



ORIGINAL ARTICLE

# Evaluation and Comparison of Molecular and Conventional Diagnostic Tests for Detecting Tuberculosis in Korea, 2013

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**Abstract**

**Objectives:** A fast and accurate diagnosis is necessary to control and eliminate tuberculosis (TB). In Korea, TB continues to be a serious public health problem. In this study, diagnostic tests on clinical samples from patients suspected to have TB were performed and the sensitivity and specificity of the various techniques were compared. The main objective of the study was to compare various diagnostic tests and evaluate their sensitivity and specificity for detecting tuberculosis.

**Methods:** From January 2013 to December 2013, 170,240 clinical samples from patients suspected to have TB were tested with smear microscopy, acid-fast bacilli culture, and real-time polymerase chain reaction (PCR). The test results were compared and data were analyzed.

**Results:** A total of 8216 cultures tested positive for TB (positive detection rate, 4.8%). The contamination rate in the culture was 0.6% and the isolation rate of nontuberculous mycobacteria was 1.0%. The sensitivity and specificity of smear microscopy were 56.8% and 99.6%, respectively. The concordance rate between the solid and liquid cultures was 92.8%. *Mycobacterium* isolates were not detected in 0.4% of the cases in the liquid culture, whereas no *Mycobacterium* isolates were detected in 6.8% of the cases in the solid culture. The sensitivity and specificity of real-time PCR for the solid culture were 97.2% and 72.4%, respectively, whereas the corresponding data for the liquid culture were 93.5% and 97.2%.

**Conclusion:** The study results can be used to improve existing TB diagnosis procedure as well as for comparing the effectiveness of the assay tests used for detecting *Mycobacterium tuberculosis* isolates.

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## 1. Introduction

A fast and accurate diagnosis is necessary to control and eliminate tuberculosis (TB). To diagnose TB, clinical tests are performed by obtaining a specimen such as sputum from the person with symptoms suggestive of TB. The tests used for diagnosing TB are microscopic examination of acid-fast bacilli (AFB) smear, culture tests, nucleic acid amplification tests, and drug-susceptibility tests. Microscopic examination of sputum samples (AFB smear staining) and their culture with subsequent drug-susceptibility testing is the standard method for diagnosing TB; however, this method has insufficient sensitivity and specificity. Nucleic acid amplification is a useful supplement to improve the rapidity and accuracy of TB diagnosis [1,2]. In Korea, TB continues to be a serious public health problem. In countries with a high prevalence of TB, patients whose sputum sample tests are positive for AFB on direct microscopic examination or those displaying chest radiographic findings suggestive of TB are generally presumed to have pulmonary TB and are treated empirically with anti-TB drugs.

In Korea, laboratory data of all suspected TB patients who visit public health centers are registered in the Korean TB Information Management System (TBnet). Based on these data, diagnostic tests on clinical samples from patients suspected to have TB were performed and the sensitivity and specificity of the various techniques were compared. The study results can be used for further improving existing TB diagnosis procedures as well as for comparing the effectiveness of the assay tests used for detecting *Mycobacterium tuberculosis* isolates.

## 2. Materials and methods

### 2.1. Study patients

From January 2013 to December 2013, 170,240 clinical samples recovered from patients in whom TB was suspected were prospectively collected from the 255 regional public health centers. The samples were tested for TB in 13 regional diagnostic centers of the Korean Institute of Tuberculosis (Table 1).

### 2.2. Smear microscopy

All samples were processed and concentrated by centrifugation (3000 g, 20 minutes). The samples were tested with smear microscopy, culture (used as reference technique), and polymerase chain reaction (PCR). For smear examination, fixed preparations were stained with auramine fluorochrome and examined under a fluorescence microscope (magnification, 200×; number of fields observed, 30). Smears tested were confirmed to be positive by the Ziehl–Neelsen (ZN) staining method.

### 2.3. AFB culture and identification

The respiratory samples were digested and decontaminated using 4% sodium hydroxide and 2% *N*-acetyl-L-cysteine sodium hydroxide. The samples were then separately cultured in solid (3% Ogawa solid egg-based medium kit; KIT, Cheongju, Korea) and liquid medium (Mycobacteria Growth Indicator Tube liquid medium; Becton Dickinson, Sparks, MD, USA) systems. Liquid cultures were monitored using the automated MGIT 960 system (Becton Dickinson) for up to 6 weeks, whereas the solid medium was monitored for up to 8 weeks. If an AFB culture tests positive for *M. tuberculosis* in either the liquid or the solid medium, an immunochromatographic assay was performed to confirm the culture result (TB Ag MPT64 RAPID; Standard Diagnostics, Yongin, Korea).

### 2.4. Real-time PCR

DNA extract from each sample was amplified using the AdvanSure TB/NTM real-time PCR kit (LG Life Science, Seoul, Korea) according to the manufacturer's protocol. Based on the instructions supplied by the manufacturer,  $Ct < 35$  was considered positive for *M. tuberculosis*.

### 2.5. Data analysis

Data management and statistical analyses were performed using IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). Sensitivity and specificity of the diagnostic tests were calculated using culture results as gold standard. A patient is confirmed to have TB if mycobacterial growth was observed on the culture and the AFB smear was confirmed to be positive by the ZN staining method. Multivariate logistic regression was used to estimate associations between the covariates and the outcomes, adjusting for potential confounders that showed a significant effect in crude odds ratios at the 5% significance level.

## 3. Results

Smear microscopy and solid culture tests were performed on all the samples collected ( $n = 170,240$ ). Table 1 presents data on the number of samples tested in various regions in Korea ( $n = 13$ ) and the rate of positivity. The positive rates of culture varied from 2.1% to 24.1% (based on location). The number of samples tested was high during the period between March and May, whereas it was low in December (Figure 1). The culture positivity rates reported each month during the study period were between 4.1% and 5.7%. Liquid culture was performed for 10,607 samples and real-time PCR was performed for 5,275 samples. The smears were positive in 5,962 cases (positive detection rate, 3.5%), with 8,216 cultures testing positive for TB (positive

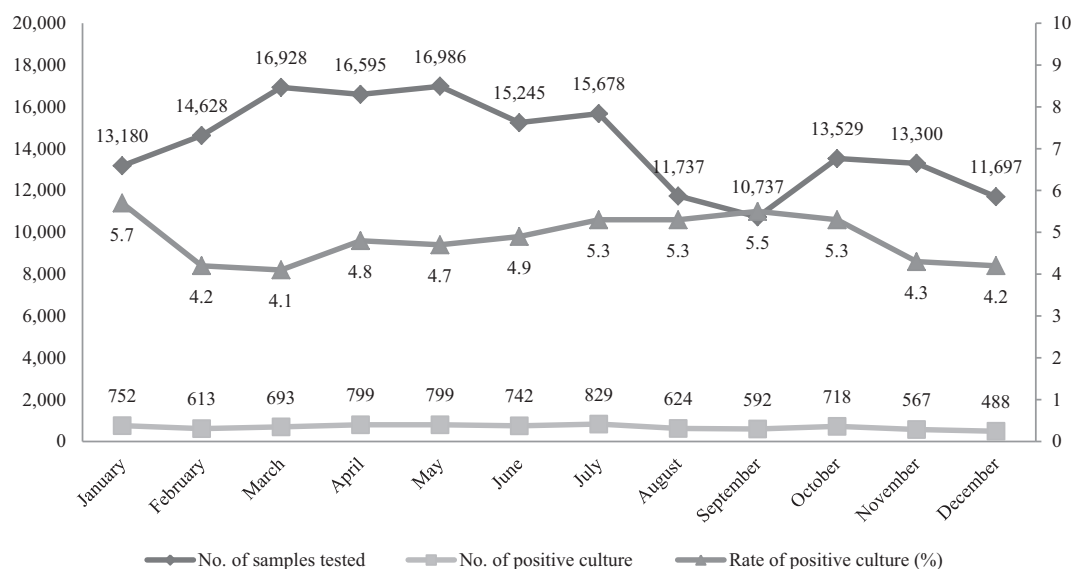
**Table 1.** Locationwise data of total samples tested and positivity rates for *Mycobacterium tuberculosis*.

Location	No. of samples tested	No. of positive cultures	Rate of positive culture (%)
Seoul	24,000	1,855	7.7
Busan	16,595	790	4.8
Incheon	9,626	483	5.0
Gyeonggi-do	26,788	1,554	5.8
Gangwon-do	13,815	295	2.1
Chungcheongbuk-do	6,230	211	3.4
Chungcheongnam-do	14,090	567	4.0
Jeollabuk-do	6,982	291	4.2
Jeollanam-do	18,319	460	2.5
Gyeongsangbuk-do	12,899	679	5.3
Gyeongsangnam-do	16,000	723	4.5
Jeju-do	4,104	117	2.9
Osong (central center)	792	191	24.1
Total	170,240	8,216	4.8

detection rate, 4.8%). The contamination rate in the cultures was 0.6% (996/170,240) and the isolation rate of nontuberculous mycobacteria (NTM) was 1.0% (1,706/170,240). The contamination rate and NTM isolation rate were 0.5% and 0.8% for the solid culture, respectively, and the corresponding rates for the liquid culture were 2.4% and 3.4%. The contamination rate and the NTM isolation rate of the liquid culture were higher than that of the solid culture. The sensitivity and specificity were calculated for each assay and the values were compared, except for contaminated cultures and NTM isolations. The sensitivity and specificity of smear microscopy were 56.8% and 99.6%, respectively. Based on solid culture results, the sensitivity and specificity of smear microscopy were 54.3% and 99.6%, respectively. However, when liquid culture was included in the

calculation, the sensitivity of the culture results improved to 62.6% and 98.6%, respectively. The concordance rate between the solid and liquid culture was 92.8%. No *Mycobacterium* isolates were detected in 0.4% and 6.8% of the cases in the liquid culture and solid culture, respectively.

Real-time PCR identified 3360 positive cases (positive detection rate, 63.7%). Compared with culture results, real-time PCR had sensitivity and specificity of 83.7% and 96.2%, respectively. The sensitivity and specificity of real-time PCR were 97.2% and 72.4% for the solid culture, and the corresponding values for the liquid culture were 93.5%, and 97.2% (Table 2). The TB detection rates of real-time PCR were 26.2% (11/42) in samples detected to be positive in solid culture but negative in liquid culture.



**Figure 1.** Monthly distribution of sample numbers tested and detections of *Mycobacterium tuberculosis* (culture results).

**Table 2.** Comparisons of smear microscopy, real-time PCR, and culture results.

	Solid culture		Liquid culture	
	Smear microscopy	Real-time PCR	Smear microscopy	Real-time PCR
No. of samples	167,538	3,692	10,144	1,583
True positive	4,633	2,185	1,524	826
False positive	684	298	105	51
True negative	158,697	1,146	7,604	649
False negative	3,524	63	911	57
Sensitivity (95% CI)	56.8% (55.7–57.9)	97.2% (96.4–97.84)	62.6% (60.6–64.5)	93.5% (91.7–95.1)
Specificity (95% CI)	99.6% (99.5–99.6)	79.4% (77.2–81.4)	98.6% (98.4–98.9)	92.7% (90.5–94.5)
<i>p</i>	<0.001	<0.001	<0.001	<0.001

CI = confidence interval; PCR = polymerase chain reaction.

### 3. Discussion

Sputum smear microscopy is the most widely available diagnostic test for pulmonary TB. A recent meta-analysis of over eight studies showed that pooled sensitivity and pooled specificity were 64% and 98%, respectively, for smear microscopy [3]. However, our results showed lower sensitivity and higher specificity than these previously reported values. It is unclear why low sensitivity values were obtained in this study. The possible explanations for low sensitivity are laboratory workload, differences in experimenter's skill, and capacity variation between laboratories. The exact reason for such a low sensitivity can be identified by collecting data over many years and analyzing them.

Although there are differences in the number of samples tested every month, the *M. tuberculosis* culture-positive rate was relatively constant. Therefore, it is obvious that the prevalence rate of TB is constant and that the various seasons do not have an impact on the prevalence of TB. When comparing the total number of samples tested regionwise, the number of samples tested was relatively higher for densely populated areas; however, in this case, the culture-positive rate differed slightly, with rates lower than the average reported in some areas. The reason of high culture-positive rate in Osong is Korea institute of Tuberculosis as central center located there. They conducted epidemiological samples suspected be infected with TB. Therefore, it is necessary to survey multilateral conditions such as quality inspection, medical referral level, and the sample state to identify these causes.

The liquid culture system showed good sensitivity and specificity in detecting the pathogen. In combination with solid culture, the sensitivity of detection is increased [4,5]. Our results also confirm the advantage of using liquid culture to detect *M. tuberculosis* isolates. Specimens of patients registered in the epidemiological and health centers were tested with the liquid culture. The smear microscopy and real-time PCR results show

that the specificity and sensitivity of the liquid culture were higher than those of the solid culture. However, further improvements are required with regard to isolation of mycobacteria and decreasing culture contamination rate by introducing necessary steps such as modifying sample pretreatment conditions before culture.

Because of the good sensitivity of real-time PCR for smear cultures, the technique can be used as a supplement for identifying positive and negative smear cultures. In a previous meta-analysis, 125 separate studies from 105 articles that reported real-time PCR results from respiratory specimens were included. The pooled sensitivity and pooled specificity in that study were found to be 85.0% and 97.0%, respectively [1]. Another study compared three molecular assays for the detection of rifampin resistance in *M. tuberculosis* and reported sensitivity for the assays to be between 84.0% and 94.0% and specificity to be 100% [2]. In this study, the specificity of real-time PCR based on solid culture was 79.4%. Therefore, there is an urgent need to identify additional techniques to decrease the number of false-positive cases detected for efficient diagnosis of TB.

### Conflicts of interest

All contributing authors declare no conflicts of interest.

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