JOURNAL OF CLINICAL MICROBIOLOGY, June 1995, p. 1435–1441 Vol. 33, No. 6
0095-1137/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Q-Beta Replicase-Amplified Assay for Detection of *Mycobacterium tuberculosis* Directly from Clinical Specimens

JYOTSNA S. SHAH,* JING LIU, DEBRA BUXTON, ANNETTE HENDRICKS, LINDA ROBINSON, GAIL RADCLIFFE, WALTER KING, DAVE LANE, D. MICHAEL OLIVE, AND JEFFREY D. KLINGER

GENE-TRAK, Framingham, Massachusetts 01701

Received 27 October 1994/Returned for modification 6 December 1994/Accepted 27 February 1995

We report the results of a study conducted to evaluate the performance of manual Q-Beta replicase-amplified *Mycobacterium tuberculosis* complex assay compared with that of culture for detecting *M. tuberculosis* directly from digested sputum pellets. A total of 261 specimens submitted to three tuberculosis testing laboratories were analyzed. Culture and acid-fast bacillus smear results were provided by the tuberculosis testing laboratories. Of these 261 specimens, 34 (13% prevalence rate) were positive for *M. tuberculosis* by culture. The samples were digested and decontaminated by the testing laboratories by using their standard digestion and decontamination procedures. An aliquot of the digested and decontaminated pellet was sent to GENE-TRAK. The digested and decontaminated pellet was neutralized by washing it with 0.067 M phosphate buffer (pH 6.8), and the bacteria present in the washed pellet were heat inactivated at 100°C for 15 min. The samples were combined with sample processing buffer containing GuSCN and were treated for 6 min in the GENE-TRAK Sample Processing Instrument to release the nucleic acids. The released rRNA was analyzed in a manual Q-Beta replicase assay format which incorporates elements of sandwich hybridization, reversible target capture, and Q-Beta replicase signal amplification technologies. In comparison with culture, the overall assay sensitivity and specificity were 97.1 and 96.5%, respectively. The positive predictive value was 80.5%, and the negative predictive value was 99.5%. After analysis of discrepant results, the assay sensitivity and specificity were 97.3 and 97.8%, respectively, and the prevalence rate was 14%. The positive predictive value and the negative predictive value were 87.8 and 99.5%, respectively. The Q-Beta replicase assay is rapid, sensitive, semiquantitative, and specific for the direct detection of *M. tuberculosis* from clinical specimens.

Tuberculosis (TB) has made a dramatic comeback in industrialized countries, in part because of the AIDS pandemic and because of the increase in the number of migrant workers, immigrants, and homeless people. In the United States there has been an 18.4% increase in TB cases since 1985 (6). The recent increase of multidrug-resistant strains of *Mycobacterium tuberculosis* has added an increasingly deadly aspect to the disease (36). There have been 13 outbreaks since 1990. More than 200 cases of multidrug-resistant *M. tuberculosis* have been reported. All of this has prompted increased interest in developing rapid methods for detecting *M. tuberculosis.*

Today, the "gold standard" for TB diagnosis is still detection of *M. tuberculosis* by culture. Traditional methods for isolating *M. tuberculosis* from human specimens by culture can take 3 to 8 weeks. Radiometric liquid culture methods such as those that use the BACTEC system (Johnson Laboratories, Towson, Md.) or the Septi-Chek AFB system (Becton Dickinson Microbiological Systems, Cockeyville, Md.) have reduced the culture time to less than 2 weeks (39). Culture confirmation can take from 2 h to several days depending on which method is used. High-performance liquid chromatography (5) or DNA probe assays require 2 to 4 h to confirm the identity of a mycobacterial isolate (13, 27). Furthermore, the *p*-nitro-∂-acetylamino-β-hydroxypropiophenone test takes 3 to 5 days, while biochemical identification takes 2 to 4 weeks (20).

PCR has been described as a non-culture-based method for detecting mycobacteria directly from respiratory specimens (3, 7, 8, 10–12, 14, 16, 25, 26, 33, 37). The reported sensitivity of PCR has ranged between 74 and 90%, with a reported specificity of greater than 98%. The sensitivity, however, is poor in samples from populations with a low prevalence of *M. tuberculosis* infection and in smear-negative, culture-positive samples (25, 34). In addition, sample inhibition of the PCR occurs in 10 to 20% of the sputum specimens tested (3, 7, 34, 38).

More recently, several investigators have evaluated the performance of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (Gen-Probe, San Diego, Calif.) on clinical specimens. The reported assay sensitivities range from 71.4 to 100%, depending on the number of smear-negative, culture-positive specimens tested (2, 9, 19, 23, 28). Performance on smear-negative specimens has been poor (2, 19). We have recently described a manual Q-Beta replicase-amplified nucleic acid hybridization assay for the direct detection and identification of *M. tuberculosis* from sputum (31). The sensitivity of this probe amplification nucleic acid hybridization assay is between 1 and 2 CFU of *M. tuberculosis* in spiked sputum.

The present study was undertaken to evaluate the performance of the manual Q-Beta replicase assay compared with that of traditional culture on human sputum specimens digested and decontaminated by standard procedures. The assay uses a simple sample processing protocol, sandwich hybridization (29), a noise reduction scheme and dual-capture reversible target capture (31, 32), and Q-Beta replicase probe amplification (21, 22). Up to 96 processed samples can be analyzed simultaneously in approximately 4 h in the Q-Beta replicase assay format (32). A total of 261 specimens were tested in collaboration with three TB testing laboratories in the United States. The sensitivity and specificity of the Q-Beta replicase assay were compared with those of culture. Sensitivity is the ability of a test to give a positive finding when the person tested truly has *M. tuberculosis* bacteria present in his or her clinical

* Corresponding author. Mailing address: GENE-TRAK, 31 New York Ave., Framingham, MA 01701. Phone: (508) 935 1241. Fax: (508) 879-6462.

specimen. Specificity is the ability of the test to give a negative finding when the specimen is free of *M. tuberculosis*. The sensitivity and specificity of the Q-Beta replicase assay compared with those of culture were 97.1 and 96.5%, respectively. After analysis of samples with discrepant results, the assay sensitivity and specificity were 97.3 and 97.8%, respectively.

MATERIALS AND METHODS

Oligo(dT)$_{14}$ or oligo(dC)$_{25}$ magnetic particles. Carboxyl-terminated ferromagnetic particles (Advanced Magnetics, Inc., Cambridge, Mass.) were derivatized with either oligo(dT) or oligo(dC) as described previously (24, 31). 5′-Amino-terminated oligo(dT)$_{14}$ or oligo(dC)$_{25}$ was synthesized by standard phosphoramidite chemistry. Oligo(dT)$_{14}$ or oligo(dC)$_{25}$ was linked to carboxyl-terminated ferromagnetic particles.

Capture probes. Two single-stranded capture probes (probes A [29-mer] and B [31-mer]), complementary to the same target sequence in the 5′ region of the *M. tuberculosis* 23S rRNA, were prepared by β-cyanoethyl phosphoramidite chemistry on a 380-B synthesizer (Applied Biosystems, Foster City, Calif.) as described previously (31). A tail of approximately 150 deoxydeazaguanosine (deaza-G) residues was added to the 3′ end of capture probe A, and a tail of approximately 150 dA residues was added to the 3′ end of capture probe B (24, 31).

Preparation of RNA detector probe. The detector probe sequence, complementary to a region of the *M. tuberculosis* 23S rRNA target a few bases upstream of the annealing site of the capture probes, was inserted into a recombinant plasmid containing the MDV sequence as described previously (31). The recombinant plasmid was linearized by digestion with *Sma*I. The RNA detector probe was prepared by transcription of this linearized recombinant plasmid with T7 RNA polymerase (Megascript kit; Ambion Inc., Austin, Tex.). The RNA detector probe was diluted in 50 mM Tris (pH 8.0)–1 mM EDTA–0.5% Nonidet P-40 (NP40) to a final concentration of 10 ng/μl (6.30×10^{10} molecules per μl).

Bacterial strains. The bacterial strain *M. tuberculosis* H37A (ATCC 25177) used in the present study was obtained from the American Type Culture Collection and was grown by standard microbiological procedures (35).

Human sputum specimens. A total of 261 digested and decontaminated human sputum pellets were received from three laboratories: 181 specimens were received from a laboratory in New York State (laboratory A), 30 specimens were received from a laboratory in Massachusetts (laboratory B), and 50 specimens were received from a laboratory in California (laboratory C). The laboratories provided acid-fast bacillus smear and culture results and, in some cases, other relevant information. All of the digested and decontaminated specimens were cultured in BACTEC vials and solid media. Specimens received from laboratory A were recently obtained (specimens were less than 1 week old) and had been stored at 4°C, specimens received from laboratory B consisted of recently obtained specimens (specimens were less than 2 weeks old) and had been stored at −70°C, and specimens from laboratory C had been stored for up to 1 year at −70°C.

(i) Sample processing. Sputum specimens were processed as outlined in Fig. 1. Specimens were first processed by the laboratories according to their standard digestion and decontamination methods. Laboratory A digested and decontaminated the sputa with 2% NaOH. Laboratories B and C used *N*-acetyl cysteine–NaOH for digestion and decontamination. An aliquot of the processed sputum pellet was sent to GENE-TRAK on dry ice or cold packs. The processed pellets were stored at −70°C. On the day of the assay, a 300-μl portion of the digested and decontaminated pellet was washed once with 800 μl of 0.067 M phosphate buffer (pH 6.8) and was suspended in 300 μl of 0.2% bovine serum albumin (BSA; Remel, Lenexa, Kans.). The suspension was heated at 100°C for 15 min, and then a 250-μl aliquot was added to a processing tube containing zirconium oxide beads and 5 M GuSCN sample processing buffer (100 mM Tris-HCl [pH 7.8], 40 mM EDTA, 5 M GuSCN, and 1.0% Sarkosyl). The organisms were lysed mechanically by shaking at 5,000 rpm for 6 min in a GENE-TRAK Sample Processor. For samples received from laboratory A, 1 g of zirconium oxide beads and 1.25 ml of 5 M GuSCN sample processing buffer were used. For samples received from laboratory B and laboratory C, a slight modification was made so that more sample could be used. Samples were combined with 0.5 g of zirconium oxide beads and 400 μl of 5 M GuSCN sample processing buffer (31).

(ii) Purified RNA. rRNA from *M. tuberculosis* ATCC 25177 was purified and quantitated as described by Barns et al. (1). A portion of either 100 or 250 μl of the purified RNA in probe dilution buffer (100 mM Tris-HCl [pH 7.8], 20 mM EDTA) was combined with 400 μl of 5 M GuSCN sample processing buffer, and the mixture was vortexed for 30 s.

Portions of 100 μl of purified RNA ($n = 6$) in 4 M GuSCN (with samples received from laboratory A) or 250-μl portions of purified RNA ($n = 6$) in 3.07 M GuSCN (with samples received from laboratories B and C) were used as assay standards. Either 100-μl portions of samples in 4 M GuSCN from laboratory A or 250-μl portions of samples in 3.07 M GuSCN from laboratories B and C were analyzed as described below ($n = 1$).

Dual-capture reversible target capture assay. The samples were analyzed by slight modifications of the method described by Shah et al. (31). The assay method is diagrammed in Fig. 2. Briefly, the target, the deaza-G-tailed capture probe A (100 ng/ml), and the MDV RNA detector probe (100 ng/ml) were hybridized for 30 min and were then captured on paramagnetic particles derivatized with (dC)$_{25}$ by diluting the GuSCN concentration to 2.20 M. The particle-bound ternary complex was washed four times with 200 μl of low-salt buffer (100 mM Tris [pH 8.1], 20 mM EDTA, 25 mM NaCl, 0.2% Sarkosyl, 0.05% BSA, 0.05% Bronopol). Following the washes, the capture probe-target hybrids were disrupted by adding 100 μl of 3.5 M GuSCN release buffer (300 mM Tris-HCl [pH 7.8], 100 mM EDTA, 3.5 M GuSCN, 0.5% Sarkosyl, 0.5% BSA). This step efficiently releases the target-detector probe complex from the deaza-G-tailed capture probe, which remains bound to the beads. The target-detector probe complex was then hybridized to dA-tailed capture probe B (100 ng/ml) for 30 min. The target-detector probe-capture probe B complex then was captured onto paramagnetic particles derivatized with (dT)$_{14}$ beads, washed three times with 200 μl of high-salt buffer (100 mM Tris [pH 8.1], 20 mM EDTA, 300 mM NaCl, 0.5% Sarkosyl, 0.5% BSA, 0.5% Bronopol), and then eluted from the (dT)$_{14}$ beads with 100 μl of the low-salt buffer, as shown in Fig. 2. The ternary complexes were subjected to two additional rounds of capture, washing, and elution on the (dT)$_{14}$ beads. Following the fourth round of capture, the ternary complexes were washed with preamplification wash buffer (50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 300 mM KCl, 0.5% NP40) and were then eluted in 200 μl of preamplification release buffer (50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 0.5% NP40).

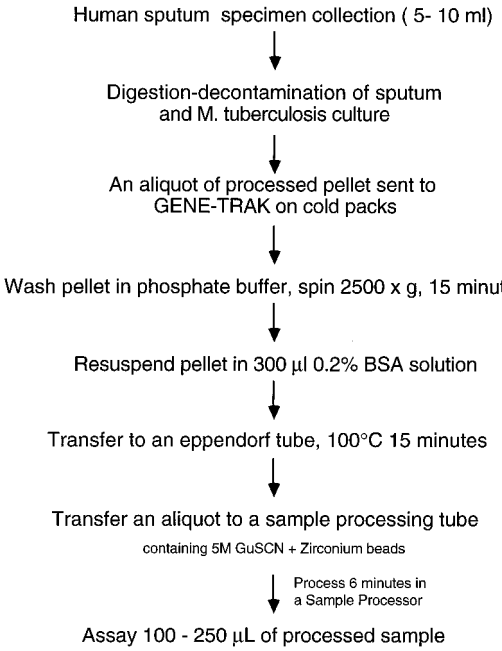

FIG. 1. Sample processing methodology.

Amplification with Q-Beta replicase. The released MDV detector probe was amplified by Q-Beta replicase as described previously (31). A 100-μl portion of the released hybrid complex was combined with 50 μl of Q-Beta replicase reaction buffer (220 mM Tris [pH 7.8], 40 mM $MgCl_2$, 1.2 mM nucleoside triphosphates [GTP, ATP, CTP, and UTP], 20 μg of propidium iodide per ml) and 50 μl of a Q-Beta replicase stock solution (containing about 110 μg of Q-Beta replicase per ml in 25% glycerol). The mixture was incubated at 37°C, and the production of RNA, as measured by increasing fluorescence because of the binding of propidium iodide, was recorded every 40 s in a prototype recording kinetic fluorometer (31). The instrument measures and records fluorescence for 96 samples simultaneously and calculates the time required to attain a preset level of fluorescence, termed the response time. In practice this corresponds to the amount of time a particular reaction takes to produce a fixed amount of RNA (approximately 3.0×10^{11} molecules) and is inversely related to the logarithm of the number of RNA detector molecules present at the start of the amplification reaction (4).

Analysis. Under the amplification conditions used in the present study, the slope range of the amplification product, generated by amplification of the detector probe by Q-Beta replicase, was between 30 and 160 relative fluorescence units (RFU) per min. Therefore, all of the samples which gave a slope of less than 30 or greater than 160 RFl units were discarded as contaminants. A cutoff was set at a response time equivalent to that for 10^3 rRNA molecules, determined from the standard dose-response curve ($n = 6$), which was run with

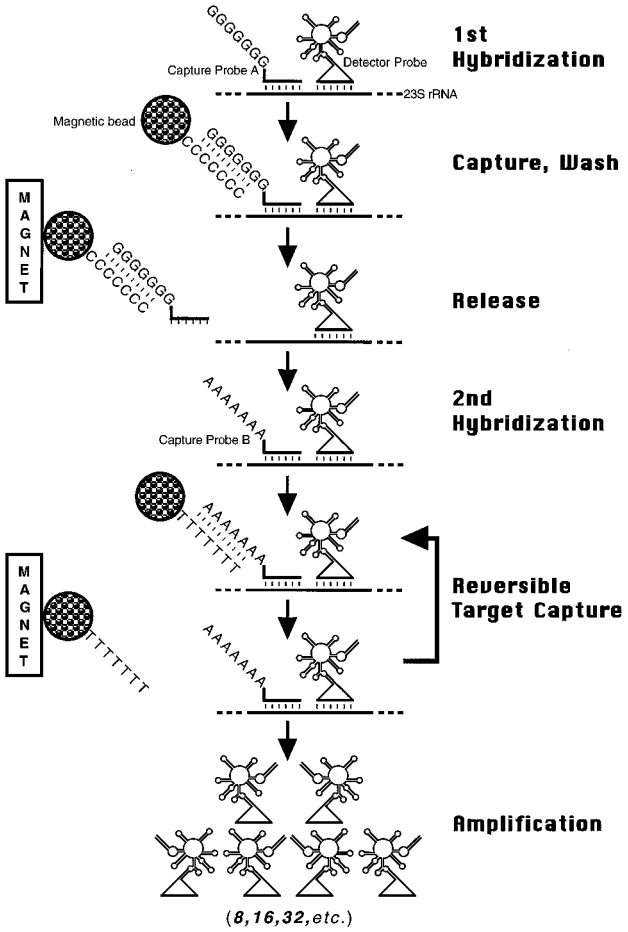

FIG. 2. Dual-capture reversible capture assay format.

each batch of specimens. Because the limit of detection was 10^3 rRNA molecules (there were fewer than 100% responders below 10^3 rRNA molecules), only specimens which gave a response time equivalent to or greater than that for 10^3 rRNA molecules were considered positive.

Several concentrations of purified *M. tuberculosis* ATCC 25177 23S rRNA molecules (0, 10^2, 10^3, 10^4, 10^5, and 10^6) were run with each batch of specimens. A graph of the logarithm of the number of rRNA molecules (x axis) versus the response time (y axis) was plotted (Fig. 3A). The response times of the Q-Beta replicase-positive test samples were compared with standard curves of response times versus the number of rRNA molecules to quantitate the number of *M. tuberculosis* 23S rRNA molecules in each specimen. The reason for running standard dose curves with each batch of specimens was that there were minor differences in the amplification cocktails, which in turn caused slight variations in response times. Assuming that the lysis of *M. tuberculosis* CFU in all clinical specimens was similar (unpublished data), the number of 23S rRNA molecules per *M. tuberculosis* CFU was estimated by comparing standard curves of response time versus the number of rRNA molecules and response time versus CFU as shown in Fig. 3A and B, respectively. Note that the mean response time for 1 *M. tuberculosis* CFU (ca. 20.5 min) corresponds to the response time generated from ca. 4.0×10^3 23S rRNA molecules.

Statistical analysis. The significance of differences in the mean response times between two sample sets was determined by the t test. If the t-test value was greater than 2, the difference in the mean response times (signal) between the two sample sets was considered statistically significant. The significance of difference between the Q-Beta replicase assay and culture sensitivity percentages was determined by the chi-square test. If the chi-square test value was greater than 3.84, the difference between the two tests was considered to be statistically significant.

RESULTS

Estimated number of rRNA targets per CFU. To determine the number of rRNA targets in *M. tuberculosis* CFU, two sets

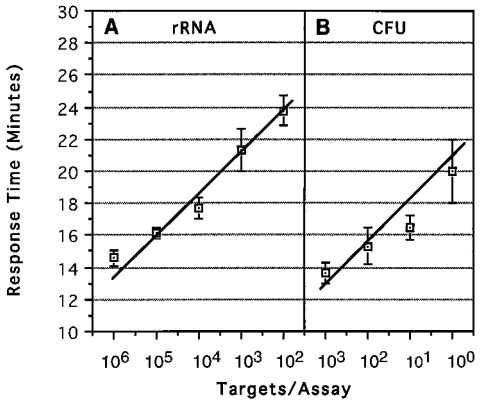

FIG. 3. *M. tuberculosis* dose response. (a) Response times of serially diluted *M. tuberculosis* rRNA. (b) Response times of *M. tuberculosis* CFU. Serial dilutions of *M. tuberculosis* rRNA equivalent to 0 to 10^6 23S rRNA molecules per assay point and *M. tuberculosis* CFU equivalent to 0 to 10^4 CFU per assay point were analyzed as described in the Materials and Methods section. The number of assay points at each concentration of rRNA or CFU was 5. None of the zero-target controls ($n = 10$) gave a response time of less than 30 min (data not shown).

of samples were prepared. Set A was for purified *M. tuberculosis* rRNA, and set B was for *M. tuberculosis* CFU. In set A, purified *M. tuberculosis* rRNA was serially diluted in probe dilution buffer to 10^7, 10^6, 10^5, 10^4, and 10^3 23S rRNA molecules per ml. In set B, six 0.2% BSA samples were spiked with 0, 10^1, 10^2, 10^3, and 10^4 CFU of *M. tuberculosis* H37A (ATCC 25177) per ml. The samples from set A and set B were processed and assayed as described in the Materials and Methods section. All of the samples ($n = 5$) containing 10^3, 10^4, 10^5, and 10^6 23S rRNA molecules per assay point gave response times of less than 30 min (Fig. 3A). At the 10^2 target level only three of five samples gave response times of less than 30 min. All of the samples ($n = 5$) containing 10^0, 10^1, 10^2, and 10^3 CFU of *M. tuberculosis* per assay point gave response times of less than 30 min (Fig. 3B). None of the controls with no target gave a response time (data not shown). On the basis of these results the limit of detection was 10^3 purified 23S rRNA molecules or 1 CFU (Fig. 3). The linearity of the curve for *M. tuberculosis* CFU suggests that the bacteria were lysed completely. Comparison with the standard curve derived from serially diluted rRNA indicated that there were approximately 4.0×10^3 23S rRNA targets per *M. tuberculosis* CFU (Fig. 3B).

Effect of increasing sample volume on signal and noise (sensitivity and specificity). *M. tuberculosis*-positive specimens can have very few *M. tuberculosis* organisms. Therefore, we felt that it would be advantageous to assay as much sample as possible. Two sets of samples, set A and set B, were prepared. Set A samples were prepared and tested in the same way as the samples from laboratory A. Set B samples were prepared and tested in the same way as the samples from laboratory B or laboratory C. Three 5-ml aliquots of *M. tuberculosis*-negative sputum samples were spiked with 0, 10^3, and 10^4 CFU of *M. tuberculosis* H37A (ATCC 25177), respectively. The spiked samples were digested and decontaminated by the *N*-acetyl-L-cysteine–NaOH method; this was followed by a phosphate buffer wash to neutralize the processed pellet prior to heat inactivation. For set A, 250 μl of the heat-inactivated sample was added to a processing tube containing 1,250 μl of 5 M GuSCN and 1.0 g of zirconium oxide beads. For set B, 250 μl of the heat-inactivated sample was added to a processing tube containing 400 μl of 5 M GuSCN and 0.5 g of zirconium oxide

TABLE 1. Effect of increasing sample volume on signal and noise

M. tuberculosis CFU/5 ml of sputum	Response time (min)[a]		*t*-test value[b]
	Set A	Set B	
10^4	15.80 ± 0.65 (5)	15.20 + 0.95 (5)	1.17
10^3	18.04 ± 0.79 (5)	15.84 + 0.99 (6)	4.01
0	NR[c] (5)	NR (5)	0

[a] Values are means ± standard deviations. Values in parentheses are the number of samples tested. Set A samples were processed as described in the text for samples from laboratory A. A 250-μl portion of heat-inactivated sample was mixed with 1,250 μl of 5 M GuSCN and 1.0 g of zirconium beads, and the mixture was processed as described in the Materials and Methods section. A 100-μl portion of the processed sample was assayed. Set B samples were processed as described in the text for samples from laboratories B and C. A 250-μl portion of heat-inactivated sample was mixed with 400 μl of 5 M GuSCN and 0.5 g of zirconium beads, and the mixture was processed as described in the Materials and Methods section. A 250-μl portion of the processed sample was assayed.

[b] If the *t*-test value was greater than 2, the difference in response time (signal) between the two sample sets was considered significant.

[c] NR, no response in 30 min.

beads. The results are presented in Table 1. There was no significant difference in the response times generated by 10^4 *M. tuberculosis* CFU per spike between the two sets (*t*-test value, 1.17). However, at the 10^3 spike level, the mean response time for set B samples was significantly faster than that for set A samples, by 2.2 min (*t*-test value, 4.01). No signal was generated within 30 min in the absence of target under both conditions. On the basis of these results, we conclude that increasing the sample volume fivefold improved the sensitivity slightly but that it had no effect on specificity. Thus, for specimens from laboratories B and C, we used fivefold more sample (~10% of the sputum sample) than the amount of sample from laboratory A.

Negative controls. There were 120 zero-target control (buffer-only) assay points. Three assay points were excluded from the final analysis because their slopes were outside of the set range. Of the remaining 117 zero-target control assay points, two gave response times indicating the presence of greater than 10^3 rRNA molecules.

Human sputum specimens. A total of 261 specimens were tested. One human sputum specimen was excluded from the final analysis because the slope was outside of the set slope range (30 to 160 RFU). Of the remaining 260 specimens, 35 were smear positive and 225 were smear negatives (Table 2). Thirty-four specimens (20 smear-positive and 14 smear-negative specimens) were positive for *M. tuberculosis* by culture (Table 2). Between 1 and 10 CFU were isolated from 9 of the 14 *M. tuberculosis*-positive, smear-negative specimens. Forty-one specimens (20 smear-positive and 21 smear-negative specimens) were considered positive by the Q-Beta replicase assay because they gave response times indicating the presence of 10^3 more rRNA molecules. Of the 41 specimens positive by the Q-Beta replicase assay, 33 were also positive by culture (Table 3). One smear-negative specimen was missed by the Q-Beta replicase assay.

TABLE 3. Comparison of Q-Beta replicase assay with culture

Assay and result	No. of specimens with the following culture result[a]:		No. of true-positive specimens with the following result[b]:	
	Positive	Negative	Positive	Negative
Q-Beta				
Positive	33 (*a*)	8 (*b*)	36	5[c]
Negative	1 (*c*)	218[d] (*d*)	1[e]	218[d]
Culture				
Positive			34	0[c]
Negative			3[e]	223[d]

[a] Sensitivity, 97.1%; specificity, 96.4%; positive predictive value, 80.4%; negative predictive value, 99.6%; *a*, *M. tuberculosis*-positive specimens by Q-Beta replicase assay and culture; *b*, false-positive specimens by Q-Beta replicase assay (type I or alpha error); *c*, false-negative specimens by the Q-Beta replicase assay (type II or beta errors); *d*, *M. tuberculosis*-negative specimens by Q-Beta replicase assay and culture. Sensitivity, which is the percentage of *M. tuberculosis*-positive specimens by the Q-Beta replicase assay, is calculated by $[a/(a + c)] \cdot 100$. Specificity, which is the percentage of *M. tuberculosis*-negative specimens by the Q-Beta replicase assay, is calculated by $[d/(b + d)] \cdot 100$. The positive predictive value, which is the percentage of *M. tuberculosis*-positive specimens correctly identified by the Q-Beta replicase assay, is calculated by $[a/(a + b)] \cdot 100$. The negative predictive value, which is the percentage of *M. tuberculosis*-negative specimens correctly identified by the Q-Beta replicase assay, is calculated by $[d/(c + d)] \cdot 100$).

[b] For the Q-Beta assay, sensitivity, 97.3%; specificity, 97.8%; positive predictive value, 87.8%; negative predictive value, 99.5%. For culture, sensitivity, 91.9%; specificity, 100%; positive predictive value, 100%; negative predictive value, 98.7%.

[c] Type I or alpha errors.

[d] Includes six *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex isolates, three *M. chelonae* isolates, two *M. xenopi* isolates, and two mycobacteria other than *M. tuberculosis* complex.

[e] Type II or beta errors.

The remaining eight Q-Beta replicase assay-positive, culture-negative specimens were smear negative (Tables 2 and 3). Three were from one patient whose past cultures were positive for *M. tuberculosis*. This patient was on antibiotic therapy at the time that these samples were taken. Therefore, these samples (Fig. 4, specimens 26, 29, and 32) were considered to be positive for *M. tuberculosis. M. xenopi* was isolated from the fourth *M. tuberculosis* culture-negative, Q-Beta replicase assay-positive specimen.

The remaining 218 specimens (15 smear-positive and 203

TABLE 2. Comparison of Q-Beta replicase assay versus culture before analysis of samples with discrepant results

Specimen	No. of specimens				Sensitivity (%)	No. of specimens MTB negative by:			Specificity (%)
	In final analysis	MTB[a] positive by:							
		Culture	Culture and Q-Beta replicase assay	Q-Beta replicase assay only		Culture	Culture and Q-Beta replicase assay	Q-Beta replicase assay only	
Total	260	34	33	8	97.1	226	218	1	96.5
Smear positive	35	20	20		100	15[b]	15	0	
Smear negative	225	14	13	8	92.9	211[c]	203	1	

[a] MTB, *M. tuberculosis*.

[b] Including three *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex organisms.

[c] Including three *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex isolates, three *M. chelonae* isolates, two *M. xenopi* isolates, and two mycobacteria other than *M. tuberculosis.*

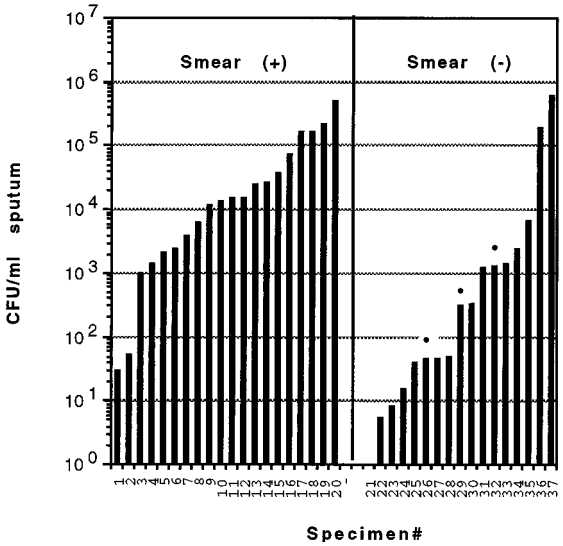

FIG. 4. Estimated number of *M. tuberculosis* CFU per milliliter of processed sputum pellet in *M. tuberculosis*-positive specimens. ●, specimens negative for *M. tuberculosis* by culture. The assumption was made that the lysis of *M. tuberculosis* CFU in all clinical specimens was similar.

smear-negative specimens) were negative by the Q-Beta replicase assay. Of these 218 specimens, 13 were positive for mycobacteria other than *M. tuberculosis* by culture. This included 10 smear-negative and 3 smear-positive specimens. All three smear-positive specimens contained *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex organisms.

Of the 10 smear-negative specimens positive for mycobacteria other than *M. tuberculosis* by culture, 3 contained *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex organisms, three contained *M. chelonae*, two contained *M. xenopi*, and two contained fast-growing mycobacteria other than *M. tuberculosis*. With the exception of the single smear-negative, *M. xenopi*-containing false-positive specimen discussed above, all of these samples were negative by the Q-Beta replicase assay.

Semiquantitative estimates of the *M. tuberculosis* concentration in sputum. Of the 260 specimens included in the final analysis, 34 were positive for *M. tuberculosis* by culture. Of these, 33 were also positive by the Q-Beta replicase assay (Tables 2 and 3). Additionally, three specimens were considered positive on the basis of the past culture and antibiotic therapy history of the patient from whom they were obtained, as discussed above (Table 3). The estimated numbers of CFU of *M. tuberculosis* per milliliter of processed sputum pellet in the 36 *M. tuberculosis*-positive specimens (33 culture-positive specimens and 3 culture-negative, Q-Beta replicase assay-positive specimens) were calculated from the standard curve derived from serially diluted rRNA. The results are presented in Fig. 4. The number of CFU per milliliter of processed sputum ranged between 1 and 10^6 on the basis of our estimate of approximately 4.0×10^3 23S rRNA targets per *M. tuberculosis* CFU (Fig. 3). Among the smear-positive, *M. tuberculosis*-positive specimens, 18 of 20 specimens had between 10^3 and 10^6 CFU/ml of processed pellet. The remaining two specimens had less than 10^2 CFU/ml of sputum. Among the smear-negative, *M. tuberculosis*-positive specimens, 9 of the 16 specimens had less than 10^3 CFU/ml of sputum. Five specimens had between 10^3 and 10^4 CFU/ml. The remaining two specimens had greater than 10^5 CFU/ml.

DISCUSSION

We have previously demonstrated that the Q-Beta replicase-amplified assay can detect *M. tuberculosis* directly in spiked human sputum specimens digested and decontaminated by either 2% NaOH or *N*-acetyl-L-cysteine procedures (31). The assay has high degrees of sensitivity and specificity (31). Here we report that the Q-Beta replicase assay, in comparison with culture, has a sensitivity and specificity greater than 97% with clinical specimens. Unlike PCR assays, up to 10% of the processed sputum sediment can be analyzed in the Q-Beta replicase assay format. Whereas detection of *M. tuberculosis* by culture can take anywhere from a few days to several weeks, the total assay time for the Q-Beta replicase assay, from the heat inactivation step to the final result, is approximately 4 h. The Q-Beta replicase assay was performed on 261 sputum specimens from patients suspected of having TB. The prevalence rate of *M. tuberculosis* disease in the present study was 13% prior to analysis of specimens with discrepant results. One specimen was discarded from the final analysis because of the presence of a contaminant. Of the remaining 260 specimens, 34 specimens were positive for *M. tuberculosis* by culture. Of these, 33 were also positive by the Q-Beta replicase assay. There were eight additional smear-negative, culture-negative specimens that were positive by the Q-Beta replicase assay (Tables 2 and 3). When duplicate samples from these same eight specimens were retested in the Q-Beta replicase assay format, all were positive. Prior to resolution of discrepant results, the overall assay sensitivity and specificity were 97.1 and 96.5%, respectively, compared with culture. The positive predictive value and the negative predictive value for the Q-Beta replicase assay were 80.5 and 99.6%, respectively (Tables 2 and 3). In comparison with culture, the assay sensitivities were 100% for the smear-positive specimens and 92.9% for the smear-negative specimens (Table 2).

One specimen (Fig. 4, specimen 21) that was smear negative and Q-Beta replicase assay negative but culture positive yielded fewer than 10 CFU by quantitative culture. Q-Beta replicase assay of a duplicate sample from the same specimen again yielded a negative result. Thus, this specimen was considered false negative. Since the Q-Beta replicase assay detects all strains of *M. tuberculosis* complex organisms (31), it is possible that this specimen was missed because of either a sampling error as a result of a low target level or because of a nonuniform distribution of microorganisms in the test suspension (19, 28). The eight specimens which were negative by smear and culture but positive by the Q-Beta replicase assay were from different laboratories. Three of these specimens (Fig. 4, specimens 26, 29, and 32) were from a patient with a past history of cultures positive for *M. tuberculosis*. This patient was on antibiotic therapy at the time that these samples were taken. It has recently been reported that in patients on antibiotic therapy, the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test, which detects rRNA directly from a specimen, remains positive for no longer than 2 weeks after the culture result reverts to negative (9). Second, the presence of RNA may be strongly suggestive of the persistence of live organisms (17). Thus, it is possible that the Q-Beta replicase assay may be more sensitive than culture and, therefore, can detect nonculturable levels of *M. tuberculosis* organisms in patients on antibiotic treatment. On this basis, three specimens were considered positive. Of the other five smear- and culture-negative specimens, one was from a patient whose past three cultures were negative for *M. tuberculosis*. This result was classified as a false positive. The second specimen was from a male who had recently immigrated to the United States. There were

no previous culture results or history for this patient. After 60 days of culture, three yellow colonies of *M. xenopi* were isolated from this patient's specimen. While it is possible that there was cross-reactivity with this particular isolate of *M. xenopi*, we were unable to obtain an isolate for further characterization. Another specimen positive for *M. xenopi* which was isolated by the same laboratory showed no cross-reactivity in the Q-Beta replicase assay.

Several mycobacterial species including *M. xenopi* ATCC 19250 at approximately 10^6 CFU per assay point also produced no signal (31). High levels of other bacteria including *Rhodococcus*, *Pseudomonas*, *Micrococcus*, and *Deinococcus* species at 10^6 CFU or more per assay point generated no signal by the Q-Beta replicase assay. The reason for the positive result for this patient is unclear. However, it is possible that the patient in question may have been coinfected with *M. tuberculosis*, which was undetected by culture. Unfortunately, final resolution was not possible. The remaining three specimens were from patients with no previous history of TB. These were also considered false positives. The other 10 mycobacterial isolates (non-*M. tuberculosis*) showed no cross-reactivity in the Q-Beta replicase assay.

After analysis of specimens with discrepant results, 37 specimens were considered true positives and 223 were considered true negatives (Table 3). This corresponds to a prevalence rate of 14%. Of the 37 true-positive specimens, 36 were positive by the Q-Beta replicase assay and 33 were positive by culture (Table 3). On the basis of these data, the sensitivities of culture and the Q-Beta replicase assay following resolution of the discrepancies were 91.9 and 97.3%, respectively. All 223 specimens truly negative for *M. tuberculosis* were also negative by culture. Of these 223 specimens, 218 were also negative by the Q-Beta replicase assay (Table 3). Therefore, there were five false-positive specimens by the Q-Beta replicase assay (Table 3). On the basis of these data the specificities of culture and the Q-Beta replicase assay following resolution of specimens with discrepant results were 100 and 97.6%, respectively.

In medical decision making type I or alpha errors are synonymous with false-positive results and type II or beta errors are synonymous with false-negative results. The Q-Beta replicase assay missed one true-positive specimen and culture missed three true-positive specimens (i.e., one beta [type II] error by the Q-Beta replicase assay and three beta errors by culture). There were five false-positive specimens by the Q-Beta replicase assay and none by culture. The Q-Beta replicase assay appeared to be more sensitive than culture because it had more alpha (type I) errors and fewer beta (type II) errors than culture. However, the observed difference in sensitivities between the Q-Beta replicase assay and culture is not significant on the basis of the chi-square test value of 0.25 (a chi-square test value of less than 3.84 is not significant). The difference may be entirely due to sampling variation.

The positive predictive value and the negative predictive value for the Q-Beta replicase assay with an *M. tuberculosis* prevalence rate of 14% were 87.8 and 99.5%, respectively (Table 3). The prevalence rate for *M. tuberculosis* varied from 2 to 15%. On the basis of the results presented above, the calculated positive and negative predictive values would be 70 and 99.8%, respectively, in a population with a 6% *M. tuberculosis* prevalence rate and 58 and 99.9%, respectively, in a population with a 3% *M. tuberculosis* prevalence rate.

Two of the 117 (1.7%) negative controls (no-target, no-matrix samples) gave response times indicating the presence of greater than 10^3 rRNA molecules. This level of noise was attributed to limitations of performing amplification assays in an open system. Ordinary manual manipulations such as pipetting and aspirations in the presence of high initial concentrations of detector probe molecules create aerosols which can cross-contaminate samples, thus giving false-positive results (32). The solution to this problem lies in the present efforts aimed at developing a fully automated closed system.

The Q-Beta replicase assay is quantitative over a 10^9-fold concentration of target (22). Because of this broad quantitative range, estimation of the infectious load can be derived by comparison with a standard curve. The range of *M. tuberculosis* CFU in *M. tuberculosis*-positive specimens varied from less than 1 to more than 10^6/ml of sputum (Fig. 4). Approximately 10^3 organisms per ml are required to produce a positive smear result (15, 30). In 90% of the smear-positive specimens analyzed in the study, the estimated number of CFU per milliliter of processed sputum was between 10^3 and 10^6. On the basis of the results of the Q-Beta replicase assay, 58.8% (10 of 17) of the smear-negative specimens had fewer than 10^3 CFU/ml of processed sputum. There were only two smear-negative specimens with more than 10^5 CFU/ml of sputum and two smear-positive specimens with fewer than 10^3 CFU/ml of sputum. The slight inconsistency could be due to a nonuniform distribution of microorganisms in the test suspension (15, 19, 28).

Recently, several investigators have reported the detection of *M. tuberculosis* in respiratory specimens by the Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test and PCR. The reported sensitivities varied from 70 to 100%. Overall sensitivities were affected by the number of smear-negative, culture-positive samples included in the studies. The reported sensitivities were considerably lower for smear-negative specimens (2, 14, 19, 25). In part, this may be due to a combination of the presence of amplification inhibitors in sputum specimens (3, 7, 19, 25, 28, 34, 38) and low sample utilization (1% or less). In the present study, the false-negative rate was less than 3%. We believe that this was not due to sample inhibition but was due to either a sampling error because of a low target level or a nonuniform distribution of microorganisms in the test suspension, as discussed above. The absence of inhibitors is due to the fact that the probe-target complex is extensively purified by reversible target capture prior to the amplification by the Q-Beta replicase. This method has been reported to decrease the level of interfering substances by 10^{12}- to 10^{16}-fold following four rounds of cycling (18, 24). Thus, the Q-Beta replicase assay not only can be performed directly on sample-processed sputum pellets without any purification but it also allows for a large sample utilization (~10%), as demonstrated in both the present and previous (31) studies.

In summary, we have demonstrated that there is no significant difference in the performances of the Q-Beta replicase assay and culture. The assay can be performed on specimens digested and decontaminated by standard methods. Because of its speed, accuracy, and high degree of sensitivity, the Q-Beta replicase assay may provide a powerful new tool for the diagnosis of TB. We are testing an automated, instrumented version of the test.

REFERENCES

1. **Barns, S. M., D. J. Lane, M. L. Sogin, C. Bibeau, and W. G. Weisburg.** 1991. Evolutionary relationship among pathogenic Candida species and relatives. J. Bacteriol. **173:**2250–2255.
2. **Bodmer, T., A. Gurtner, K. Schopfer, and L. Mater.** 1994. Screening of respiratory tract specimens for the presence of *Mycobacterium tuberculosis* by using Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J. Clin. Chem. **32:**1483–1487.
3. **Brisson-Noel, A., B. Gicquel, D. Lecossier, V. Levy-Frebault, X. Nassif, and A. J. Hance.** 1989. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet **ii:**1069–1071.
4. **Burg, J. L.** Personal communication.
5. **Butler, W. R., and J. O. Kilburn.** 1988. Identification of major slowly growing pathogenic mycobacteria and *Mycobacterium gordonae* by high-performance

liquid chromatography of their mycolic acids. J. Clin. Microbiol. **26:**50–63.
6. **Centers for Disease Control.** 1992. Tuberculosis morbidity—United States, 1991. Morbid. Mortal. Weekly Rep. **41:**240.
7. **Clarridge, J. E., III, R. B. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine microbiology laboratory. J. Clin. Microbiol. **31:**2049–2056.
8. **Cousins, D. V., S. D. Wilton, B. R. Francis, and B. L. Gow.** 1992. Use of the polymerase chain reaction for rapid diagnosis of tuberculosis. J. Clin. Microbiol. **30:**255–258.
9. **Della-Latta, P., E. Waithe, S. Whittier, O. Lungu, I. Weitzman, and S. Silverstein.** 1994. Direct detection of *Mycobacterium tuberculosis* from sequential specimens by the Gen-Probe Amplified System and PCR, abstr. C-336, p. 549. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
10. **De Wit, D., L. Steyn, S. Shoemaker, and M. Sogin.** 1990. Direct detection of *Mycobacterium tuberculosis* in clinical specimens by DNA amplification. J. Clin. Microbiol. **28:**2437–2441.
11. **Eisenach, K. D., M. D. Cave, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
12. **Eisennach, K. D., M. D. Gifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
13. **Ellner, P. D., T. E. Kiehn, R. Cammarata, and M. Hosmer.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26:**1349–1352.
14. **Forbes, B. A., and K. E. S. Hicks.** 1993. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol. **31:**1688–1694.
15. **Gullans, C. R., Sr.** 1992. Digestion-decontamination procedures, p. 3.4.1–3.4.7. *In* H. D. Isenburg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
16. **Hermans, P. W. M., A. R. J. Schuitema, D. van Sooligen, C. P. H. J. Verstynen, E. M. Bik, J. E. R. Thole, A. H. J. Kolk, and J. D. A. van Embden.** 1990. Specific detection of *Mycobacterium tuberculosis* complex strains by polymerase chain reaction. J. Clin. Microbiol. **28:**2051–2058.
17. **Holland, S. M., A. P. Hudson, L. Bobo, J. A. Whittum-Hudson, R. P. Viscidi, T. C. Quinn, and H. R. Taylor.** 1992. Demonstration of chlamydial RNA and DNA during a culture-negative state. J. Clin. Microbiol. **60:**2040–2047.
18. **Hunsaker, W. R., H. Badri, M. Lombardo, and M. L. Collins.** 1989. Nucleic acid hybridization assays employing dA-tailed capture probes. II. Advanced capture methods. Anal. Biochem. **181:**360–370.
19. **Jonas, V., M. J. Alden, I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
20. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control, Atlanta.
21. **Kramer, F., and P. Lizardi.** 1989. Replicatable RNA reporters. Nature (London) **339:**401–402.
22. **Lizardi, P. M., C. Guerra, H. Lomeli, I. Tussie-Luna, and F. Kramer.** 1988. Exponential amplification of recombinant-RNA hybridization probes. Biol. Technol. **6:**1197–1202.
23. **Miller, N. M., S. D. Hernandez, and T. J. Cleary.** 1994. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Chem. **32:**393–397.
24. **Morrissey, D. V., M. Lombardo, J. K. Eldridge, K. R. Kearney, E. P. Groody, and M. L. Collins.** 1989. Nucleic acid hybridization assays employing dA-tailed capture probes. Anal. Biochem. **181:**345–359.
25. **Nolte, F. S., B. Metchock, J. E. McGowen, Jr., A. Edwards, O. Okwumabua, C. Thurmond, P. S. Mitchell, B. Plikaytis, and T. Shinnick.** 1993. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31:**1777–1782.
26. **Noordhoek, G. T., A. H. J. Kolk, G. Bjune, D. Catty, J. W. Dale, P. E. M. Fine, P. Godfrrey-Faussett, S. Cho, T. Shinnick, S. B. Svenson, S. Wilson, and J. D. A. van Embden.** 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32:**227–284.
27. **Peterson, E. M., R. Lu, C. Floyd, A. Nakasone, G. Friedly, and L. M. de la Maza.** 1989. Direct identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* from amplified primary cultures in BACTEC media using DNA probes. J. Clin. Microbiol. **27:**1543–1547.
28. **Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber.** 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by target-amplified test system. J. Clin. Microbiol. **32:**918–923.
29. **Ranki, M., A. Palva, M. Virtanen, M. Laaksonen, and H. Soderlund.** 1983. Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples. Gene **21:**77–85.
30. **Roberts, G. D., E. W. Koneman, and Y. K. Kim.** 1992. *Mycobacterium*, p. 304–339. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenburg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
31. **Shah, J., J. Liu, D. Buxton, D. M. Olive, W. King, and J. D. Klinger.** 1995. Detection of *Mycobacterium tuberculosis* directly from spiked human sputum by a Q-Beta replicase amplified assay. J. Clin. Microbiol. **33:**322–328.
32. **Shah, J., J. Liu, J. Smith, S. Poppof, G. Serpe, B. O'Brian, G. Radcliffe, D. M. Olive, and W. King.** 1994. A novel ultra sensitive, Q-Beta replicase amplified hybridization assay for the detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **32:**2718–2724.
33. **Sjobring, V., M. Mecklenburg, A. B. Anderson, and H. Miorner.** 1990. Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **28:**2200–2204.
34. **Soini, H., M. Skurnil, K. Liippo, E. Tala, and M. K. Vilganen.** 1992. Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32-kilodalton protein. J. Clin. Microbiol. **30:**2025–2028.
35. **Sommers, H. M., and R. C. Good.** 1985. *Mycobacterium*, p. 216–248. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
36. **Sudre, P., G. en Dam, and A. Kochi.** 1992. Tuberculosis: a global overview of the situation today. Bull. W. H. O. **70:**149–159.
37. **Veringa, E., B. van Harrelaar, and P. Hermans.** 1992. Polymerase chain reaction to detect *Mycobacterium tuberculosis* in a clinical microbiology laboratory. J. Microbiol. Methods **16:**139–147.
38. **Victor, T., R. du Toit, and P. D. van Helden.** 1992. Purification of sputum samples through sucrose improves detection of *Mycobacterium tuberculosis* by polymerase chain reaction. J. Clin. Microbiol. **30:**1515–1517.
39. **Whittier, P. S., K. Westfall, S. Setterquist, and R. L. Hopfer.** 1992. Evaluation of the Septi-Chek AFB System in the recovery of mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **11:**915–918.