

Use of Smear-Positive Samples To Assess the PCR-Based Genotype MTBDR Assay for Rapid, Direct Detection of the *Mycobacterium tuberculosis* Complex as Well as Its Resistance to Isoniazid and Rifampin[∇]

Akos Somoskovi,¹ Jillian Dormandy,¹ Dimitra Mitsani,^{1,2} Jeremy Rivenburg,¹ and Max Salfinger^{1,2*}

Wadsworth Center, New York State Department of Health, Albany, New York,¹ and Albany Medical College, Department of Medicine, Albany, New York²

Received 20 July 2006/Returned for modification 11 September 2006/Accepted 4 October 2006

Isoniazid (INH) and rifampin (RIF) are two of the most important antituberculosis drugs, and resistance to both of these drugs can often result in treatment failure and fatal clinical outcome. Resistance to these two first-line drugs is most often attributed to mutations in the *katG*, *inhA*, and *rpoB* genes. Historically, the identification and testing of the susceptibility of *Mycobacterium tuberculosis* complex (MTBC) strains takes weeks to complete. Rapid detection of resistance using the PCR-based Genotype MTBDR assay (Hain Lifescience GmbH, Nehren, Germany) has the potential to significantly shorten the turnaround time from specimen receipt to reporting of results of susceptibility testing. Therefore, the aim of the present study was to determine (i) the sensitivity and accuracy of the Genotype MTBDR assay for the detection of MTBC strains and (ii) the ability of the assay to detect the presence of INH and RIF resistance-associated mutations in *katG* and *rpoB* from samples taken directly from smear-positive clinical specimens. The results were compared with those obtained with the reference BACTEC 460TB system combined with standard DNA sequencing analysis methods for *katG*, *inhA*, and *rpoB*. A total of 92 drug-resistant and 51 pansusceptible smear-positive specimens were included in the study. The Genotype MTBDR assay accurately and rapidly detected MTBC strains in 94.4% of the 143 specimens and showed a sensitivity of 94.4% for *katG* and 90.9% for *rpoB* when used directly on smear-positive specimens. The assay correctly identified INH resistance in 48 (84.2%) of the 57 specimens containing strains with resistance to high levels of INH (0.4 µg/ml) and RIF resistance in 25 (96.2%) of the 26 specimens containing RIF-resistant strains.

The greatest concern of tuberculosis control programs is the appearance of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant tuberculosis, which encodes resistance not only to isoniazid (INH) and rifampin (RIF) but also to at least three of the six main classes of second-line drugs (3). The cornerstones for the effective control of drug-resistant tuberculosis are the immediate isolation of patients and rapid detection of drug-resistant strains, followed by prompt implementation of an adequate antituberculosis therapy that is based on laboratory findings. Although the use of the liquid medium-based, semiautomated, radiometric BACTEC 460TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD.) has significantly reduced the turnaround time for growth detection and testing of susceptibility of *Mycobacterium tuberculosis* complex (MTBC) strains, the determination of drug resistance in MTBC strains still requires the generation of a viable, pure culture (6). Recently, several nonradiometric, fully automated systems that are suitable alternatives to the reference radiometric system were introduced (18). However, despite the technical and safety advantages, these systems did not reduce the overall turnaround times (18). The clinical consequence of these extended assay times for susceptibility

testing is that patients with drug-resistant tuberculosis may not be adequately treated and therefore could remain infectious for longer times than patients infected with susceptible strains.

Importantly, the increased understanding of the molecular basis of resistance to antituberculosis drugs, and the consequent optimization of molecular methods, has significantly shortened the turnaround time for the detection of the presence of MTBC strains and mutations encoding drug resistance directly in clinical specimens without the need for a viable and large biomass (4, 9, 14, 17, 20). INH and RIF are the most important antituberculosis drugs, and resistance to these drugs often results in treatment failure and fatal clinical outcome (5, 13). Collective observations have shown that a variety of mutations within the catalase peroxidase (*katG*) gene (in strains showing 60 to 90% resistance), the enoyl-acyl carrier protein reductase (*inhA*) gene (15 to 43%), or the RNA polymerase β-subunit (*rpoB*) gene (>96%) are found in INH- and RIF-resistant strains (17, 22, 24). The multiplex PCR-based solid-phase reverse hybridization Genotype MTBDR line probe assay (Hain Lifescience GmbH, Nehren, Germany) has been shown to be a rapid and accurate method to detect the most common mutations of *katG* and *rpoB* from MTBC growth-positive cultures, either liquid or solid, and it has the potential to shorten the overall turnaround time from specimen receipt to reporting of results of susceptibility testing (7, 11).

The aims of the present retrospective study were to determine the sensitivity and accuracy of the Genotype MTBDR

* Corresponding author. Mailing address: Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509. Phone: (518) 474-2196. Fax: (518) 474-6964. E-mail: salfinger@wadsworth.org.

[∇] Published ahead of print on 11 October 2006.

assay when it is used directly on smear-positive clinical specimens and to compare the results with those obtained by the reference BACTEC 460TB system and direct DNA sequencing analysis of *katG*, *inhA*, and *rpoB*. The additional analysis of *inhA* by DNA sequencing allowed the determination of whether the inclusion of this gene in a modified Genotype MTBDR assay would further improve the performance of the test.

MATERIALS AND METHODS

Clinical specimens. One hundred forty-three acid-fast bacillus smear-positive sputum specimens were analyzed. These bacterials included clinical specimens that had been received for routine mycobacterial testing between January 2000 and October 2005 and that had been shown to be MTBC positive by Amplified Mycobacterium Tuberculosis Direct tests (Gen-Probe Incorporated, San Diego, CA). Only one specimen per patient was analyzed. The specimens were initially digested and decontaminated by using a modified Petroff's NaOH method (23). After decontamination, the concentrated sediment was suspended in 3.0 ml sterile phosphate-buffered saline (pH 6.8), and smears were prepared with 0.1 ml of sediment using the Ziehl-Neelsen acid-fast staining method (10). After inoculation for growth detection, the leftover sediment was stored at -80°C . This leftover sediment was later thawed and used for Genotype MTBDR testing.

Growth detection and conventional susceptibility testing. Routine media including a BACTEC 12B vial, a Lowenstein-Jensen slant, and a Middlebrook 7H10/7H11 selective biplate were also inoculated, incubated at 37°C , and monitored for 8 weeks before being reported as negative. When growth was detected, the mycobacteria were identified by the DNA AccuProbe (Gen-Probe Incorporated, San Diego, CA) and conventional methods, with susceptibility testing performed using the BACTEC 460TB system as reported previously (8, 10, 21). For INH and RIF susceptibility, all strains were tested at two concentrations: 0.1 and 0.4 $\mu\text{g/ml}$ for INH and 0.5 and 2.0 $\mu\text{g/ml}$ for RIF. If MTBC strains were identified, final identification to the species level was made using PCR-based deletion analysis (15). When the strain was found to be drug resistant, the BACTEC susceptibility results were confirmed by the proportion method using Middlebrook 7H10 agar as described elsewhere previously (10).

Genotype MTBDR assay. The Genotype MTBDR line probe assay (Hain Lifescience GmbH, Nehren, Germany) was carried out according to the manufacturer's instructions and as described previously with the use of a modified amplification protocol (7). Briefly, 1 ml of the decontaminated and concentrated specimens was centrifuged at $10,000 \times g$ for 15 min, the supernatant was discarded, and the pellet was resuspended in 300 μl sterile distilled water. The specimen was then heat killed at 95°C for 20 min in a heat block. This was followed by a 15-min sonication step. After sonication, 5 μl of the supernatant was used immediately for amplification, while the remainder was stored at -20°C . Amplification was done according to the following modified amplification protocol: denaturation at 95°C for 15 min; 10 cycles of denaturation at 95°C for 30 s and elongation at 58°C for 120 s; an additional 30 cycles of denaturation at 95°C for 25 s, annealing at 53°C for 40 s, and elongation at 70°C for 40 s; and a final extension step at 70°C for 8 min.

The biotin-labeled PCR product was denatured and hybridized to a strip with specific oligonucleotide probes. One probe is complementary with an MTBC-specific region of the 23S rRNA gene (Tub), and one probe is specific for the *rpoB* gene (*rpoB*-Uni) and should be always positive for all MTBC strains, while five wild-type (WT) probes (WT1 to WT5) encompass the region of the *rpoB* gene encoding amino acids 509 to 534. Four other probes are specific for the most common mutations: D516V, H526Y, H526D, and S531L (probes *rpoB* MUT D516V, *rpoB* MUT H526Y, *rpoB* MUT H526D, and *rpoB* MUT S531L, respectively). One probe detects a *katG*-specific gene region and should always be positive for all MTBC strains. Three other probes are specific for the S315 region of *katG*. One is the wild-type probe (*katG* WT), while two others (*katG* MUT1 and MUT2) are designed to detect the AGC-to-ACC (S315T) and the AGC-to-ACA (S315T) mutations. Hybridized PCR products were detected, and the Genotype MTBDR results were evaluated as described elsewhere previously (7).

DNA sequencing of *katG*, *inhA*, and *rpoB*. Primers Tb86 (5'-GAAACAGCG GCGCTGATCGT-3') and Tb87 (5'-GTTGTCCATTTTCGTCGGGG-3') flanking the region encoding amino acid Ser315 of *katG* were used to amplify a 209-bp product. Primers inhA-1 (5'-CCTCGCTGCCAGAAAGGA-3') and inhA-2 (5'-ATCCCCGGTTTCCTCCGGT-3') were used to amplify a 250-bp fragment of *inhA* and its upstream promoter region. Primers rpo95 (5'-CCAC

CCAGGACGTGGAGGCGATCACACCG-3') and rpo397 (5'-GTCAACCCG TTCGGGTTTCATCGAAACG-3') were used to amplify a 329-bp product, which included the relevant segment of *rpoB*. The same primers were used for DNA sequencing of both strands of both genes using the automated Applied Biosystems 3700 DNA sequencer (Applied Biosystems, Foster City, CA.) as described previously (16, 23). The DNA sequencing was carried out by the Molecular Genetics Core Facility at the Wadsworth Center.

RESULTS

BACTEC 460TB susceptibility results. Following growth detection of the 143 smear-positive specimens, a total of 92 specimens showed resistance to INH and/or RIF. Of these 92 specimens, 26 were MDR, including 24 specimens resistant to a high level (0.4 $\mu\text{g/ml}$) and 2 specimens resistant to a low level (0.1 $\mu\text{g/ml}$) of INH; 33 were resistant to a high level of INH; and 33 were resistant to only a low level of INH. Fifty-one specimens were pansusceptible according to the BACTEC 460TB system.

Sensitivity of the Genotype MTBDR assay for MTBC *katG* and *rpoB*. Of the 143 MTBC-containing specimens, 135 (94.4%) were correctly identified by the MTBC-specific TUB capture probe. With respect to the assay for the resistance-associated genes, amplification of *katG* provided conclusive results, i.e., successful amplification for 135 (94.4%) of the 143 specimens, and amplification of *rpoB* provided conclusive results for 130 (90.9%) of the 143 specimens. Although amplification of *katG* was successful in a total of 142 strains, *katG* results could not be validated for seven specimens due to the lack of a positive hybridization signal with the MTBC-specific probe.

Performance of the Genotype MTBDR assay on specimens with strains resistant to high (0.4 $\mu\text{g/ml}$) and low (0.1 $\mu\text{g/ml}$) levels of INH and with INH-susceptible strains. A total of 57 (40.1%) specimens contained strains that were resistant to high (0.4 $\mu\text{g/ml}$) levels of INH by the BACTEC 460TB assay. Twenty-four (42.1%) of the 57 strains were MDR. Results of the Genotype MTBDR assay for MDR strains are summarized in Table 1. In 48 (84.2%) of the 57 specimens, the Genotype MTBDR assay results were in agreement with results from BACTEC 460TB susceptibility testing and the DNA sequencing results (Table 1). For one (1.8%) of these 48 specimens, the Genotype MTBDR assay indicated the presence of strains that were both resistant and susceptible to INH (weak *katG* WT with strong *katG* MUT1 mutation-specific bands) (Table 1). DNA sequencing confirmed an S315T mutation in this strain. In one (1.8%) specimen, DNA sequencing revealed a rare S315I mutation of *katG* that was missed by the Genotype MTBDR test. Although an oligonucleotide probe specific for this mutation is not present in the Genotype MTBDR test, the positive hybridization signal with the *katG* WT probe falsely indicated susceptibility to INH. For 7 (12.3%) of the 57 specimens, the Genotype test indicated INH susceptibility (no detectable mutation at codon S315), while amplification was unsuccessful for three genes (MTBC-specific 23S rRNA, *katG*, and *rpoB*) in one (1.8%) specimen by the assay (Table 1). However, DNA sequencing was also unable to find any S315 *katG* mutations in these eight (14%) strains. DNA sequencing revealed mutations in *inhA* in only 2 (3.5%) of the 57 strains (both MDR; a T-to-G and a T-to-A point mutation at position -8 upstream of the translation start site of *inhA*) (Table 1).

TABLE 1. Performance of the Genotype MTBDR assay with isoniazid-resistant and -susceptible strains

Resistance or susceptibility by BACTEC 460TB	No. (%) of specimens						
	Genotype MTBDR			<i>katG315</i> sequencing		<i>inhA</i> sequencing	
	Mutation +	Mutation -	Unsuccessful amplification	Mutation +	Mutation -	Mutation +	Mutation -
High-level INH resistant (0.4 µg/ml) (<i>n</i> = 57) ^a	48 (84.2) ^c	8 (14) ^d	1 (1.8) ^e	49 (86)	8 (14)	2 (3.5)	55 (96.5)
Low-level INH resistant (0.1 µg/ml) (<i>n</i> = 35) ^b	2 (5.7)	30 (85.7)	3 (8.6) ^f	2 (5.7)	33 (94.3)	18 (51.4)	17 (48.6)
INH susceptible (<i>n</i> = 51)	0	47 (92.2)	4 (7.8) ^f	0	51 (100)	0	51 (100)

^a A total of 42% of these strains were multidrug resistant.

^b A total of 5.7% of these strains were multidrug resistant.

^c One strain showed a wild-type and mutation probe-positive hybridization pattern together.

^d In one of these strains, DNA sequencing identified a rare S315I mutation.

^e Amplification was unsuccessful for three genes (*M. tuberculosis* complex-specific 23S rRNA, *katG*, and *rpoB*).

^f Amplification was unsuccessful for two genes (*M. tuberculosis* complex-specific 23S rRNA and *rpoB*).

A total of 35 (24.6%) specimens contained strains that were resistant to low levels of INH (0.1 µg/ml), two (5.7%) strains of which were MDR (Table 1). Amplification was unsuccessful for two genes (MTBC-specific 23S rRNA and *rpoB*) in three (8.6%) specimens (Table 1). Although amplification of *katG* was successful in all three specimens, because of an unsuccessful amplification for the MTBC, these results could not be validated. DNA sequencing did not identify *katG* mutations in these strains (Table 1). In 2 (5.7%) of the 35 strains, the Genotype MTBDR assay indicated the presence of a *katG* mutation (*katG* positive but negative for WT, MUT1, and MUT2 probes) (Table 1). DNA sequencing revealed two rare (S315G and S315N) *katG* mutations in these strains. Neither the Genotype MTBDR test nor DNA sequencing was able to detect any *katG* mutations in 30 (85.7%) of the 35 specimens (Table 1). Interestingly, analysis of DNA sequences of *inhA* showed mutations in 18 (51.4%) of the 35 strains that were resistant to low levels of INH (a C-to-G point mutation at position -15 in 16 strains, a T-to-C point mutation at position -8 in 1 MDR strain, and a double mutation of a C-to-G point mutation at position -15 plus a T-to-C mutation at position -8 in 1 strain).

In 4 (7.8%) of the 51 INH-susceptible, as well as pansusceptible, specimens, amplification was unsuccessful for the MTBC and *rpoB*. Although the assay successfully amplified *katG* and correctly indicated susceptibility to INH in these specimens, these results could not be validated because of the inability of

the assay to detect the presence of the MTBC. The Genotype MTBDR assay predicted susceptibility to INH in all the remaining 47 (92.2%) control specimens.

Performance of the Genotype MTBDR assay for specimens with RIF-resistant and -susceptible strains. A total of 26 (18.3%) specimens contained RIF-resistant strains according to the BACTEC 460TB assay. All strains were MDR. Results with the Genotype MTBDR assay for MDR strains are summarized in Table 2. RIF resistance was correctly predicted by the Genotype MTBDR test in 25 (96.2%) specimens, while amplification was unsuccessful by the assay for the *rpoB* gene in one (3.8%) specimen (Table 2). For 3 (11.5%) of these 26 specimens, the Genotype MTBDR assay indicated the presence of both RIF-resistant and -susceptible strains (two strains with MUT H526Y plus *rpoB* WT probes, and one strain with MUT S531L and *rpoB* WT probes). Of the 26 RIF-resistant strains, 13 (44.8%) carried mutation H526Y, 12 (41.4%) carried mutation S531L, and 1 (3.4%) carried mutation Del526.

The Genotype MTBDR test correctly indicated RIF susceptibility in 102 (87.2%) of the RIF-susceptible strains (43.6% of strains were pansusceptible, 28.2% of strains were resistant to high levels of INH, and 28.2% of strains were resistant to low levels of INH) (Table 2). However, in 1 of these 102 specimens, the test failed to detect the presence of a silent (no amino acid change) R528R *rpoB* mutation (all *rpoB* WT probes were positive). In addition, amplification was unsuccessful for three genes (*M. tuberculosis* complex-specific 23S rRNA, *katG*, and

TABLE 2. Performance of the Genotype MTBDR assay with rifampin-resistant and -susceptible strains

Resistance or susceptibility by BACTEC 460TB	No. (%) of specimens				
	Genotype MTBDR			<i>rpoB</i> sequencing	
	Mutation +	Mutation -	Unsuccessful amplification	Mutation +	Mutation -
Rifampin resistant (<i>n</i> = 26) ^a	25 (96.2) ^c	0	1 (3.8) ^e	26 (100)	0
Rifampin susceptible (<i>n</i> = 117) ^b	3 (2.6) ^d	102 (87.2)	12 (10.2) ^f	3 (2.6)	114 (97.4)

^a All strains were multidrug resistant.

^b Fifty-one (43.6%) strains were pansusceptible, 33 (28.2%) strains were resistant to a high level (0.4 µg/ml) of isoniazid, and 33 (28.2%) strains were resistant to a low level (0.1 µg/ml) of isoniazid.

^c Three strains showed a wild-type and mutation probe-positive hybridization pattern together.

^d These strains showed susceptibility to RIF at concentrations of 2.0 µg/ml (reportable concentration) and resistance at concentrations of 0.5 µg/ml by the BACTEC 460TB system.

^e Amplification was unsuccessful for one gene (*rpoB*).

^f Amplification was unsuccessful for three genes (*M. tuberculosis* complex-specific 23S rRNA, *katG*, and *rpoB*) in one strain, for two genes (*M. tuberculosis* complex-specific 23S rRNA and *rpoB*) in seven strains, and for one gene (*rpoB*) in four strains.

rpoB) in one (0.9%) strain, for two genes (*M. tuberculosis* complex-specific 23S rRNA and *rpoB*) in seven (5.9%) strains, and for one gene (*rpoB*) in four (3.4%) strains (Table 2). Finally, three (2.6%) specimens showed susceptibility to RIF at 2.0 µg/ml (reportable concentration) and resistance at 0.5 µg/ml by the BACTEC 460TB system (Table 2). For all three strains, the Genotype MTBDR silent (no amino acid change) test indicated the presence of an *rpoB* mutation (*rpoB*-Uni probe positive but no signal with WT1 or WT2 bands); however, it could not determine the type of mutation. DNA sequencing revealed a rare mutation of L511P in two strains and a rare mutation of D516Y in one strain.

Age of frozen specimens. Eighteen (19.6%) of the 92 resistant strains were received in 2000, 17 (18.5%) were received in 2001, 14 (15.2%) were received in 2002, 15 (16.3%) were received in 2003, 23 (25%) were received in 2004, and 5 (5.4%) were received in 2005. Of the 51 susceptible strains, 3 (5.9%) were received in 2000, 31 (60.8%) were received in 2004, and 17 (33.3%) were received in 2005. Unsuccessful amplification with the Genotype MTBDR test did not show any association with the length of time of freezing.

DISCUSSION

If we are to meet the goal of controlling the spread of drug-resistant tuberculosis, the time frame of many weeks required for detection, identification, and drug susceptibility testing of MTBC strains by growth detection must be shortened. This is best achieved by the introduction of modern molecular methods that can be applied directly to clinical specimens. The findings of the present study show that the recently developed Genotype MTBDR multiplex PCR assay is a suitable molecular method for this purpose. The assay offers a simple protocol that is compatible with routine work flow and can be completed within 24 h.

The results of the present study also indicate that the Genotype MTBDR assay has an overall good performance and sensitivity (successful amplification for all strains) for the rapid detection of the MTBC (94.4%) and for mutations in the *katG* (94.4) and *rpoB* (90.9%) genes when used directly on smear-positive specimens. The sensitivity of the test for MTBC strains has previously been shown to be comparable to those of the Amplicor (Roche Molecular Systems, Branchburg, NJ) PCR assay, the Amplified Mycobacterium Tuberculosis Direct test transcription-mediated amplification method, and the BDProbeTec (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) strand displacement amplification technology (19). The Genotype MTBDR assay correctly predicted INH resistance in 84.2% of the strains resistant to high levels of INH and correctly predicted RIF resistance in 96.2% of the RIF-resistant strains. For seven of the strains encoding resistance to high levels of INH, and for a majority of the strains encoding resistance to low levels of INH, neither the Genotype MTBDR assay nor DNA sequencing detected a mutation in *katG*. This finding points to a weakness of the test, namely, that the assay relies on the occurrence of certain INH resistance-related mutations. Thus, there is a need to continue testing via growth-based assays until all INH resistance-related mutations can be identified. However, the rapid diagnostic predictions made by the assay justify its use for establishing preliminary

patient treatment regimens until results are confirmed by the empirical growth-based assays. Our findings also indicate that one solution to this problem can be the inclusion of *inhA* into the Genotype MTBDR test by the manufacturer. In our sample set, molecular analysis of *inhA* served to increase the capability of predicting INH resistance by 51.5% among strains with low levels of INH resistance and by 11.5% in the MDR strains. However, the clinical significance of low-level INH resistance and the effectiveness of INH in patients with low-level INH resistance is unclear (2).

In general, interpretation of the test strips was easy, but it is noteworthy that the intensities of the different hybridization bands varied. Another important finding of the study was that the performance of the test was not influenced by the length of freezing time (up to 6 years), thus allowing rapid validation within a laboratory using stored specimens with known results. In line with our experiences, the Genotype MTBDR test was also found to be reliable for the prediction of INH and RIF resistance directly in smear-positive specimens in two very recent studies (1, 12). However, those studies were based on significantly fewer specimens.

In conclusion, the Genotype MTBDR assay is a rapid and reliable tool for the routine direct detection of MTBC strains and of strains resistant to INH and RIF in smear-positive, highly infectious patients. The rapid turnaround time of the test should enable the optimization of the therapy of these patients before confirmatory culture results are available. The test does not require viable organisms and thus reduces the biohazard risk in the laboratory.

ACKNOWLEDGMENTS

We thank Keith Derbyshire for critical review of the manuscript, Hain Lifescience for providing some of the Genotype MTBDR kits, and the Molecular Genetics Core Facility of the Wadsworth Center for performing the sequencing analyses.

REFERENCES

- Bang, D., A. B. Andersen, and V. O. Thomsen. 2006. Rapid genotypic detection of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* directly in clinical specimens. *J. Clin. Microbiol.* **44**:2605–2608.
- Blumberg, H. M., W. J. Burman, R. E. Chaisson, C. L. Daley, S. C. Etkind, L. N. Friedman, P. Fujiwara, M. Grzemska, P. C. Hopewell, M. D. Iseman, R. M. Jasmer, V. R. Koppaka, R. I. Menzies, R. J. O'Brian, R. R. Reves, L. B. Reichman, P. M. Simone, J. R. Starke, and A. A. Vernon. 2003. Treatment of tuberculosis. *Am. J. Respir. Crit. Care Med.* **167**:603–662.
- Centers for Disease Control and Prevention. 2006. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs worldwide, 2000–2004. *Morb. Mortal. Wkly. Rep.* **55**:301–305.
- De Beenhouwer, H., Z. Lhiang, G. Jannes, W. Mijls, L. Machtelinckx, R. Rossau, H. Traore, and F. Portaels. 1995. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. *Tuber. Lung Dis.* **76**:425–430.
- Goble, M., M. D. Iseman, L. A. Madsen, D. Waite, L. Ackerson, and C. R. Horsburgh, Jr. 1993. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *N. Engl. J. Med.* **328**:527–532.
- Hale, Y. M., E. P. Desmond, K. C. Jost, Jr., and M. Salfinger. 2000. Access to newer laboratory procedures: a call for action. *Int. J. Tuberc. Lung Dis.* **4**:S171–S175.
- Hillemann, D., M. Weizenegger, T. Kubica, E. Richter, and S. Niemann. 2005. Use of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. *J. Clin. Microbiol.* **43**:3699–3703.
- Inderlied, C. B., and M. Salfinger. 1999. Antimycobacterial agents and susceptibility tests, p. 1601–1623. In P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. ASM Press, Washington, D.C.
- Johansen, I. S., B. Lundgren, A. Sosnovskaja, and V. Ø. Thomsen. 2003. Direct detection of multidrug-resistant *Mycobacterium tuberculosis* in clinical specimens in low- and high-incidence countries by line probe assay. *J. Clin. Microbiol.* **41**:4454–4456.

10. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology. A guide for a level III laboratory. Center for Disease Control, Atlanta, Ga.
11. **Makinen, J., H. J. Marttila, M. Marjamaki, M. K. Viljanen, and H. Soini.** 2006. Comparison of two commercially available DNA line probe assays for detection of multidrug-resistant *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **44**:350–352.
12. **Miotto, P., F. Piana, V. Penati, F. Canducci, G. B. Migliori, and D. M. Cirillo.** 2006. Use of Genotype MTBDR assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. *J. Clin. Microbiol.* **44**:2485–2491.
13. **Mitchison, D. A., and A. J. Nunn.** 1986. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **133**:423–430.
14. **Moore, D. F., J. A. Guzman, and L. T. Mikhail.** 2005. Reduction in turn-around time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. *Diagn. Microbiol. Infect. Dis.* **52**: 247–254.
15. **Parsons, L. M., R. Brosch, S. T. Cole, A. Somoskovi, A. Loder, G. Bretzel, D. Van Soolingen, Y. M. Hale, and M. Salfinger.** 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *J. Clin. Microbiol.* **40**:2339–2345.
16. **Parsons, L. M., M. Salfinger, A. Clobridge, J. Dormandy, L. Mirabello, V. L. Polletta, A. Sanic, O. Sinyavskiy, S. C. Larsen, J. Driscoll, G. Zickas, and H. W. Taber.** 2005. Phenotypic and molecular characterization of *Mycobacterium tuberculosis* isolates resistant to both isoniazid and ethambutol. *Antimicrob. Agents Chemother.* **49**:2218–2225.
17. **Parsons, L. M., A. Somoskovi, R. Urbanczik, and M. Salfinger.** 2004. Laboratory diagnostic aspects of drug resistant tuberculosis. *Front. Biosci.* **9**:2086–2105.
18. **Piersimoni, C., A. Olivieri, L. Benacchio, and C. Scarparo.** 2006. Current perspectives on drug susceptibility testing of *Mycobacterium tuberculosis* complex: the automated nonradiometric systems. *J. Clin. Microbiol.* **44**: 20–28.
19. **Piersimoni, C., and C. Scarparo.** 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. *J. Clin. Microbiol.* **41**:5355–5365.
20. **Ruiz, M., M. J. Torres, A. C. Llanos, A. Arroyo, J. C. Palomares, and J. Aznar.** 2004. Direct detection of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* in auramine-rhodamine-positive sputum specimens by real-time PCR. *J. Clin. Microbiol.* **42**:1585–1589.
21. **Somoskovi, A., J. E. Hotaling, M. Fitzgerald, V. Jonas, D. Stasik, L. M. Parsons, and M. Salfinger.** 2000. False-positive results for *Mycobacterium celatum* with the AccuProbe *Mycobacterium tuberculosis* complex assay. *J. Clin. Microbiol.* **38**:2743–2745.
22. **Somoskovi, A., L. M. Parsons, and M. Salfinger.** 2001. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*. *Respir. Res.* **2**:164–168.
23. **Somoskovi, A., Q. Song, J. Mester, C. Tanner, Y. M. Hale, L. M. Parsons, and M. Salfinger.** 2003. Use of molecular methods to identify the *Mycobacterium tuberculosis* complex (MTBC) and other mycobacterial species and to detect rifampin resistance in MTBC isolates following growth detection with the BACTEC MGIT 960 system. *J. Clin. Microbiol.* **41**:2822–2826.
24. **Zhang, Y., and A. Telenti.** 2000. Genetics of drug resistance in *Mycobacterium tuberculosis*, p. 235–254. *In* G. F. Hatful and W. R. Jacobs, Jr. (ed.), *Molecular genetics of mycobacteria*. ASM Press, Washington, D.C.