JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2009, p. 481–484 Vol. 47, No. 2
0095-1137/09/$08.00+0 doi:10.1128/JCM.01253-08

Evaluation of an Immunochromatographic Assay Kit for Rapid Identification of *Mycobacterium tuberculosis* Complex in Clinical Isolates▽

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Received 2 July 2008/Returned for modification 16 August 2008/Accepted 22 November 2008

We evaluated a new immunochromatographic assay (ICA) using mouse monoclonal anti-MPT64 antibody for rapid discrimination between *Mycobacterium tuberculosis* and nontuberculous mycobacteria in clinical isolates. A study with mycobacteria and other organisms showed excellent sensitivity (≅99%) and specificity (100%) and an appropriate detection limit (10^5 CFU/ml) when tested with *M. tuberculosis* H37Rv. This ICA can simplify the identification of *M. tuberculosis* in clinical laboratories.

Tuberculosis is a global problem and the single most common cause of death from any bacterial agent (15, 22). *Mycobacterium tuberculosis* and nontuberculous mycobacteria (NTM) are different clinically, so prompt detection, isolation, and discrimination are essential for appropriate management (3, 7).

The MPT64 protein is highly specific for *M. tuberculosis* complex, including *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, and some, although not all, substrains of *M. bovis* BCG (1, 6, 16, 18, 23), and can be detected in culture isolates and biopsy samples (1, 7, 17, 18, 20). Recently, Standard Diagnostics (SD, Yongin, Korea) developed a simple and rapid assay using a mouse monoclonal anti-MPT64 antibody to discriminate between *M. tuberculosis* complex and NTM by immunochromatography. Mouse monoclonal anti-MPT64 antibodies (SD Bioline TB Ag MPT64; SD) are immobilized on a nitrocellulose membrane as the capture material. Another antibody, which recognizes another epitope of MPT64 and has been conjugated with colloidal gold particles, is used for antigen capture and detection in a sandwich-type assay.

We evaluated the clinical usefulness of the kit using mycobacteria and other organisms. To determine specificity, 137 bacterial isolates (68 species), 20 fungal isolates (10 species), 53 reference mycobacterial isolates (40 species), and 51 NTM isolates from clinical samples were tested (Tables 1 to 3). To determine sensitivity, 159 *M. tuberculosis* complex strains grown on 3% Ogawa medium (isolated at Pusan National University Hospital), 60 strains from Bactec MGIT 960 culture tubes (isolated at Kosin University Gospel Hospital), and one reference strain, *M. tuberculosis* H37Rv, were tested. All bacterial, fungal, and mycobacterial isolates were stock cultures that had been kept in a −4°C refrigerator or a −72°C deep freezer for as long as 18 months. The cultured mycobacteria were identified by acid-fast bacillus stain, nucleic acid amplification, and DNA microarray (10, 14). Finally, to determine the detection limit, a series of diluted suspensions of *M. tuberculosis* H37Rv were inoculated onto Middlebrook 7H10 agar and the resulting colonies were counted (19). One hundred microliters of sample taken from liquid medium was applied directly to the sample well without preparation. Three or four colonies were scraped from the solid medium and suspended in 300 μl of extraction buffer (SD); then, 100 μl of the suspension was added to the sample well. If there was condensation fluid in egg-based medium, 100 μl of the fluid was applied directly to the sample well, instead of using extraction buffer. Tests were interpreted 15 min after sample application. The presence of a control band alone indicates a negative result, whereas the presence of two color bands (control and test bands), no matter which band appears first, indicates a positive result. A color band of any intensity was read as a positive reaction (Fig. 1). If the control band was not visible after 15 min, the result was considered invalid, and the sample was retested.

All bacterial, fungal, and NTM isolates, including reference strains, were negative by the immunochromatographic assay (ICA) (specificity, 100%). One hundred fifty-eight of 159 *M. tuberculosis* complex strains grown on solid medium and 59 of 60 strains from liquid medium were positive by the ICA (overall sensitivity, 98.6%). The 1:128-diluted suspension (5.5×10^5 CFU/ml) revealed 10% reaction intensity, and the band intensity gradually weakened with serial dilutions until the 1:1,024 suspension (6.8×10^4 CFU/ml) was negative. The detection limit thus was determined to approximate 10^5 CFU/ml.

Although most culture-positive mycobacteria are *M. tuberculosis* in regions where tuberculosis is highly prevalent, NTM isolates have been increasing gradually, such that now 20 to 30% of mycobacteria found in clinical specimens in Korea are NTM (13). These organisms trigger diseases and true infections and thus can be important clinically (21). Because of the complexity of test methods, many small hospital laboratories do not discriminate between *M. tuberculosis* and NTM (4, 11, 12), meaning that NTM are inappropriately managed with first-line antituberculosis drugs (12, 24), worsening the pa-

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▽ Published ahead of print on 3 December 2008.

TABLE 1. List of bacterial and fungal strains

Species (no. of strains)
Bacteria
Acinetobacter baumannii (4)
Aeromonas caviae (2)
Aeromonas hydrophila (3)
Aeromonas veronii (2)
Aeromonas veronii biovar sobria (1)
Alcaligenes faecalis (2)
Alcaligenes xylosoxidans (2)
Bacillus cereus (2)
Bordetella bronchiseptica (2)
Branhamella catarrhalis (2)
Brevundimonas vesicularis (2)
Chryseobacterium indologenes (2)
Chryseobacterium meningosepticum (2)
Citrobacter freundii (4)
Comamonas acidovorans (2)
Escherichia coli (2)
Enterobacter aerogenes (2)
Enterobacter agglomerans (2)
Enterobacter cloacae (2)
Enterobacter intermedius (2)
Enterococcus avium (2)
Enterococcus casseliflavus (2)
Enterococcus faecalis (2)
Enterococcus faecium (2)
Enterococcus gallinarum (2)
Enterococcus raffinosis (2)
Flavimonas oryzihabitans (2)
Flavobacterium indologenes (2)
Klebsiella ornithinolytica (2)
Klebsiella oxytoca (2)
Klebsiella ozaenae (2)
Klebsiella pneumoniae (2)
Kocuria rosea (1)
Kocuria varians (2)
Leclercia adecarboxylata (2)
Morganella morganii (2)
Myroides spp. (2)
Neisseria gonorrhoeae (2)
Ochrobactrum anthropi (2)
Pichia anomala (2)
Pichia ohmeri (2)
Plesiomonas shigelloides (2)
Proteus vulgaris (2)
Providencia rettgeri (3)
Providencia stuartii (2)
Pseudomonas aeruginosa (2)
Pseudomonas fluorescens (2)
Ralstonia pickettii (2)
Salmonella group D (1)
Salmonella paratyphi A (2)
Serratia marcescens (2)
Shigella sonnei (2)
Sphingobacterium spiritivorum (2)
Sphingomonas paucimobilis (2)
Staphylococcus aureus (4)
Staphylococcus epidermidis (2)
Staphylococcus haemolyticus (2)
Staphylococcus saprophyticus (2)
Staphylococcus, coagulase negative (2)
Stenotrophomonas maltophilia (2)
Streptococcus agalactiae (2)
Streptococcus dysgalactiae (2)
Streptococcus mitis (2)
Streptococcus pneumoniae (2)
Streptococcus pyogenes (2)
Streptococcus viridans group (2)
Subtotal (137)
Fungi
Candida albicans (3)
Candida dubliniensis (2)
Candida glabrata (2)
Candida krusei (1)
Candida pelliculosa (2)
Candida tropicalis (2)
Cryptococcus humicolus (2)
Cryptococcus neoformans (2)
Trichosporon asahii (2)
Trichosporon beigelii (2)
Subtotal (20)
Total (157)

TABLE 2. List of reference mycobacterial strains

Mycobacterium species and strain
M. abscessus
ATCC 19977
ATCC 23003
M. acapulcensis KTCC9501
M. agri KTCC9502
M. asiaticum KTCC9503
M. austroafricanum KTCC9504
M. avium ATCC 25291
M. branderi ATCC 51788
M. celatum ATCC 51131
M. chelonae KTCC9505
M. diernhoferi KTCC9506
M. flavescens
ATCC 14474
ATCC 23008
M. fortuitum
KTCC1122
KTCC9510
M. gastri ATCC 15754
M. gilvum KTCC9512
M. gordonae
KTCC3036
KTCC9513
M. interjectum ATCC 51457
M. intermedium ATCC 51846
M. intracellulare
KIT41105
KTCC9514
M. kansasii KTCC9515
M. marinum
ATCC 11564
ATCC 927
M. malmoense ATCC 29571
M. morikaense KTCC9516
M. mucogenicum
KTCC19088
ATCC 49650
M. neoaurum ATCC 25795
M. nonchromogenicum ATCC 19530
M. peregrinum
KTCC9615
ATCC 14467
M. phlei KTCC2192
M. porcinum KTCC9517
M. pulveris KTCC9518
M. scrofulaceum
KTCC9519
ATCC 19981
M. senegalense ATCC 35796
M. shimoidei ATCC 27962
M. simiae
ATCC 15080
ATCC 25275
M. smegmatis KTCC1057
M. sphagni ATCC 33027
M. szulgai KTCC9520
M. terrae
KTCC9614
ATCC 15755
M. triviale
ATCC 23290
ATCC 23292
M. tuberculosis H37Rv
M. vaccae
KTCC19087
ATCC 15483
M. xenopi ATCC 19250

tient's condition and raising the risk of drug resistance. Thus, exact and rapid identification of mycobacteria is important, and a simple, sensitive, and specific identification method is necessary. Direct staining of a colony is simple and fast but does not discriminate between *M. tuberculosis* and NTM, and traditional biochemical tests not only can produce equivocal results but also take a long time (2). Chemiluminescent DNA probes, nucleic acid amplification, high-performance liquid chromatography, and sequencing of 16S rRNA genes are more

TABLE 3. List of mycobacteria isolated from clinical specimens

Medium (total no. of strains)	Species (no. of strains)
Solid (3% Ogawa medium) (200)	*M. abscessus* (5) *M. avium-intracellulare* complex (21) *M. fortuitum* (2) *M. kansasii* (4) *M. szulgai* (9) *M. tuberculosis* complex (159)
Liquid (MGIT) (70)	*M. avium-intracellulare* complex (7) *M. szulgai* (1) Mycobacteria, unidentifiable (2) *M. tuberculosis* complex (60)

sophisticated methods that require expensive equipment (2, 5, 10, 14). Although our study was conducted with culture specimens and needs further direct testing with clinical specimens, the ICA was shown to be rapid and easy and to have high sensitivity and specificity.

In this study, one isolate of *M. tuberculosis* complex from solid medium was negative. This organism was subcultured twice on 3% Ogawa medium with failure of growth, suggesting that the culture was kept too long in a slant culture format in a refrigerator before testing and lost its viability. The negative isolate that was grown in liquid medium was subcultured on 3% Ogawa medium, and a repeat test also was negative. According to a recent study, MPT64, once secreted into the medium, is stable, as the test remains positive even if performed 1 year after the detection of growth in either solid or liquid medium (7). This suggests another reason for the negative test results besides storage. Some *M. bovis* BCG substrains lack MPT64 production (1, 6, 16, 18), and this could have been a nonproducing strain. Another possible explanation for the negative test results is that the strain had mutations within the *mpt64* gene, leading to the production of an incomplete protein. By sequencing, Hirano et al. identified several such mutations, including deletion of nucleotides, point mutations, and an IS*6110* insertion mutation at nucleotide position 501 (8, 9).

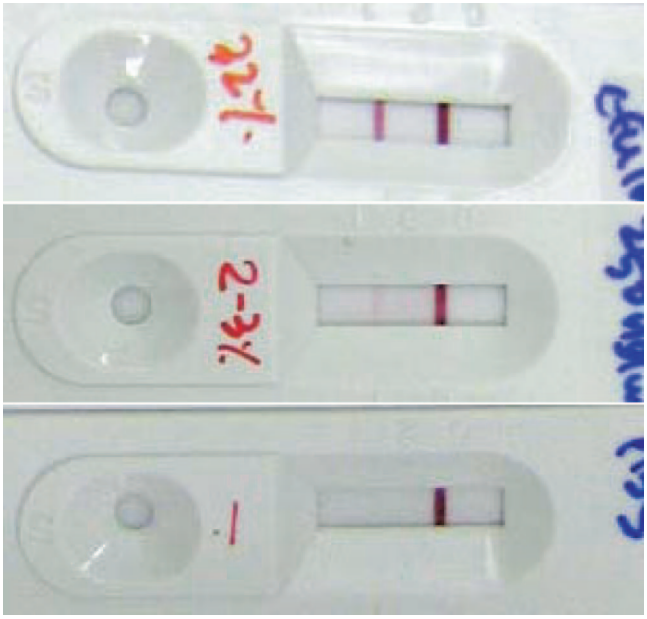

FIG. 1. Identification of the *M. tuberculosis* complex by the MPT64 ICA kit. Top, strong positive; middle, weak positive; bottom, negative.

The ICA is rapid and easy, is applicable for specimens from both liquid and solid media, and does not require any special equipment. It showed excellent sensitivity (≅99%) and specificity (100%) and an appropriate detection limit (10^5 CFU/ml). It can simplify the identification of *M. tuberculosis* complex strains, avoiding the technical complexity of PCR and similar identification techniques in clinical laboratories.

C. L. Chang participated in the development of the MPT64 ICA kit as a scientific consultant.

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