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Performance of the SD Bioline TB Ag MPT64 Rapid test for quick confirmation of *Mycobacterium bovis* isolates from animals

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Mycobacterium (*M.*) *bovis*, a bacterium in the *M. tuberculosis* complex, is a causative agent of bovine tuberculosis, a contagious disease of animals. Mycobacterial culture is the gold standard for diagnosing bovine tuberculosis, but this technique is laborious and time-consuming. In the present study, performance of the SD Bioline TB Ag MPT4 Rapid test, an immunochromatographic assay, was evaluated using reference bacterial strains and *M. bovis* field isolates collected from animals. The SD MPT64 Rapid test produced positive results for 95.5% (63/66) of the *M. bovis* isolates from cattle and 97.9% (46/47) of the isolates from deer. Additionally, the test had a sensitivity of 96.5% (95% CI, 91.2-99.0), specificity of 100% (95% CI, 96.7-100.0), positive predictive value of 100% (95% CI, 96.7-100.0), and negative predictive value of 92.9% (95% CI, 82.7-98.0) for *M. bovis* isolates. In conclusion, the SD MPT64 Rapid test is simple to use and may be useful for quickly confirming the presence of *M. bovis* in animals.

Keywords: animals, *Mycobacterium bovis*, SD MPT64 Rapid test

Introduction

Bovine tuberculosis is a contagious disease of animals caused by *Mycobacterium* (*M.*) *bovis*, which is part of the *M. tuberculosis* complex [2,21]. Bovine tuberculosis is responsible for major agricultural economic loss and is a public health concern [7,18]. In most countries, control and eradication of bovine tuberculosis involves testing and slaughter [17].

The intradermal tuberculin skin test is widely used to identify infected animals and has contributed to the control of bovine tuberculosis [8]. In Korea, the incidence of bovine tuberculosis was approximately 15% in the 1940s; this was reduced to 0.15% in 2005 by a bovine tuberculosis eradication program involving the intradermal tuberculin skin test [6]. However, the current incidence has increased to 0.25% (approximately 1,000 cattle per year) [24]. An efficient and effective diagnostic system or strategy is urgently needed to eradicate bovine tuberculosis. Serological tests using *M. bovis*-specific antigens and the interferon-gamma (IFN-γ) assay are currently being considered or evaluated in Korea. These assays use mycobacterial cultures as the gold standard for diagnosing bovine tuberculosis. Culturing takes 3 to 6 weeks while the presence of *M. bovis* can be confirmed using acid-fast bacilli staining and biochemical tests [22]. The Ziehl-Neelsen stain is simple and rapid, but cannot differentiate *M. bovis* from nontuberculous mycobacteria. Furthermore, conventional biochemical tests are laborious and time-consuming.

The SD Bioline TB Ag MPT64 Ag MPT64 Rapid test (SD

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Table 1. Reference bacteria strains used in this study and results of the SD MPT64 Rapid test

Species	Reference number	MPT64-ICA
M. abscessus	ATCC 19977	−
M. africanum	ATCC 25420	+
M. avium	ATCC 25291	−
M. avium	ATCC 35719	−
M. bovis AN5		+
M. bovis BCG Danish		−
M. bovis BCG Pasteur		−
M. celatum	ATCC 51130	−
M. chelonae	ATCC 19237	−
M. fortuitum	ATCC 49403	−
M. gastri	ATCC 25027	−
M. gordonae	ATCC 14470	−
M. intracellulare	ATCC 13950	−
M. kansasii	ATCC 12478	−
M. marinum	ATCC 927	−
M. microtii	ATCC 19422	−
M. mucogenicum	ATCC 49649	−
M. peregrinum	ATCC 14467	−
M. senegalense	ATCC 35796	−
M. septicum	ATCC 700731	−
M. smegmatis	ATCC 19420	−
M. szulgai	ATCC 35796	−
M. terrae	ATCC 15755	−
M. tuberculosis H37Rv	ATCC 27294	+
M. ulcerans	ATCC 19423	−
Citrobacter freundii	ATCC 8090	−
Enterococcus faecalis	ATCC 29212	−
E. faecium	ATCC 35667	−
Escherichia coli	ATCC 25922	−
Haemophilus influenza	ATCC 49247	−
Klebsiella pneumonia	ATCC 13883	−
Listeria monocytogenes	ATCC 35152	−
Pseudomonas aeroginosa	ATCC 27853	−
Salmonella typhi	ATCC 19430	−
Shigella sonnei	ATCC 25931	−
Staphylococcus aureus	ATCC 29213	−
Yersinia enterocolitica	ATCC 9610	−

M.: *Mycobacterium*, ATCC: American Type Culture Collection.

MPT64 Rapid test), a simple immunochromatographic test (ICT) for the *M. tuberculosis* complex, has been developed and uses monoclonal antibodies to detect MPT64 protein [1,9]. MPT64 is a protein specifically secreted by members of the *M. tuberculosis* complex including *M. tuberculosis*, *M. bovis*, and *M. africanum* as well as some strains of *M. bovis* bacilli Calmette-Guérin (BCG) [12]. The SD MPT64 Rapid Test is used widely to confirm the identity of *M. tuberculosis* isolates from humans. Furthermore, the test is highly sensitive and specific. The SD MPT64 Rapid test is potentially useful for identifying *M. bovis*, a member of the *M. tuberculosis* complex, because the MPT64 antigen is expressed by all members of the *M. tuberculosis* complex [10]. However, few reports have evaluated the performance of this test for analyzing *M. bovis* isolates from animals. We therefore assessed the ability of the SD MPT64 Rapid test to identify *M. bovis* isolates from cattle and deer.

Materials and Methods

Bacterial strains

Reference bacterial strains consisting of 25 mycobacteria and 12 other bacteria were obtained from American Type Culture Collection (ATCC, USA) and used for the present investigation (Table 1). *M. bovis* AN5 was grown in Middlebrook 7H9 broth media (Difco, USA) supplemented with 10% Middlebrook OADC enrichment medium (BBL, USA) to determine the detection limit of the test. *Mycobacterium* species grown on Löwenstein-Jensen medium (UNION LAB, Korea) and other bacterial strains grown on Luria-Bertani agar plates (Difco) were used for the SD MPT64 Rapid test. This study evaluated 113 *M. bovis* field strains isolated from cattle and deer in Korea along with 25 *M. avium* and 27 *M. intracellulare* clinical strains isolated from humans (Table 2). All mycobacterial isolates were identified by Ziehl-Neelsen staining and biochemical tests as previously described [22]. AccuProbe (Gen-Probe, USA), REBA Myco-ID (M&D, Korea), and multiplex PCR were performed to identify *Mycobacterium* species and distinguish *M. bovis* from other members of the *M. tuberculosis* complex [14,15,20].

SD MPT64 Rapid test

SD MPT64 Rapid test was performed according to the manufacturer's guidelines. Three or four colonies of mycobacterial strains grown in Löwenstein-Jensen media and other bacterial strains grown on Luria-Bertani agar plates were emulsified in 200 μL of extraction buffer. Next, 100 μL were placed in sample wells of the test and the results were visually assessed based on color development after incubating at room temperature for 15 min. Non-reactive *M. bovis* isolates in the test was confirmed by sequencing of the *mpt*64 gene.

To determine the detection limit of the SD MPT64 Rapid test, a series of diluted *M. bovis* AN5 suspension from 2.1×10^3 to 2.0×10^6 cultured in Middlebrook 7H9 medium with 10% enrichment medium were analyzed with the SD MPT64 Rapid test and colony-forming unit (CFU) counted using Middlebrook 7H10 (Difco) plates with 10% enrichment medium.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined using

Table 2. Results of the SD MPT64 Rapid test for *M. bovis* isolates from animals

Strains	Isolates (n)	MPT64-ICA	
		Positive [n (%)]	Negative [n (%)]
M. bovis isolates from cattle	66	63 (95.5)	3 (4.5)
M. bovis isolates from deer	47	46 (97.9)	1 (2.1)
M. avium isolates*	25	0 (0)	25 (100)
M. intracellulare isolates*	27	0 (0)	27 (100)
Total	165	109 (66.1)	56 (33.9)

*Strains isolated from humans.

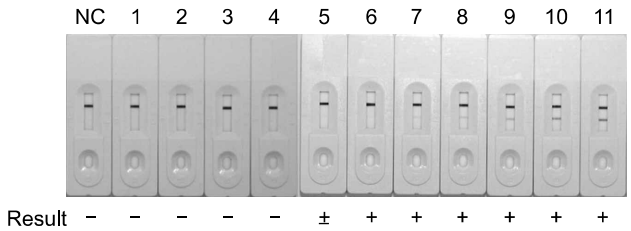

Fig. 1. Detection limit of the SD MPT64 Rapid test for *Mycobacterium* (*M.*) *bovis*. The test was used to analyze a series of diluted suspensions of *M. bovis* AN5 cultured in Middlebrook 7H9 broth with enrichment. A representative image of three tests is shown. The results were interpreted as positive (+), negative (−), or weak (±). NC, negative control; 1, 2.1×10^3 CFU/mL of *M. bovis*; 2, 4.2×10^3 CFU/mL of *M. bovis*; 3, 8.5×10^3 CFU/mL of *M. bovis*; 4, 1.7×10^4 CFU/mL of *M. bovis*; 5, 3.4×10^4 CFU/mL of *M. bovis*; 6, 6.8×10^4 CFU/mL of *M. bovis*; 7, 1.3×10^5 CFU/mL of *M. bovis*; 8, 2.7×10^5 CFU/mL of *M. bovis*; 9, 5.4×10^5 CFU/mL of *M. bovis*; 10, 1.0×10^6 CFU/mL of *M. bovis*; 11, 2.0×10^6 CFU/mL of *M. bovis*.

GraphPad Prism software (ver. 4.0; GraphPad Software, USA). Agreement of test results was estimated based on kappa values using the SPSS 13.0 (SPSS, USA).

Results

SD MPT64 Rapid test results for the reference strains

The SD MPT64 Rapid test was performed for 25 reference strains of mycobacteria and 12 other bacteria to identify members of the *M. tuberculosis* complex. The SD MPT64 Rapid test produced strong results for *M. bovis*, *M. africanum*, and *M. tuberculosis*, but not *M. bovis* BCG Pasteur or *M. bovis* BCG Danish (Table 1). No positive signal was observed for non-tuberculous mycobacteria (NTM) bacilli or any other bacteria tested. These results imply that the SD MPT64 Rapid test can identify members of the *M. tuberculosis* complex, including *M. bovis*, with a high degree of specificity.

Detection limit of the SD MPT64 Rapid test for *M. bovis*

Ten-fold dilutions of *M. bovis* AN5 were used to measure the detection limit of the SD MPT64 Rapid test. As shown in Fig. 1, the detection limit of the test for *M. bovis* was estimated to be 5.5×10^4 CFU/mL. The test produced a strong positive signal (purple line) for samples containing more than 5.4×10^5 CFU/mL of *M. bovis* and a clear positive signal for samples containing 1.3×10^5 CFU/mL or 2.7×10^5 CFU/mL of *M. bovis*. The test signal was weakly positive for the sample containing 6.8×10^4 CFU/mL of *M. bovis*, and weak but determinable as positive with careful examination for the sample containing 3.4×10^4 CFU/mL of *M. bovis*.

MPT64 Rapid test results for *M. bovis* isolates from animals

In total, 113 *M. bovis* isolates from animals and 52 NTM isolates were used to evaluate ability of the SD MPT64 Rapid test to identify *M. bovis* isolates from animals (Table 2). Among the *M. bovis* isolates, 109 (96.5%) had a positive SD MPT64 Rapid test result; all *M. avium* and *M. intracellulare* isolates produced negative results. Positive results were obtained for 95.5% (63/66) of the *M. bovis* isolates from cattle and 97.9% (46/47) of the isolates from deer. Sensitivity was 96.5% (95% confidence interval [CI], 91.2-99.0), specificity was 100% (95% CI, 96.7-100.0), the positive predictive value was 100.0% (95% CI, 96.7-100.0), the negative predictive value was 92.9% (95% CI, 82.7-98.0), and the kappa value was 0.945.

Discussion

The purpose of this study was to evaluate the SD MPT64 Rapid test, an ICT for *M. tuberculosis*. We assessed the ability of the test to confirm the identity of *M. bovis* isolates from animals. ICT is widely used to diagnose infectious diseases because of its simple and rapid nature. The SD MPT64 Rapid test has a high level of sensitivity and specificity for detecting *M. tuberculosis* in humans [1,9,19]. A few groups included *M. bovis* isolates in their evaluation of the SD MPT64 Rapid test and found that some *M. bovis* isolates did not produce a reaction [5,11,16]. The present study evaluated the ability of SD MPT64 Rapid test to detect *M. bovis* isolates from animals using a considerable numbers of specimens.

The sensitivity (96.5%) and specificity (100.0%) of the SD MPT64 Rapid test for *M. bovis* isolates observed in the present investigation were comparable to those of previous reports (sensitivity of 92～99% and specificity of 97～100%) for *M. tuberculosis* [3,4,19]. These results indicate that the SD MPT64 Rapid test is as specific and sensitive for *M. bovis* as it is for *M. tuberculosis*. The detection limit for *M. bovis* was similar to that reported in studies that measured the detection limit for *M. tuberculosis* [1,19].

In this investigation, four out of the 113 *M. bovis* isolates (3.5%) were nonreactive in the SD MPT64 Rapid test. This finding is comparable to data from other studies in which the MPT64 Rapid test was used to detect *M. tuberculosis* isolates from humans. The false-negative rate of the MPT64 Rapid test is 3.1% (12 out of 384) according to Hirano *et al*. [13] and 1.4% (2 out of 146) according to Wang *et al*. [23]. These reports indicate that the performance of the SD MPT64 Rapid test is similar for *M. tuberculosis* isolates from humans and *M. bovis* from animals. For three out of the four nonreactive *M. bovis* isolates, two contained a deletion between 512 and 688 base pairs (bp) in the *mpt*64 gene, and the remaining one had a point mutation at position 402 (G to A) that created a stop codon. These findings were similar to ones in a report by Hirano *et al*. [13] and imply that these changes might have produced false-negative results in the SD MPT64 Rapid test.

A mutation was identified by sequencing the *mpt*64 gene in three of the four nonreactive *M. bovis* isolates (data not shown). This might have resulted in the false-negatives obtained from the SD MPT64 Rapid test as also reported by Hirano *et al*. [13]. Because incorrect identification of *M. bovis* can have serious consequences, conventional or molecular tests to confirm the presence of this microorganism should be performed when microbiological findings are suggestive of *M. bovis* but the SD MPT64 Rapid test result is negative.

Results of this study suggest that the SD MPT64 Rapid test might be a useful method for quick identification of *M. bovis* isolates from animals. The test could possibly replace conventional confirmation assays. The greater simplicity and lower cost of the SD MPT64 Rapid test compared to other methods make this technique a good choice for confirming *M. bovis* isolates. Using this test for identifying *M. bovis* isolates from animals might contribute to the establishment of an eradication strategy for bovine tuberculosis. In conclusion, the SD MPT64 Rapid test is a simple and reliable method that may serve as a diagnostic tool for confirming the presence of *M. bovis* in animals.

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Conflict of Interest

There is no conflict of interest.

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