellulare* isolates. There were no false-positive results at this DPR level. The false-negative results from probing the sediment from the BACTEC 12B bottle could not solely be attributed to the number of organisms present, the growth index, or antimicrobial therapy.

Over the past 10 years, several methods have been described that aid in the rapid detection and identification of *Mycobacterium* spp., particularly *Mycobacterium tuberculosis* and *Mycobacterium avium-M. intracellulare*. These methods have been targeted at reducing not only the time required to initially detect a *Mycobacterium* sp. but also the time needed to identify an isolate once detected. Using conventional isolation methods in a clinical setting, Kirihara et al. (5) reported that the detection time for *M. tuberculosis* was 9 to 58 days and that the detection time for *M. avium* was 7 to 67 days. This is similar to results of our clinical laboratory, where our average detection time with conventional methods is 21 days for *M. tuberculosis* and 28 days for *M. avium-M. intracellulare*. The radiometric BACTEC system (Becton Dickinson and Co., Towson, Md.) has greatly reduced this detection time, especially with the *M. avium-M. intracellulare* species, for which positive cultures can be detected on the average in 7 to 8 days (5). However, once detected these isolates can take up to 4 to 6 weeks for final identification. The introduction of computer-assisted gas-liquid chromatography, radiometric techniques, and nucleic acid hybridization has greatly reduced this identification time. The last method, using both whole chromosomal DNA probes (8) and short DNA ribosomal probes (1, 3, 4, 6), has been used for *M. tuberculosis*, *M. avium*, and *M. intracellulare* identifications. While both types of probes proved to be highly specific and sensitive, the DNA probes to rRNA are commercially available and utilize a rapid liquid hybridization taking 2 h to complete, while hybridization using the longer probes requires up to 48 h. Ellner et al. (2) recently reported that by using both radiometry and probe technology the time to a final identification was greatly reduced. Kiehn and Edwards (4), examining isolates of *M. avium* and *M. intracellulare*, tested 23 isolates directly from the BACTEC bottle with DNA probes (Gen-Probe, San Diego, Calif.) and found no false-positive specimens but four false-negative specimens. In this investigation, we directly assayed 190 positive BACTEC bottles with three DNA probes (Gen-Probe) directed at the *M. tuberculosis* complex, *M. avium*, and *M. intracellulare*.

MATERIALS AND METHODS

Specimens. Specimens included in the study were those sent to the Medical Microbiology Laboratory at the University of California Irvine Medical Center with requests for mycobacterial cultures. During a 7-month period (August 1987 through February 1988), there were 1,988 specimens received which were included in the study. The sources of the specimens were sputum (n = 721), body tissues or fluids (n = 550), blood (n = 213), bronchoalveolar lavage (n = 193),

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urine (n = 168), stools (n = 39), lymph nodes (n = 29), and wounds or abscesses (n = 75).

Specimen processing. Upon receipt in the laboratory, specimens were processed for culture within 24 h. Specimens were inoculated into BACTEC 12B bottles (Becton Dickinson) as well as onto conventional media, including Lowenstein-Jensen medium (Cal Scott Laboratories, Carson, Calif.) and a biplate of Middlebrook 7H11 agar (Remel Laboratories, Lenexa, Kans.) containing carbenicillin (50 μg/ml) and trimethoprim lactate (20 μg/ml).

Blood specimens were received in Isolator tubes (Du Pont Co., Wilmington, Del.) which were centrifuged at 3,000 × g for 30 min, and the pellets were resuspended according to the instructions of the manufacturer. Specimens, including blood, that did not require further decontamination or digestion were inoculated directly into the media. Each BACTEC 12B bottle received 0.5 ml of specimen, and each conventional medium received 0.1 ml.

Specimens requiring decontamination or digestion or both were treated with *N*-acetyl-L-cysteine and 2% sodium hydroxide, according to standard procedures (7). Subsequently, treated specimens were suspended in phosphate-buffered saline (0.01 M, pH 7.2) containing penicillin (50 U/ml). BACTEC 12B bottles supplemented with antimicrobial agents (polymyxin B, 50 U/ml; amphotericin B, 5 μg/ml; nalidixic acid, 20 μg/ml; trimethoprim, 5 μg/ml; and azlocillin, 10 μg/ml) were inoculated with 0.4 ml of treated specimen, and conventional media were inoculated with 0.1 ml.

Culture interpretation. Conventional media incubated at 37°C and 5% CO_2 were read weekly up to 6 weeks. Organisms were identified by standard biochemical methods (7). In addition, differentiation of *M. avium* from *M. intracellulare* was accomplished by performing DNA-RNA hybridization (Gen-Probe), using the directions of the manufacturer for organisms grown on solid media.

BACTEC 12B bottles were read twice a week by using the BACTEC 460 (Becton Dickinson). When a growth index (GI) of ≥100 was detected, medium from the BACTEC bottle was withdrawn. A 0.01-ml sample was inoculated into chocolate agar to check for contamination; a semiquantitative plate count (0.1 ml of a 10^{-3} dilution) was performed, using a 7H11 agar plate, and each of three microfuge tubes received 1 ml of medium. These tubes were centrifuged at 15,000 × g for 15 min, the supernatants were decanted, and the pellets were suspended in 0.1 ml of distilled water and stored at 4°C for up to 7 days before being used in a probe assay.

DNA probe assay. Suspended pellets from the BACTEC bottles were used in a hybridization assay within 7 days, using the instructions of the manufacturer (Gen-Probe) for the hybridization technique. Since these probes were designed to probe organisms from solid media, the specimen preparation used in this study with the BACTEC material varied from that suggested by the manufacturer. Three probes, directed at the *M. tuberculosis* complex, *M. avium*, and *M. intracellulare*, were used with each specimen.

For direct testing of the BACTEC material, a method different from that suggested by the manufacturer was used to calculate the final result. For each probe assay, a hybridization value was obtained by dividing the hybridization counts by the total input counts per minute and multiplying by 100. For each patient, a derived patient ratio (DPR) was calculated from the three resulting hybridization values as follows. The hybridization value corresponding to the most reactive probe was divided by the average hybridization value obtained with the remaining two probes. When a *Mycobacterium* sp. other than the three species recognized by the probes was isolated, as well as for cultures with non-acid-fast bacillus (AFB) bacterial contaminants and mixed cultures containing two *Mycobacterium* spp., the highest percent hybridization was also divided by the average value for the other two probes to obtain the final DPR. Therefore, the following formula was used to calculate the DPR: DPR = $a/[1/2(b + c)]$, where a equals the percent hybridization of the probe corresponding to the most reactive probe and b and c are the other two probe hybridization values.

The specificity and sensitivity values for direct testing of organisms grown in BACTEC media were calculated, using the identification obtained by conventional culture as the reference.

RESULTS

Overall results. During the 7-month study period, of 1,988 specimens submitted for mycobacterial cultures, 165 (8.3%) cultures reached a GI of 100 in the BACTEC 12B bottles, grew a *Mycobacterium* sp., and were therefore included in the study protocol. As determined by conventional culture, there were 64 isolates of *M. tuberculosis* from 23 patients, 61 *M. avium* isolates from 39 patients, 14 *M. intracellulare* isolates from 12 patients, 1 isolate unable to be assigned to a species within the *M. avium-M. intracellulare* complex, and 29 other *Mycobacterium* spp. The 29 other *Mycobacterium* spp. included *M. gordonae* (n = 21), *M. fortuitum* (n = 2), *M. marinum* (n = 2), *M. kansasii* (n = 2), *M. avium-M. intracellulare-M. scrofulaceum* complex (n = 1), and a rapid-grower *Mycobacterium* sp. (n = 1). Of the 165 specimens containing a *Mycobacterium* sp., 4 were mixed; 2 contained *M. tuberculosis* and *M. avium*, and the other 2 contained *M. kansasii* with *M. avium* in one and *M. tuberculosis* in the other. In addition, from 11 specimens containing *M. tuberculosis* and 18 specimens containing *M. avium*, bacteria other than AFB were also isolated. Also included in the testing were 25 specimens which reached a GI of >100 and grew non-AFB contaminants.

Since each specimen was hybridized with the three separate probes, one specific for each of the *M. tuberculosis* complex, *M. avium*, and *M. intracellulare*, hybridization results from the BACTEC material for the 190 cultures were derived by dividing the hybridization value obtained with the most reactive probe by the average of the other two hybridizations; this ratio was termed DPR. For example, with a culture that gave a hybridization value with the *M. tuberculosis* probe that was higher than the other two probe values, this value would be divided by the average hybridization value of the result for this specimen obtained with the *M. avium* and *M. intracellulare* probes. For cultures containing other *Mycobacterium* spp. not recognized by the probes and for specimens containing only non-AFB contaminants, here too the highest hybridization signal was divided by the average of the other two probe results. The overall distribution of the DPR hybridization results, compared with the identifications obtained by conventional culture, can be seen in Table 1.

In Table 2, the overall specificity, sensitivity, and predictive values can be seen for DPRs of 3, 4, and 5. Calculations were done at these values since these were the lowest DPRs with which the specificity was 100%. The sensitivities with a DPR of 3 obtained for the *M. tuberculosis*, *M. intracellulare*, and *M. avium* probes were 83, 86, and 92%, respectively. One isolate, however, which had a DPR of 2, was not

TABLE 1. Distribution of isolates by the DPR

Organism[a]	No. of isolates	No. of isolates with factor above patient background of:																	
		<1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	>10
M. tuberculosis	64	5	2	2	2		2	4		1	2	1	1	1	2		3		1	35
M. avium	61[b]		1	4								1		1		1				53
M. intracellulare	14[b]		1		1	1				1		2		2			1			5
Other mycobacteria	27[c]	10	12	5																
Bacteria (non-AFB)	25[c]	7	15	3																

[a] Identified by conventional methods.
[b] Excludes one isolate that had a DPR of 2 and could not be assigned to a species within the *M. avium-M. intracellulare* complex.
[c] Includes isolates that were recovered in pure culture and excludes isolates recovered along with *M. tuberculosis*, *M. avium*, or *M. intracellulare*.

included in the calculation. This isolate could not be identified by conventional methods, including biochemicals and gas-liquid chromatography, to the species level within the *M. avium-M. intracellulare* complex. In addition, when tested from solid media, the isolate did not hybridize with any of the probes and was therefore excluded from data analysis.

Resolution of discrepancies. In an attempt to determine the factor(s) that might have contributed to the false-negative direct hybridization assays of positive BACTEC bottles, the final DPRs were correlated with the GIs, colony counts, times of storage of the pellets obtained from the BACTEC media before use in hybridization, and the antimicrobial therapies of selected patients.

The GIs from cultures growing *M. tuberculosis*, *M. avium*, and *M. intracellulare* were correlated with the DPRs (Table 3). Here a DPR of 3 was used, and of the false-negative hybridizations, 73% (8 of 11) of the cultures containing *M. tuberculosis*, 80% (4 of 5) of the cultures containing *M. avium*, and 50% (1 of 2) of the cultures containing *M. intracellulare* had GIs of <500; however, so did 62% (33 of 53) of the true-positive *M. tuberculosis* hybridizations, 47% (26 of 56) of the *M. avium* hybridizations, and 25% (3 of 12) of the *M. intracellulare* hybridizations. Therefore, the GI did not show a direct correlation with the hybridization result.

In Table 4, the distribution of colony counts with respect to a DPR of 3 can be seen. All false-negative *M. tuberculosis* hybridizations had $\leq 10^5$ CFU/ml of BACTEC 12B broth, while 41% (21 of 51) of the subcultures containing *M. tuberculosis* with a DPR of ≥3 also grew $\leq 10^5$ CFU/ml. The majority of cultures giving the false-negative *M. avium* and *M. intracellulare* hybridizations grew $>10^6$ CFU/ml, a level that should have been detected by the probes used.

When a GI of ≥100 was reached, medium from the BACTEC bottle was centrifuged, and the resulting pellets were suspended in 0.1 ml of distilled water and stored at 4°C. The time interval between assaying these pellets was 1 to 7 days; therefore, results using a DPR of ≥3 as a positive cutoff were analyzed with respect to the length of storage at 4°C. When true-positive and false-negative probe results for *M. tuberculosis*-, *M. avium*-, and *M. intracellulare*-positive cultures were compared, there was no significant difference in the interval between being stored at 4°C and being tested by the probe assay ($P > 0.1$). Therefore, this factor did not appear to account for the false-negative probe results.

A group of patients who had more than one culture-positive specimen within the study period and who also had true-positive and false-negative direct BACTEC probe results were studied to determine whether antimicrobial therapy correlated with false-negative results. There was no apparent correlation with treatment in that with specimens collected on the same day, although all grew a *Mycobacterium* sp., direct probe results were obtained that were both positive and negative (Table 5). This was seen with both patients 1 and 2. It is true, however, that BACTEC probe-positive specimens from the same day as the false-negative BACTEC probe results, in general, had low positive DPRs. It is also important to note in Table 5 that for the two patients (2 and 3) from whom a mixed culture of *M. tuberculosis* and *M. avium* was grown, only *M. avium* was detected from probing the BACTEC pellet.

TABLE 2. Identification by DNA-RNA hybridization of *M. tuberculosis*, *M. avium*, and *M. intracellulare* from BACTEC 12B bottles, using DPRs

Isolate and DPR	Parameter (%)			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
M. tuberculosis				
3	83	100	100	92
4	73	100	100	88
5	73	100	100	88
M. avium[a]				
3	92	100	100	96
4	92	100	100	96
5	92	100	100	96
M. intracellulare[a]				
3	86	100	100	99
4	79	100	100	98
5	79	100	100	98

[a] Excluded from the calculations is one isolate that had a DPR of 2 and that could not be assigned to a species within the *M. avium-M. intracellulare* complex.

TABLE 3. Correlation of GI with DPR

Organism[a]	DPR	No. of isolates	No. (%) of isolates with GI of:			
			<200	200–500	>500–998	≥999
M. tuberculosis	<3	11	4 (36)	4 (36)	0	3 (27)
	≥3	53	15 (28)	18 (34)	8 (15)	12 (23)
M. avium	<3	5	4 (80)	0	1 (20)	0
	≥3	56	11 (20)	15 (27)	9 (16)	21 (37)
M. intracellulare	<3	2	1 (50)	0	1 (50)	0
	≥3	12	1 (8)	2 (17)	3 (25)	6 (50)

[a] Identified by conventional methods.

TABLE 4. Correlation of colony count from BACTEC 12B broth at time of hybridization with DPR

Organism[a]	DPR	No. of specimens	No. (%) of specimens with colony count (CFU/ml) of:			
			$<10^4$	10^4–10^5	$>10^5$–10^6	$>10^6$
M. tuberculosis	<3	8[b]	3 (38)	5 (62)	0	0
	≥3	51	5 (10)	16 (31)	22 (43)	8 (16)
M. avium	<3	5	0	2 (40)	0	3 (60)
	≥3	51[b]	1 (2)	5 (10)	15 (29)	30 (59)
M. intracellulare	<3	2	0	1 (50)	0	1 (50)
	≥3	11[c]	0	0	5 (45)	6 (55)

[a] Identified by conventional methods.
[b] Excludes five isolates due to quantitative plate contamination.
[c] Excludes one isolate due to quantitative plate contamination.

DISCUSSION

The advantage in terms of time that the radiometric method of detection and DNA probe technology of identification have given to the area of clinical mycobacteriology has been clearly documented (1–6). In this study, we combined both methodologies in an effort to further decrease the time to final identification for cultures positive for mycobacteria. By probing positive BACTEC broth sediment directly with the three DNA probes, we were able to identify the majority of the *M. tuberculosis* isolates within 18 days of receipt of the specimen and within 7 days for the members of the *M. avium-M. intracellulare* complex.

In this study, all BACTEC bottles with GIs of >100 were tested with the three probes currently produced by GenProbe. Since we were not probing organisms from solid media, which is the original intent of the commercially available probe kit, we assumed that the number of organisms in the sediment from the BACTEC bottle would be lower than that outlined in the package insert for testing from solid media. Therefore, taking this into account in analyzing the data, we decided to use the patient specimen as the base line in calculating the percent hybridization. In doing this we were able to lower the parameters for the determination of a positive specimen while not jeopardizing the specificity of the assay.

Ellner et al. (2) used a similar approach in combining radiometric and DNA probe technologies. However, in their study, when BACTEC bottles reached GIs of ≥80, 2 ml of medium was pelleted at 2,000 × *g* for 30 min, the pellet was resuspended, and a smear was made for AFB. In their study, the smear needed to be positive for the sediments to be used in hybridization reactions. In our study all sediments were tested, regardless of smear results. In addition, in their study, for a hybridization reaction to be positive, it needed to be 10% or greater. If a 10% cutoff had been used in our study, our sensitivity values would have dropped dramatically to 55% for *M. tuberculosis*, 87% for *M. avium*, and 36% for *M. intracellulare*.

Although we were able to rapidly identify 83, 92, and 86%, respectively, of all *M. tuberculosis*, *M. avium*, and *M. intracellulare* isolates, we were interested to see if we could determine why some specimens were negative when tested directly from the BACTEC bottle. We first looked at the number of organisms present and found that the colony

TABLE 5. Correlation of false-negative direct BACTEC probe results with other culture results and treatment

Patient no.	Isolate[a]	Specimen source[b]	Date of collection (mo-day-yr)	Direct AFB smear result[c]	Direct BACTEC probe result (DPR)	Therapy (start date, mo-day-yr)[d]
1	Mtb	Sp	11-18-87	1+	+ (4)	RIF, CLO, CY, EMB (5-13-87)
	Mtb	Sp	11-18-87	4+	− (2)	
	Mtb	Sp	12-16-87	1+	+ (9)	
	Mtb	Sp	12-16-87	1+	+ (31)	
	Mtb	Sp	12-16-87	1+	+ (33)	
	Mtb	Sp	1-14-88	4+	+ (6)	
	Mtb	Sp	1-14-88	4+	− (1)	
2	Mtb	Sp	7-24-87	2+	+ (58)	INH, RIF, PZA (7-3-87)
	Mtb	Sp	7-26-87	2+	+ (17)	
	Mtb	Sp	7-28-87	1+	+ (6)	
	Mtb	Sp	7-28-87	2+	− (2.5)	
	Mtb, Ma	Sp	8-3-87	2+	−Mtb, +Ma (20)	
	Mtb	Sp	8-3-87	2+	− (2.5)	
	Mtb	Sp	8-3-87	1+	+ (3.5)	
	Mtb	Sp	8-3-87	3+	+ (20)	
3	Mtb, Ma	Sp	8-3-87	2+	−Mtb, +Ma (23)	INH, RIF, EMB (7-3-87)
	Mtb	Sp	8-3-87	2+	− (1.5)	
	Mtb	Sp	8-5-87	3+	+ (7)	
4	Mtb	Sp	8-8-87	Neg	− (1)	None
	Mtb	Sp	8-11-87	Neg	+ (5)	
5	Ma	Bld	10-13-87	Neg	+ (8)	RIF, EMB, AMK, CIP (9-87)
	Ma	Bld	10-30-87	Neg	+ (31)	
	Ma	BM	11-2-87	Neg	− (2)	
	Ma	Bld	11-19-87	Neg	+ (37)	

[a] Mtb, *M. tuberculosis*; Ma, *M. avium*.
[b] Sp, Sputum; Bld, blood; BM, bone marrow.
[c] Direct smear (400×) of concentrated original specimen. Neg, No organisms seen; 1+, 1 to 9 AFB per 100 fields; 2+, 1 to 9 AFB per 10 fields; 3+, 1 to 9 AFB per field; 4+, >9 AFB per field.
[d] AMK, Amikacin; CLO, clofazimine; CIP, ciprofloxacin; CY, cycloserine; EMB, ethambutol; INH, isoniazide; PZA, pyrazinamide; RIF, rifampin.

count distribution for the false-negative specimens was similar to that for the true-positive specimens and therefore may not have been the explanation for the false-negative results. However, we cannot rule out that the organisms may have been unevenly distributed due to clumping in the samples used in the hybridizations and colony counts.

We also were interested in establishing a threshold GI at which we could test the sample by hybridization. For that reason we analyzed the data, comparing the GIs with the hybridization results. Again, as with the colony counts, the distribution of the false-negative and false-positive specimens with respect to the GI was not significantly different except for the specimens containing *M. avium*, and here the GIs for four of the five false-negative samples were lower than those for most of the true-positive specimens with *M. avium*.

Whether treatment, especially with a drug such as rifampin, which inhibits RNA chain initiation, affects the final probe result was also examined for organisms isolated from patients on treatment. This information would also be extremely important, especially in a laboratory that services mainly patients on treatment. We approached this problem by looking at patients in our study who had both true-positive and true-negative specimens while on treatment. From this limited analysis, while it appears that many of the true-positive specimens had relatively low DPRs, these same patients also had high values for specimens collected on the same day; therefore, treatment did not appear to solely account for the false-negative specimens in this study.

The specificity of the probes in this study was 100%, as has been previously reported by other investigators (1, 6). Results from this study also determined that in BACTEC cultures which contained non-AFB or *Mycobacterium* spp. other than *M. tuberculosis* or *M. avium-M. intracellulare* there was no interference with the final probe result.

In our study, we encountered an isolate that was negative with all three probes yet biochemically and phenotypically resembled a member of the *M. avium-M. intracellulare* complex. By gas-liquid chromatography, this isolate was also identified as a member of this complex. De Girolami et al. (P. C. De Girolami, C. Sisson, A. Grumney, K. Eichelberger, and A. Y. Tsang, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1358, 1987) and Musial et al. (6) have reported on similar isolates that were negative by the Gen-Probe *M. avium-M. intracellulare* probes yet biochemically appeared to be *M. avium-M. intracellulare* isolates.

There were two mixed cultures from which both *M. avium* and *M. tuberculosis* were recovered from solid media. In probing the positive BACTEC bottle sediments and applying our formula for calculating hybridization results, only the *M. avium* probe was positive with the sediments. Most likely this is due to the faster growth rate of *M. avium* in the BACTEC media (5).

In conclusion, we have presented a method combining radiometry and DNA probes for the rapid identification of specimens containing *M. tuberculosis* or *M. avium-M. intracellulare* complexes. The specificity of this method was 100%; however, due to the sensitivity of this method, we strongly recommend that conventional cultures be done in parallel with the rapid technique. Using this strategy allows >83% of specimens containing clinically relevant mycobacterial organisms to be reported within 7 days for *M. avium-M. intracellulare* isolates and within 18 days for *M. tuberculosis* isolates. From the standpoint of patient care, this is a significant improvement even over the use of the BACTEC combined with conventional identification methods.

LITERATURE CITED

1. **Drake, T. A., J. A. Hindler, O. G. W. Berlin, and D. A. Bruckner.** 1987. Rapid identification of *Mycobacterium avium* complex in culture using DNA probes. J. Clin. Microbiol. **25**:1442–1445.
2. **Ellner, P., T. E. Kiehn, R. Cammarata, and M. Hosner.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26**:1349–1352.
3. **Gonzalez, R., and B. A. Hanna.** 1987. Evaluation of Gen-Probe DNA hybridization systems for the identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*. Diagn. Microbiol. Infect. Dis. **8**:69–78.
4. **Kiehn, T. E., and F. F. Edwards.** 1987. Rapid identification using a specific DNA probe of *Mycobacterium avium* complex from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. **25**:1551–1552.
5. **Kirihara, J. M., S. L. Hillier, and M. B. Coyle.** 1985. Improved detection times for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* with the BACTEC radiometric system. J. Clin. Microbiol. **22**:841–845.
6. **Musial, C. E., L. S. Tice, L. Stockman, and G. D. Roberts.** 1988. Identification of mycobacteria from culture using the Gen-Probe rapid diagnostic system for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **26**: 2120–2123.
7. **Roberts, G. D.** 1985. Mycobacteria and nocardia, p. 379–418. *In* J. A. Washington II (ed.), Laboratory procedures in clinical microbiology. Springer-Verlag, New York.
8. **Roberts, M. C., C. McMillan, and M. B. Coyle.** 1987. Whole chromosomal DNA probes for rapid identification of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. J. Clin. Microbiol. **25**:1239–1243.