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Evaluation of the AID TB Resistance Line Probe Assay for Rapid Detection of Genetic Alterations Associated with Drug Resistance in *Mycobacterium tuberculosis* Strains

C. Ritter,[a,b] K. Lucke,[a,b]* F. A. Sirgel,[c] R. W. Warren,[c] P. D. van Helden,[c] E. C. Böttger,[a,b] G. V. Bloemberg[a]

Institut für Medizinische Mikrobiologie, Universität Zürich, Zurich, Switzerland[a]; Nationales Zentrum für Mykobakterien, Zurich, Switzerland[b]; DST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Health Science, Stellenbosch University, Cape Town, South Africa[c]

The rapid accurate detection of drug resistance mutations in *Mycobacterium tuberculosis* is essential for optimizing the treatment of tuberculosis and limiting the emergence and spread of drug-resistant strains. The TB Resistance line probe assay from Autoimmun Diagnostika GmbH (AID) (Strassburg, Germany) was designed to detect the most prevalent mutations that confer resistance to isoniazid, rifampin, streptomycin, amikacin, capreomycin, fluoroquinolones, and ethambutol. This assay detected resistance mutations in clinical *M. tuberculosis* isolates from areas with low and high levels of endemicity (Switzerland, $n = 104$; South Africa, $n = 52$) and in selected *Mycobacterium bovis* BCG 1721 mutant strains ($n = 5$) with 100% accuracy. Subsequently, the line probe assay was shown to be capable of rapid genetic assessment of drug resistance in MGIT broth cultures, the results of which were in 100% agreement with those of DNA sequencing and phenotypic drug susceptibility testing. Finally, the line probe assay was assessed for direct screening of smear-positive clinical specimens. Screening of 98 clinical specimens demonstrated that the test gave interpretable results for >95% of them. Antibiotic resistance mutations detected in the clinical samples were confirmed by DNA sequencing. We conclude that the AID TB Resistance line probe assay is an accurate tool for the rapid detection of resistance mutations in cultured isolates and in smear-positive clinical specimens.

The diagnosis and treatment of multidrug-resistant (MDR) tuberculosis (TB), resistant to at least isoniazid and rifampin, represent a major challenge for tuberculosis control programs worldwide. In 2010, there were an estimated 650,000 cases of MDR-TB among the world's 12.0 million prevalent cases of TB (1). Extensively drug-resistant (XDR) *Mycobacterium tuberculosis* strains are defined as showing resistance to at least isoniazid and rifampin, to any fluoroquinolone, and to any of the 3 second-line injectable drugs (amikacin, capreomycin, and kanamycin) (2). Early rapid detection of resistance to both first- and second-line drugs is essential to ensure appropriate chemotherapy and to prevent the spread and amplification of resistance.

Antibiotic resistance in *M. tuberculosis* is mediated mostly through genetic changes in the chromosome, such as point mutations and deletions. The involvement of different mutational alterations and genes associated with drug resistance (3), resulting in different levels of resistance, complicates culture-based drug susceptibility testing (DST). Molecular methods offer fast accurate detection of resistance mutations by line probe assays, DNA sequencing, or multiplex (real-time) PCR assays. One of the few commercially available tests is the Genotype MTBDR*plus* assay (Hain Lifescience GmbH, Germany), which was endorsed by the World Health Organization (WHO) in 2008 and was recently extended for the detection of second-line drug resistance mutations (Genotype MTBDRsl) (4–8). The more recently endorsed Xpert MTB/RIF assay (Cepheid, USA) facilitates rapid detection of the *M. tuberculosis* complex (9) but is limited to detection of rifampin resistance mutations in *rpoB* (10, 11). Similarly, the INNO-LiPA RIF.TB line probe assay (Fujirebio Inc., Ghent, Belgium) is limited to the detection of *rpoB* mutations associated with rifampin resistance but also is able to detect the emergence of rifampin-resistant populations due to the presence of wild-type (wt) and mutant probes (12). Despite seminal improvements in PCR and DNA sequencing technologies (13, 14), line probe assays will remain the method of choice for simultaneous detection of multiple drug resistance mutations in *M. tuberculosis*, at least in the short term. Line probe assays are fast, efficient (testing for multiple chromosomal targets), accurate, and easy to handle in the laboratory. Results are usually obtained within 1 day. Performance of line probe assays does not require expensive laboratory equipment, although certain equipment to perform DNA extraction, PCR amplification, and DNA hybridization and the expertise to prevent amplicon cross-contamination are required.

Given the importance of line probe assays in clinical diagnostic testing, Autoimmun Diagnostika GmbH (AID) (Strassburg, Germany) has developed a line probe assay for the detection of chromosomal mutations associated with resistance to first-line (isoniazid and rifampin) and second-line (streptomycin, amikacin, capreomycin, fluoroquinolones, and ethambutol; ethambutol is considered a first-line drug in the United States) TB drugs. The

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Address correspondence to G. V. Bloemberg, bloemberg@imm.uzh.ch.
* Present address: K. Lucke, Unilabs Zurich, Zurich, Switzerland.
C.R. and K.L. contributed equally to this article.
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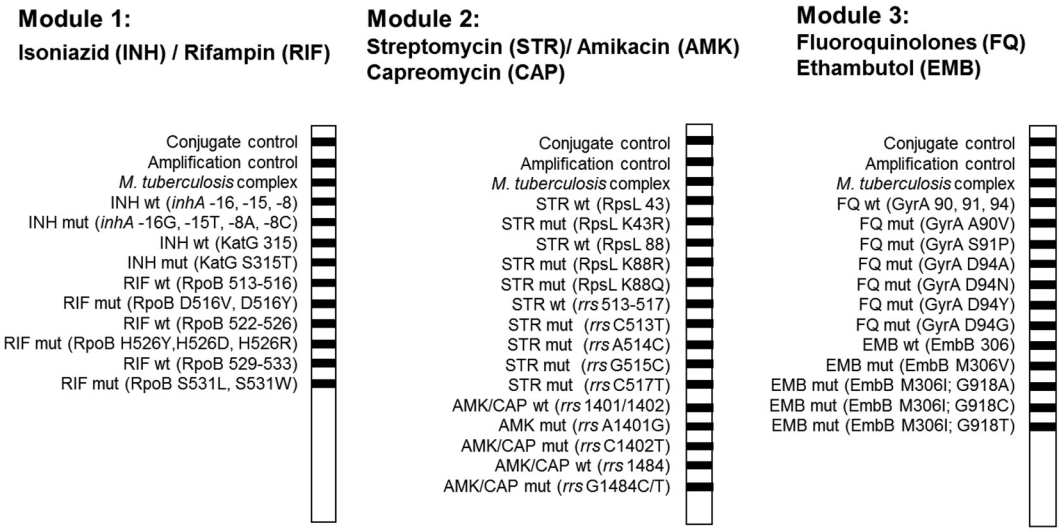

Note:
1. RpoB numbering according *E. coli*; all other protein / gene numbering according *M. tuberculosis* strain H37Rv.
2. *rrs M. tuberculosis* H37Rv positions 513, 514, 515, 517, 1401, 1402 and 1484 correspond to *rrs E. coli* positions 522, 523, 524, 526, 1408, 1409 and 1491, respectively.

FIG 1 Schematic representation of the three-module *Mycobacterium tuberculosis* antibiotic resistance line probe assay (AID, Strassburg, Germany) for the detection of resistance mutations in *Mycobacterium tuberculosis*.

aim of this study was to evaluate the specificity and sensitivity of the AID TB Resistance line probe assay for the detection of resistance mutations in cultured isolates and in smear-positive patient specimens.

MATERIALS AND METHODS

Clinical specimens, decontamination, microscopy, and culture. Clinical specimens were decontaminated using the *N*-acetyl-L-cysteine-sodium hydroxide method (15). Auramine-rhodamine fluorochrome staining was used for acid-fast bacteria (AFB) microscopic examination; AFB-positive results were confirmed for specificity using Ziehl-Neelsen staining (16). Mycobacteria were recovered on standard culture media (7H11 plates and BBL MGIT [Becton, Dickinson and Company]) by incubation at 37°C for a maximum of 7 weeks.

Clinical isolates. Clinical strains were obtained from the Institut für Medizinische Mikrobiologie (IMM), Universität Zürich (Zürich, Switzer-

TABLE 1 Evaluation of module 1 (isoniazid and rifampin) of the AID TB Resistance line probe assay using clinical *M. tuberculosis* strains ($n = 70$, Switzerland)

Isoniazid[a]													
inhA			KatG			Rifampin, RpoB							
	AID module 1			AID module 1			AID module 1						
No. of isolates	wt (−16, −15, −8)	Mutant (−16, −15, −8)	DNA sequencing	wt 315	mut315	DNA sequencing	wt 513–516	mut516	wt 522–526	mut526	wt 529–533	mut531	DNA sequencing
1	−	+	C-15T	−	+	S315T	+	−	−	+	+	−	H526D
1	−	+	C-15T	−	+	S315T	+	−	+	−	−	+	S531L
1	−	+	C-15T	+	−	wt	−	+	+	−	−	+	D516Y/S531L
3	−	+	C-15T	+	−	wt	+	−	+	−	−	+	S531L
5	−	+	C-15T	+	−	wt	+	−	+	−	+	−	wt
1	−	+	T-8C	−	+	S315T	+	−	−	+	+	−	H526Y
10	+	−	wt	−	+	S315T	+	−	+	−	+	−	wt
1	+	−	wt	−	+	S315T	−	+	+	−	+	−	D516Y
1	+	−	wt	−	+	S315T	−	+	−	+	+	−	D516Y/H526D
6	+	−	wt	−	+	S315T	+	−	−	+	+	−	H526D
18	+	−	wt	−	+	S315T	+	−	+	−	−	+	S531L
1	+	−	wt	+	−	wt	−	+	+	−	+	−	D516V
2	+	−	wt	+	−	wt	+	−	−	+	+	−	H526Y
6	+	−	wt	+	−	wt	+	−	+	−	−	+	S531L
13	+	−	wt	+	−	wt	+	−	+	−	+	−	wt

[a] −, no signal (band); +, signal (band); wt, wild type.

land), and the DST/NRF Centre of Excellence for Biomedical TB Research/MRC Centre for Molecular and Cellular Biology, Stellenbosch University (Cape Town, South Africa).

DNA extraction. DNA was extracted from decontaminated samples (0.5 ml) using a respiratory specimen preparation kit (Roche Diagnostics, Rotkreuz, Switzerland), according to the manufacturer's instructions (17). Chromosomal DNA of cultured isolates was obtained using InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. Mycobacteria were identified by 16S rRNA gene sequence analysis, as previously described (18) and analyzed using SmartGene IDNS software (SmartGene GmbH, Zug, Switzerland).

Genotypic resistance testing. *Mycobacterium tuberculosis* clinical isolates were analyzed for antibiotic resistance mutations by DNA sequencing. For sequencing, the corresponding chromosomal loci were amplified by PCR (see Table S1 in the supplemental material). Automated DNA sequence determinations were performed using the amplification primers and the BigDye Terminator v1.1 cycle sequencing kit, on an ABI Genetic Analyzer 3100 system (Applied Biosystems, Rotkreuz, Switzerland). Sequences were analyzed using DNASTAR Lasergene version 8 software (DNASTAR, Inc., Madison, WI USA).

For isoniazid resistance, mutations in *katG* at amino acid position 315 (19) and in the *fabG1-inhA-hemZ* operon promoter at positions −16, −15, and −8 (20) were determined. For rifampin resistance, mutations in *rpoB* at amino acid positions 516, 526, and 531 (corresponding to *Escherichia coli* RpoB positions) were analyzed (21). For streptomycin resistance, mutations in *rpsL* (K43R, K88R, and K88Q) (22, 23) and *rrs* (C513T, A514C, G515C, and C517T, corresponding to *E. coli rrs* positions 522, 523, 524, and 526, respectively) (18) were examined. Amikacin or capreomycin resistance was determined by screening the *rrs* gene for the mutations A1401G, C1402T, and G1484C/T (corresponding to *E. coli rrs* positions 1408, 1409, and 1491, respectively) (22, 24, 25). For fluoroquinolone resistance, GyrA amino acid mutations A90V, S91P, D94A, D94N, D94Y, and D94G were determined. The presence of the EmbB mutations M306V and M306I were regarded as indicative of ethambutol resistance (26).

AID TB Resistance line probe testing using module 1 (isoniazid and rifampin), module 2 (streptomycin, amikacin, and capreomycin), and module 3 (fluoroquinolones and ethambutol) (Fig. 1) was performed following the instructions of the manufacturer (Autoimmun Diagnostika GmbH). For testing of clinical specimens with the AID TB Resistance line probe assay, DNA was extracted with a Roche respiratory specimen preparation kit. *Mycobacterium tuberculosis* strain H37Rv was used as a positive control for gene amplification.

***In vitro* generation and selection of *Mycobacterium bovis* BCG *rrs* mutant strains resistant to aminoglycosides.** Selection of *Mycobacterium bovis* strain BCG 1721 (RpsL K43R) (27) for *rrs* mutations conferring resistance to kanamycin, amikacin, and capreomycin was done by plating *M. bovis* BCG 1721 (1×10^8 or 1×10^7 CFU) on 7H10 agar plates supplemented with amikacin (4 or 20 mg/liter), capreomycin (10 or 50 mg/liter), or a combination of capreomycin (10 mg/liter) and kanamycin (5 mg/liter). Plates were incubated at 37°C for 3 to 6 weeks. Single colonies were analyzed for the presence of mutations by *rrs* sequence analysis, as described above.

Drug susceptibility testing. Conventional culture-based drug susceptibility testing (DST) (isoniazid, rifampin, streptomycin, amikacin, capreomycin, moxifloxacin, and ethambutol) was done using the automated MGIT 960 platform, in conjunction with EpiCenter software equipped with the TB eXiST module (Becton, Dickinson and Company, Sparks, MD, USA), and methods described previously (28). In general, DST was performed for one culture per clinical specimen.

RESULTS

Analytical assessment of the various detectable mutations of the AID TB resistance kit. The TB Resistance line probe assay was evaluated against the susceptible model strain *M. tuberculosis*

TABLE 2 Evaluation of module 2 (aminoglycosides) of the AID TB Resistance assay using clinical *M. tuberculosis* strains (Switzerland, $n = 4$; South Africa, $n = 18$) and *in vitro*-selected *M. bovis* BCG mutant strains ($n = 5$)

No. of isolates from:		Streptomycin[a]												Amikacin-capreomycin, *rrs*[b]						
		RpsL						*rrs*												
		AID module 2						AID module 2						AID module 2						
Switzerland	South Africa	wt 43	K43R	wt 88	K88R	K88Q	DNA sequencing	wt 513–517	C513T	A514C	G515C	C517T	DNA sequencing	wt 1401/1402	A1401G	C1402T	wt 1484	G1484C/T	DNA sequencing
2	3	−	+	+	−	−	K43R	+	−	−	−	−	ND	−	+	−	+	−	A1401G
	2	−	+	+	−	−	K43R	+	−	−	−	−	wt	+	−	−	+	−	wt
3[b]		−	+	+	−	−	K43R	+	−	−	−	−	ND	−	−	+	+	−	C1402T
2[b]		−	+	+	−	−	K43R	+	−	−	−	−	ND	+	−	−	−	+	G1484T
1		+	−	−	+	−	K88R	+	−	−	−	−	ND	+	−	−	+	−	ND
	4	+	−	+	−	−	ND	−	−	+	−	−	A514C	−	+	−	+	−	A1401G
	2	+	−	+	−	−	wt	−	−	+	−	−	A514C	−	+	−	+	−	A1401G
	1	+	−	+	−	−	wt	−	−	+	−	−	A514C	+	−	−	+	−	wt
1		+	−	+	−	−	wt	−	−	−	−	+	C517T	+	−	−	+	−	ND
	6	+	−	+	−	−	wt	+	−	−	−	−	wt	+	−	−	+	−	wt

[a] −, no signal (band); +, signal (band); wt, wild-type; ND, not determined.
[b] *Mycobacterium bovis* BCG 1721 (RpsL K43R) derivatives selected for kanamycin-capreomycin resistance.

TABLE 3 Evaluation of module 3 (fluoroquinolones and ethambutol) of the AID TB Resistance line probe assay using clinical *M. tuberculosis* strains (Switzerland, $n = 30$; South Africa, $n = 34$)

No. of isolates from:		Fluoroquinolones, GyrA[a]								Ethambutol, EmbB					
		AID module 3								AID module 3					
Switzerland	South Africa	wt 90, 91, 94	A90V	S91P	D94A	D94N	D94Y	D94G	DNA sequencing	wt306	M306V, ATG to GTG	M306I, ATG to ATA	M306I, ATG to ATC	M306I, ATG to ATT	DNA sequencing
15	3	+	−	−	−	−	−	−	wt	+	−	−	−	−	wt
8	1	+	−	−	−	−	−	−	wt	−	+	−	−	−	M306V, ATG to GTG
3		+	−	−	−	−	−	−	wt	−	−	+	−	−	M306I, ATG to ATA
1	3	+	−	−	−	−	−	−	wt	−	−	−	+	−	M306I, ATG to ATC
1		+	−	−	−	−	−	−	wt	−	−	−	−	−	M306L, ATG to CTG
	2	−	+	−	−	−	−	−	A90V	−	+	−	−	−	M306V, ATG to GTG
	3	−	+	−	−	−	−	−	A90V	−	−	+	−	−	M306I, ATG to ATA
	1	−	+	−	−	−	−	−	A90V	−	−	−	+	−	M306I, ATG to ATC
	2	−	−	+	−	−	−	−	S91P	−	+	−	−	−	M306V, ATG to GTG
	1	−	−	+	−	−	−	−	S91P	−	−	+	−	−	M306I, ATG to ATA
	2	−	−	+	−	−	−	−	S91P	−	−	−	+	−	M306I, ATG to ATC
	2	−	−	−	+	−	−	−	D94A	−	+	−	−	−	M306V, ATG to GTG
	2	−	−	−	−	+	−	−	D94N	−	+	−	−	−	M306V, ATG to GTG
	1	−	−	−	−	+	−	−	D94N	−	−	−	+	−	M306I, ATG to ATC
1		−	−	−	−	−	+	−	D94Y	+	−	−	−	−	wt
	3	−	−	−	−	−	+	−	D94Y	−	−	+	−	−	M306I, ATG to ATA
1	2	−	−	−	−	−	−	+	D94G	+	−	−	−	−	wt
	1	−	−	−	−	−	−	+	D94G	−	+	−	−	−	M306V, ATG to GTG
	2	−	−	−	−	−	−	+	D94G	−	−	+	−	−	M306I, ATG to ATA
	1	−	−	−	−	−	−	+	D94G	−	−	−	+	−	M306I, ATG to ATC
	1	−	−	−	−	−	−	−	D94V (GTC)	+	−	−	−	−	wt
	1	−	−	−	−	−	−	−	D94H (CAC)	−	+	−	−	−	M306V, ATG to GTG

[a] −, no signal (band); +, signal (band); wt, wild-type.

H47Rv and a series of cultured clinical *M. tuberculosis* isolates ($n = 156$), which had been well characterized for drug resistance by phenotypic drug susceptibility testing and DNA sequence analyses. For *M. tuberculosis* strain H47Rv, only wt signals were observed for all three modules.

Module 1 (isoniazid and rifampin) was tested against 70 clinical *M. tuberculosis* strains isolated in Switzerland (Table 1). The set of strains allowed testing of all probes included in module 1. Mutations in the *inhA* promoter were detected at position −15 (11/70 strains) or −8 (1/70 strains). A *katG* 315 mutation was detected in 39/70 strains. A total of 42/70 strains were shown to harbor mutations in *rpoB* conferring resistance to rifampin; *rpoB* mutations in amino acid positions 516, 526, and 531 were observed in 4/70, 11/70, and 29/70 strains, respectively. Two strains showed double mutations in *rpoB* (Table 1); one strain contained mut516 and mut531 mutations, and the other strain contained mut516 and mut526 mutations. All mutations revealed by module 1 testing were confirmed by targeted DNA sequencing. Cross-reactivity signals resulting in false-positive results were not observed.

Module 2 (streptomycin, amikacin, and capreomycin) was tested against 22 clinical *M. tuberculosis* strains isolated in Switzerland ($n = 4$) or South Africa ($n = 18$). The RpsL K43R mutation conferring streptomycin resistance was detected in 7/22 strains, and the RpsL K88R mutation was detected in 1/22 strains. A strain with the RpsL K88Q mutation was not available. The *rrs* mutations A514C and C517T, which also confer resistance to streptomycin, were detected in 7/22 strains and 1/22 strains, respectively. The *rrs* mutation A1401G, which confers resistance to amikacin and capreomycin, was detected in 11/22 strains. Five BCG 1721 derivative strains with *rrs* point mutations (C1402T or G1484T) were also included for testing with module 2, as none of the clinical isolates harbored these mutations (Table 2). These strains were derived from *Mycobacterium bovis* strain BCG 1721, which contains the RpsL K43R mutation. All mutations identified by module 2 testing were confirmed by DNA sequencing. Cross-reactivity resulting in false-positive signals was not observed for module 2.

Module 3 (fluoroquinolones and ethambutol) was tested against 64 clinical *M. tuberculosis* isolates (Switzerland, $n = 30$; South Africa, $n = 34$) (Table 3). A total of 35/64 *M. tuberculosis* strains were *gyrA* wt and 29/64 had *gyrA* mutations. Among these, the line probe assay detected *gyrA* mutations in 27/29 of the fluoroquinolone-resistant strains (Table 3). Sequencing data revealed that the two strains that showed no *gyrA* wt or mutant signals had GyrA D94V (GAC to GTC) and GyrA D94H (GAC to CAC) mutations, for which probes were not included in the assay. A total of 23/64 *M. tuberculosis* strains were *embB* wt and 41/64 had *embB* mutations; 40/41 of the *emb*B resistance mutations were detected by the line probe assay. One ethambutol-resistant strain was not identified due to the presence of the EmbB M306L (ATG to CTG) mutation, which was not covered by the assay. An *M. tuberculosis* strain containing the EmbB M306I (ATG to ATT) mutation was not available to validate the corresponding probe in the module 3 line probe. Module 3 showed 100% accuracy for detection of resistance mutations, as confirmed by DNA sequencing (Table 3). No cross-reactivity resulting in false-positive results was observed for module 3.

Implementation of the AID TB resistance kit in routine diagnostic testing. To determine the feasibility of implementing the AID TB line probe resistance assay in a diagnostic workflow, we evaluated the suitability of the assay for the rapid detection of resistance in early positive MGIT cultures. All positive clinical *M. tuberculosis* cultures ($n = 50$) obtained in January through April 2012 were subjected to the AID TB Resistance line probe assay. The 50 cultures were from sputum ($n = 18$), tracheal bronchial secretion ($n = 12$), aspirate ($n = 7$), tissue ($n = 5$), gastric fluid

TABLE 4 Prospective analysis of clinical *M. tuberculosis* strains ($n = 50$) using module 1 of the AID TB resistance assay

No. of isolates	Isoniazid[a]				Rifampin, RpoB					
	inhA		KatG							
	wt (−16, −15, −8)	Mutant (−16, −15, −8)	wt 315	mut315	wt 513–516	mut516	wt 522–526	mut526	wt 529–533	mut531
47	+	−	+	−	+	−	+	−	+	−
1[b]	+	−	−	+	+	−	+	−	−	+
1[c]	+	−	−	+	+	−	+	−	−	+
1[d]	−	+	−	+	−	+	+	−	+	−

[a] All detected signals were confirmed by DNA sequencing. The three strains showing resistance mutations with module 1 were tested using modules 2 and 3. −, no signal (band); +, signal (band); wt, wild-type.
[b] MDR isolate 1.
[c] MDR isolate 2.
[d] MDR isolate 3.

($n = 4$), bronchoalveolar lavage fluid ($n = 2$), lymph node ($n = 1$), and urine ($n = 1$) specimens.

The AID line probe assay indicated that 47 of the 50 isolates lacked mutations in the *inhA*, *katG*, and *rpoB* regions (Table 4). These results correlated with phenotypic drug susceptibility testing results for rifampin and isoniazid with the MGIT 960 system (see Table S2 in the supplemental material). The remaining 3 isolates showed mutations in *inhA*, *katG*, and *rpoB* (Table 4) and were subsequently subjected to module 2 screening. Two of the 3 strains had a mutation in *rspL* (K43R), while a mutation in *rrs* (A514C) was detected in the third strain (Table 5). Mutations in *rrs* that are associated with amikacin and capreomycin resistance were not detected. Module 3 of the line probe assay did not show *gyrA* or *embB* mutations (Table 6). Complete agreement was observed between the AID TB line probe results and the results of subsequent DNA sequence analyses. Phenotypic DST of the cultured isolates showed 100% congruence with the genetic predictions (see Table S2 in the supplemental material).

Direct detection of antibiotic resistance mutations in patient specimens. Clinical specimens were used to evaluate the performance of the AID TB Resistance line probe assay for direct detection of resistance mutations. As the modules were sequentially developed over time, different sets of samples were used to exclude the possibility of quality loss of extracted DNA through repeated freezing and thawing and prolonged storage.

To analyze the performance of module 1 for direct detection of resistance mutations in clinical specimens, 98 smear-positive clinical samples were tested (Table 7). All smear-positive samples (100%) gave interpretable results. Nine samples showed mutation signals for *inhA*, *katG*, or *rpoB*, which were all confirmed by DNA sequencing. For one sample, which lacked hybridization signals for both *rpoB* wt 513 to 516 and the *rpoB* mutant probes, DNA sequencing revealed mutations resulting in L511P and D516G. All other samples showed wt signals, which were in complete agreement with the results of phenotypic DST of the corresponding cultures that were subsequently grown.

A set of 35 smear-positive clinical samples was used to evaluate modules 2 and 3. For module 2, 34/35 (97.1%) smear-positive samples (respiratory, $n = 30$; lymph node, $n = 2$; abscess, $n = 1$; aspirate, $n = 1$; tissue, $n = 1$) showed interpretable results. Only one tissue sample produced an uninterpretable result, due to high background signals. Only wild-type signals were observed for the 34 smear-positive samples, which were in complete agreement with the DST results for the corresponding cultures.

For module 3 (fluoroquinolones and ethambutol), 29/29 smear-positive samples (respiratory, $n = 24$; aspirate, $n = 2$; lymph node, $n = 1$; cerebrospinal fluid, $n = 1$; urine, $n = 1$) produced interpretable results. Only wild-type signals were detected for *gyrA*. One clinical sample showed the EmbB M306I mutation, and 28/29 samples showed wild-type signals for EmbB. The signals observed were in complete agreement with DNA sequencing results and the DST results for the corresponding cultures.

DISCUSSION

Rapid detection of *M. tuberculosis* resistance against first-line and second-line drugs is essential to identify monoresistant, MDR, or XDR *M. tuberculosis* strains. Based on epidemiological analyses of mutations associated with drug resistance in MDR-TB and

TABLE 5 Prospective analysis of clinical *M. tuberculosis* strains using module 2 of the AID TB resistance assay

No. of isolates	Streptomycin[a]										Amikacin-capreomycin, *rrs*				
	RpsL					*rrs*									
	wt 43	K43R	wt 88	K88R	K88Q	wt 513–517	C513T	A514C	G515C	C517T	wt 1401/1402	A1401G	C1402T	wt 1484	G1484C/T
1[b]	−	+	+	−	−	+	−	−	−	−	+	−	−	+	−
1[c]	−	+	+	−	−	+	−	−	−	−	+	−	−	+	−
1[d]	+	−	+	−	−	−	−	+	−	−	+	−	−	+	−

[a] All detected signals were confirmed by DNA sequencing. −, no signal (band); +, signal (band); wt, wild-type.
[b] MDR isolate 1.
[c] MDR isolate 2.
[d] MDR isolate 3.

TABLE 6 Prospective analysis of clinical *M. tuberculosis* strains using module 3 of the AID TB resistance assay

No. of isolates	Fluoroquinolones, GyrA[a]							Ethambutol, EmbB				
	wt 90, 91, 94	A90V	S91P	D94A	D94N	D94Y	D94G	wt 306	M306V, ATG to GTG	M306I, ATG to ATA	M306I, ATG to ATC	M306I, ATG to ATT
3[b]	+	−	−	−	−	−	−	+	−	−	−	−

[a] All detected signals were confirmed by DNA sequencing. −, no signal (band); +, signal (band).
[b] MDR isolates 1, 2, and 3.

XDR-TB strains, the TB Resistance line probe assay (Autoimmun Diagnostika GmbH, Strassburg, Germany) was designed to cover high-confidence resistance mutations for the following drugs: isoniazid (KatG 315 and *inhA* −16, −15, and −8), rifampin (RpoB 516, 526, and 531), streptomycin (*rpsL* K43R, K88R and K88Q and *rrs* C513T, A514C, G515C, and C517T), amikacin-capreomycin (*rrs* A1401G, C1402T, and G1484C/T), fluoroquinolones (GyrA A90V, S91P, D94A, D94N, D94Y, and D94G), and ethambutol (EmbB M306V and M306I) (Fig. 1). Notably, the AID line probe assay includes probes for detection of streptomycin resistance, i.e., *rpsL* A43G, A88G, and A88C and *rrs* C513T, A514C, G515C, and C517T. Mutations in *pncA* and *ethA*, which are associated with resistance to pyrazinamide and ethionamide, respectively, were not suitable for inclusion in the line probe assays. Resistance to these antibiotics is conferred by a diverse range of mutations scattered throughout the relatively large *pncA* and *ethA* genes (29, 30), which hampers the design of an appropriate line probe assay.

In this study, we have evaluated the performance of the TB Resistance line probe assay (AID) for the detection of resistance to first- and second-line drugs. Using clinical strains and laboratory-generated mutant strains, the specificity of the assay was shown to be excellent for all three modules (Tables 1, 2, and 3). No false-positive or false-negative results were observed. Less common mutations are indicated by the absence of signals for both wt and mutant probes. For example, two *Mycobacterium tuberculosis* strains lacking signals for *gyrA* were shown to contain mutations resulting in D94V and D94H (Table 3). A clinical culture lacking signals for *embB* was shown to harbor the EmbB M306L (ATG to CTG) mutation (Table 3). In addition, one of the clinical samples lacking a signal for *rpoB* was shown by sequencing to contain the RpoB L511P mutation (Table 7). A small prospective study, including 50 clinical isolates cultured in our diagnostic laboratory, confirmed the specificity shown by sequence analysis (Table 2) and the successful implementation of the line probe assay for routine diagnostic testing.

Evaluation of modules 1, 2, and 3 for direct screening of smear-positive respiratory and nonrespiratory specimens showed high percentages of interpretable results, i.e., 100%, 97.1%, and 100%, respectively. Mutations for isoniazid and/or rifampin resistance were detected in 9/98 samples (Table 7); these results were confirmed by DNA sequencing and were in agreement with the results of phenotypic testing of the corresponding cultured isolates. No mutations involved in aminoglycoside and fluoroquinolone resistance were found, reflecting their low prevalence in Switzerland. For all three modules, complete agreement (100%) between hybridization signals and drug susceptibility testing results for the corresponding cultured isolates was observed.

Our study addressing the suitability of the AID line probe assay for early positive MGIT cultures and smear-positive samples is biased since we had to focus on the samples available to us, i.e., specimens from an area with low disease prevalence and mostly drug-susceptible TB. We conclude that the TB Resistance line probe assay (AID) is a rapid method for accurate reliable detection of the most common mutations resulting in drug resistance in *M. tuberculosis*. The assay is easy to use, has a short time to results (producing results within 1 day), and can be readily implemented in a diagnostic laboratory for testing of cultured isolates as well as for direct testing of smear-positive clinical specimens. The usefulness of the assay in areas with high disease prevalence rates and large proportions of drug-resistant TB needs to be addressed in future studies.

TABLE 7 Evaluation of AID TB resistance line probe assay module 1 for detection of isoniazid and rifampin resistance in smear-positive clinical samples ($n = 98$)

Smear result	No. of clinical samples	Isoniazid[a]						Rifampin, RpoB						
		inhA			KatG									
		AID module 1			AID module 1			AID module 1						
		wt (−16, −15, −8)	Mutant (−16, −15, −8)	DNA sequencing	wt 315	mut315	DNA sequencing	wt 513–516	mut516	wt 522–526	mut526	wt 529–533	mut531	DNA sequencing
Positive	1[b]	−	+	C15T	−	−	wt	+	−	+	−	−	+	S531L
Positive	1[c]	−	+	C15T	+	−	ND	+	−	+	−	+	−	ND
Positive	6[d]	+	−	wt	−	+	S315T	+	−	+	−	+	−	ND
Positive	1[e]	+	−	wt	−	+	S315T	−	−	+	−	+	−	L511P, D516G
Positive	89[f]	+	−	ND	+	−	ND	+	−	+	−	+	−	ND

[a] In cases in which no mutations for isoniazid and/or rifampin were detected, wt signals were confirmed by DST only. ND, not done; −, no signal (band); +, signal (band); wt: wild-type.
[b] Respiratory specimen ($n = 1$).
[c] Respiratory specimen ($n = 1$).
[d] Respiratory ($n = 4$), biopsy ($n = 1$), and abscess ($n = 1$) specimens.
[e] Respiratory specimen ($n = 1$).
[f] Respiratory ($n = 78$), tissue ($n = 3$), wound ($n = 1$), lymph node ($n = 3$), abscess ($n = 2$), biopsy ($n = 1$), and unspecified ($n = 1$) specimens.

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