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Performance Assessment of the DR. TBDR/NTM IVD Kit for Direct Detection of *Mycobacterium tuberculosis* Isolates, Including Rifampin-Resistant Isolates, and Nontuberculous Mycobacteria

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We evaluated the performance of the DR. TBDR/NTM IVD kit, which was designed to detect *Mycobacterium tuberculosis*, rifampin-resistant *M. tuberculosis*, and nontuberculous mycobacteria, for detecting 110 positive and 50 negative cultures in *Mycobacterium* Growth Indicator Tubes. The accuracy rate of this kit for identification of *Mycobacterium* species was 95.5% (105/110).

Mycobacterium species have long been divided into *Mycobacterium tuberculosis* complex (MTC) and nontuberculous mycobacteria (NTM). An increasing number of reports on infections due to NTM, including pulmonary, soft tissue, bone, bloodstream, and central nervous system infections, as well as intra-abdominal and genitourinary tract infections, have emerged over the past decade (5, 8, 12, 13, 18). Among NTM infections, pulmonary disease warrants special attention (5). Individualized treatment options tailored to each NTM species have been suggested to optimize treatment response (5).

Recently, the DR. Chip Corporation in Taiwan developed the DR. TBDR/NTM IVD kit. The kit is designed to target MTC, rifampin-resistant *M. tuberculosis* (likely multidrug-resistant *M. tuberculosis* [MDR-*M. tuberculosis*]), and 15 species of NTM, including *Mycobacterium abscessus*, *Mycobacterium asciaticum*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium gordonae*, *Mycobacterium kansasii*, *Mycobacterium lentiflavum*, *Mycobacterium malmoense*, *Mycobacterium marinum*, *Mycobacterium scrofulaceum*, *Mycobacterium shimodei*, *Mycobacterium szulgai*, and *Mycobacterium xenopi*.

A total of 110 cultures that tested positive (≤42 days of incubation) and 50 cultures that tested negative (after 42 days of incubation) for *Mycobacterium* species in the *Mycobacterium* Growth Indicator Tube (MGIT) (Bactec MGIT 960 system; Becton, Dickinson Diagnostic Instrument Systems, Sparks, MD) from consecutive clinical respiratory specimens were collected at the National Taiwan University Hospital from January to October 2011. All respiratory specimens were processed and pretreated as previously described (15, 20). These processed respiratory specimens were also inoculated onto Lowenstein-Jensen (LJ) agar slants and cultured at 35°C in a 5% CO_2 incubator (15).

The DR. TBDR/NTM IVD kit integrates nucleic acid amplification and specific probe hybridization methods for identification of species in the MTC, species resistant to rifampin, and identification of 15 NTM species. Multiplex PCR was used to amplify the 16S-23S rRNA gene internal transcribed spacer (ITS), the RNA polymerase B subunit (*rpoB*) gene, and PCR positive-control genes. The ITS ranges in size from approximately 270 to 360 bp and has been found to be a suitable probe for obtaining additional phylogenetic information (17). After amplification, species-specific and genotype-specific probes on the chip hybridize to target-amplified DNA sequences for identification.

The protocol of the DR. TBDR/NTM IVD kit was as follows. First, 500 μl of each MGIT-positive culture was added to a 0.5-ml portion of E1 (phosphate buffer solution) buffer and centrifuged. With the supernatant removed, the pellet was then resuspended in 1 ml of E1 buffer, centrifuged, and again resuspended with 50 μl of E2 buffer (Tris-HCl solution with Triton X-100). After heating for 20 min and cooling on ice for 5 min followed by centrifugation, the supernatant containing extracted DNA was transferred to a new microcentrifuge vial. Then, 5 μl of extracted DNA was transferred to the PCR tube for amplification. The amplicons from specimen DNA were mixed with DR. Hyb buffer, denatured, and then transferred to a chiller rack at −20°C. A 100-μl aliquot of DR. Hyb buffer was then added to the DR. TBDR/NTM chip, and then 5 μl of PCR product (10 μl/well) was added to each well. The DR. AiM Reader (600 dots per in. [dpi]) was used to read the pattern that developed at the bottom of the well. The template "TBDR/NTM" was used to analyze data. Figure 1 demonstrates the patterns that developed at the bottom of the well of the DR. TBDR/NTM IVD kit.

The results of mycobacterial species identification by the DR. TBDR/NTM IVD kit and by the conventional biochemical identification method were initially evaluated and compared. When there was a difference in the species identification results obtained by the DR. TBDR/NTM IVD kit and by the conventional methods, 16S rRNA gene sequencing analysis was used for further species identification of the isolates that had produced different results (9). Sequencing analysis of the 16S rRNA gene (1,464 bp) was performed using two primers (8FPL and 1492) as previously described (9).

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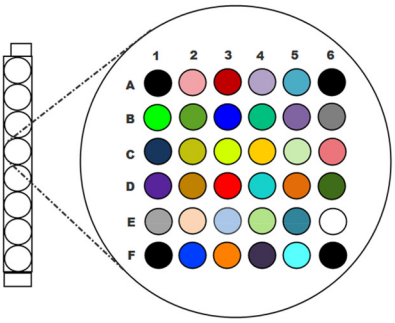

A1, A6, F1, F6	●	Hybridization Controls			
B1	●	*M. abscessus*	A4	●	PCR control
C1	●	*M. asiaticum*	B4	●	MTBC-mutation 516b
D1	●	*M. avium*	C4	●	MTBC-mutation 526c
E1	●	*M. intracellulare*	D4	●	MTBC-mutation 526a
A2	●	*M. chelonae*	E4	●	MTBC-mutation 531a
B2	●	*M. fortuitum*	F4	●	Negative control
C2	●	*M. gordonae*	A5	●	MTBC-wild type 512
D2	●	*M. kansasii*	B5	●	MTBC-wild type 516
E2	●	*M. lentiflacum*	C5	●	MTBC-wild type 523
F2	●	*M. malmoense*	D5	●	MTBC-wild type 526
A3	●	*M. marinum*	E5	●	MTBC-wild type 531
B3	●	*M. scrofulaceum*	F5	●	MTBC
C3	●	*M. shimoidei*	B6	●	MTBC-mutation 516a
D3	●	*M. szulgi*	D6	●	MTBC-mutation 526b
E3	●	*M. xenopi*	E6	●	MTBC-mutation 531b
F3	●	*Mycobacterium* spp.			

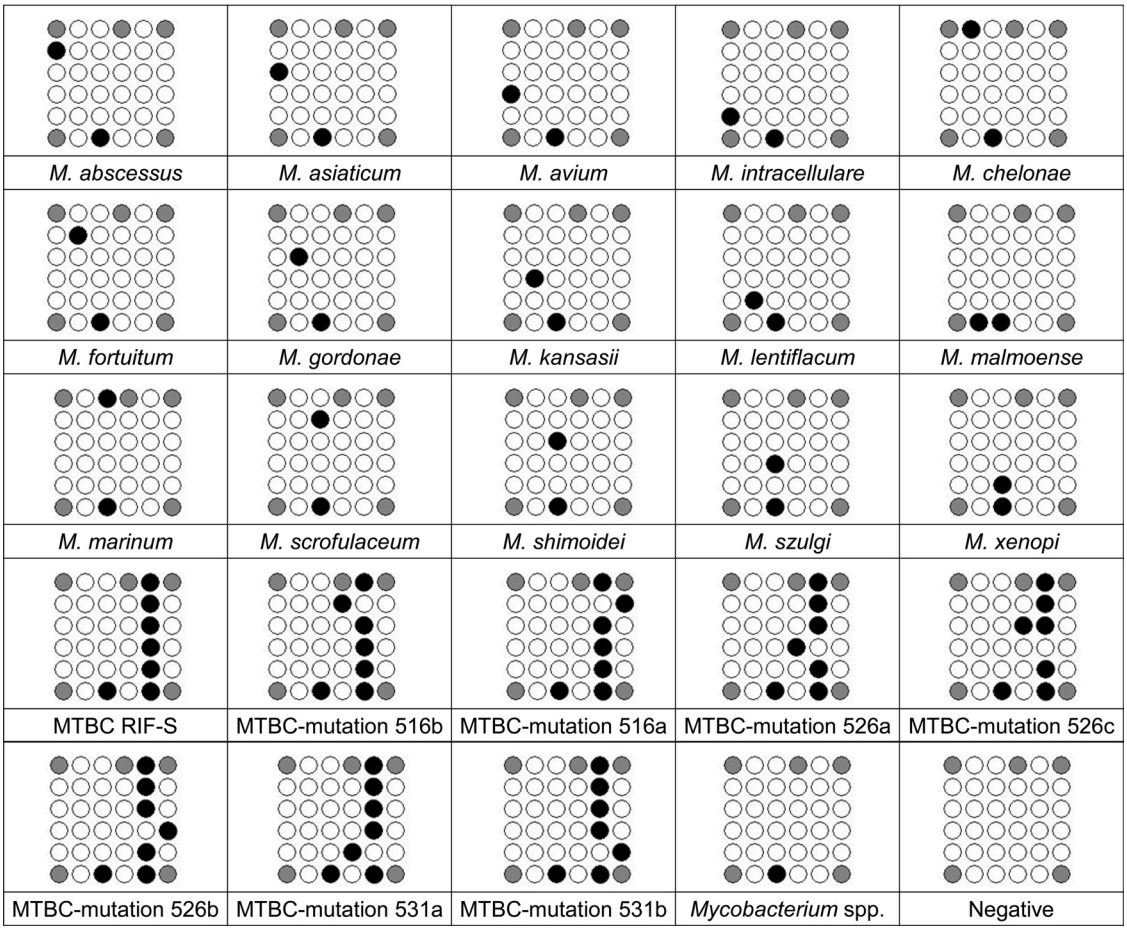

FIG 1 DR. TBDR/NTM IVD kit for identification of *M. tuberculosis* and NTM species. MTBC, *M. tuberculosis* complex.

All 50 MGIT negative cultures had negative results by the DR. TBDR/NTM IVD kit. Growth on the LJ agar slants for the 50 specimens was also negative after incubation for 2 months. Table 1 shows the results of species identification by conventional identification methods and the DR. TBDR/NTM IVD kit and 16S rRNA gene sequencing analysis of 110 MGIT cultures. The results obtained by the DR. TBDR/NTM IVD kit are illustrated in Fig. 2.

Though concerns exist regarding the efficiency of the 16S

TABLE 1 Results obtained by conventional biochemical methods and the DR. TBDR/NTM IVD kit for positive cultures of *Mycobacterium* Growth Indicator Tubes (MGIT) and by 16S rRNA sequencing analysis of the isolates with discrepant results of species identification by conventional biochemical methods and the DR. TBDR/NTM IVD kit[a]

Mycobacterial species (no. of specimens) identified by conventional methods for positive MGIT cultures	Mycobacterial species (no. of specimens) identified by DR. TBDR/NTM IVD kit for positive MGIT cultures	Mycobacterial species (no. of specimens) identified by 16S rRNA sequencing analysis of isolates identified by conventional methods
M. tuberculosis, non-multidrug-resistant *M. tuberculosis* (MDR-*M. tuberculosis*) (16)	*M. tuberculosis* (14)	
	***M. abscessus* (1)**	*M. tuberculosis*
	***Mycobacterium* species (1)**	*M. tuberculosis*
MDR-*M. tuberculosis* (3)	MDR-*M. tuberculosis* (3)	
M. avium-M. intracellulare complex (28)	*M. avium* (3)	*M. avium* (3)
	M. intracellulare (19)	*M. intracellulare* (19)
	M. avium/*M. intracellulare* (2)	*M. avium*
	M. lentiflavum (1)	*M. lentiflavum*
	M. abscessus/*M. avium*/*M. intracllulare* (2)	*M. avium* (2)
	Mycobacterium species (1)	*M. intracellulare* (1)
M. abscessus (38)	*M. abscessus* (36)	
	M. abscessus/*M. kansasii* (1)	*M. abscessus* (1)
	M. abscessus/*M. avium*/*M. intracellulare* (1)	*M. abscessus* (1)
***M. chelonae* (2)**	***M. abscessus*/*M. intracellulare* (2)**	***M. chelonae*** (2)
M. fortuitum (10)	*M. fortuitum* (7)	
	M. fortuitum/*M. intracellulare* (1)	*M. fortuitum* (1)
	M. abscessus/*M. fortuitum* (1)	*M. fortuitum* (1)
	Mycobacterium species (1)	*Mycobacterium* species
M. gordonae (6)	*M. gordonae* (6)	
M. kansasii (4)	*M. kansasii* (3)	
	M. avium/*M. kansasii* (1)	*M. kansasii* (1)
M. tuberculosis and *M. avium* (1)	*M. avium*/*M. intracellulare* (1)	*M. avium* (1)
M. abscessus and *M. fortuitum* (2)	*M. abscessus*/*M. fortuitum* (2)	*M. abscessus*/*M. fortuitum* (2)

[a] Isolates marked in bold indicate discrepant results obtained from the three identification methods. The discrepant results were defined as differences in mycobacterial species or complexes between the results from the DR. TBDR/NTM IVD Kit and 16S rRNA sequencing analysis.

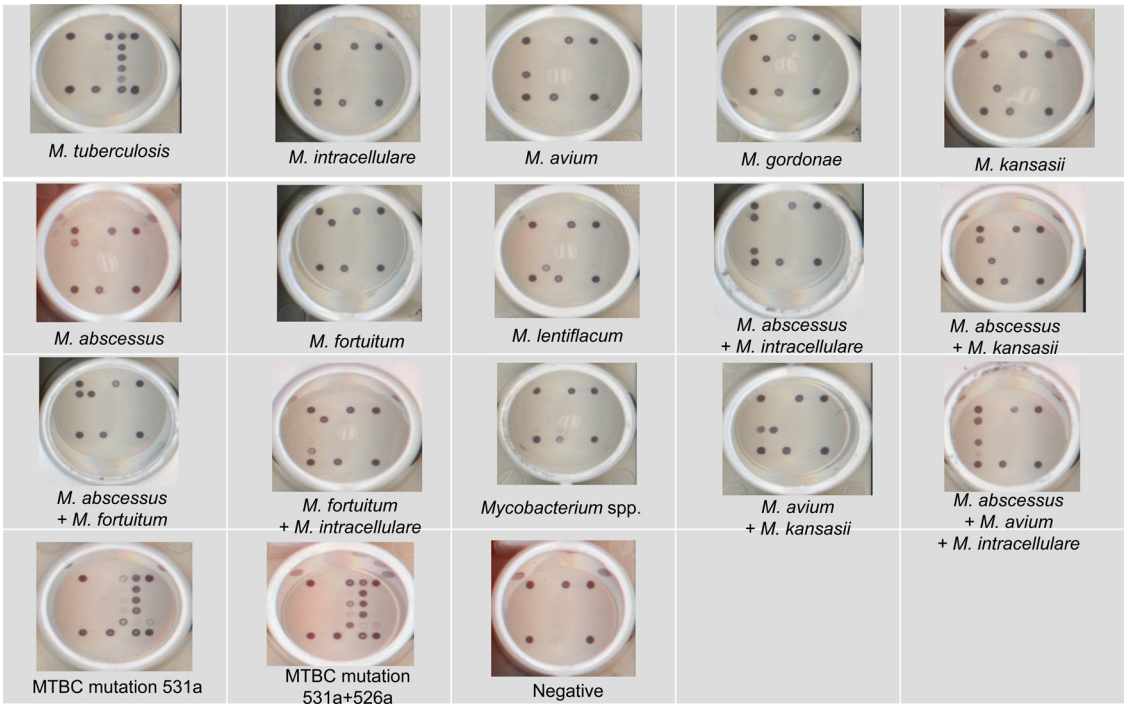

FIG 2 The performances of selected mycobacterial species for identification by the DR. TBDR/NTM IVD kit among 110 positive cultures in *Mycobacterium* Growth Indicator Tubes.

rRNA sequencing method in identifying mycobacteria, it is still widely used as the gold standard in various studies assessing the performance of kits for identification of novel mycobacteria (11, 19, 21). The accuracy rate for identifying *M. tuberculosis* was 89.5% (17/19). Three rifampin-resistant *M. tuberculosis* isolates identified by the DR. TBDR/NTM IVD kit were resistant to both isoniazid (1 μg/ml) and rifampin (1 μg/ml) (MDR-*M. tuberculosis*) as determined using the conventional agar proportion method. In identifying NTM species, the DR. TBDR/NTM IVD kit correctly identified all isolates of *M. abscessus*, *M. fortuitum*, *M. gordonae*, and *M. kansasii* species. In contrast, it misidentified the two *M. chelonae* species. The DR. TBDR/NTM IVD kit identified 27 (96.4%) of 28 *M. avium* complex (MAC) isolates.

The strengths of the DR. TBDR/NTM IVD kit may lie in the correct species identification of NTM. The major flaw of the DR. TBDR/NTM IVD kit is its limited ability to correctly identify all *M. tuberculosis* isolates. This chip also failed to differentiate between *M. abscessus* (*sensu stricto*), *M. massiliense*, and *M. bolletii* (2, 4, 16). This may be clinically important, since *M. abscessus* (*sensu stricto*) and *M. bolletii* have induced macrolide resistance whereas *M. massiliense* does not (2, 6, 7). Furthermore, *M. chelonae* is a rare cause of NTM lung disease, so the lack of identification of *M. chelonae* is not of great importance from a practical standpoint.

Not all less-common *Mycobacterium* species that are known to cause various clinical infections are included in the identification list of the DR. TBDR/NTM IVD kit (1, 3, 9, 14). The clinical significance of less-common NTM species, however, is being criticized (5). Furthermore, it can be difficult to determine which NTM species are important to include in the identification list due to the rapidly emerging database as well as changing taxonomic status of NTM species (5, 10).

In summary, we have tested a novel commercially available kit designed to rapidly identify *M. tuberculosis*, rifampin-resistant *M. tuberculosis*, and 15 NTM species from positive MGIT cultures. Though the test specimen number may be relatively small, this kit was highly sensitive at identifying common NTM species in our study. This technology could serve as a rapid and effective method for identifying *Mycobacterium* species among positive MGIT cultures and as an important epidemiologic tool for diagnosis of NTM disease. Further corroboration of the utility of this technology in population-based studies will hopefully be forthcoming.

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