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Comparison of the Xpert MTB/RIF and Cobas TaqMan MTB Assays for Detection of *Mycobacterium tuberculosis* in Respiratory Specimens

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The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a fully automated, cartridge-based real-time PCR assay designed to detect *Mycobacterium tuberculosis* and rifampin resistance within 2 h. The performance of the Xpert assay has been evaluated in various clinical settings. However, there are few data comparing the Xpert assay to the Cobas TaqMan MTB test (Roche Diagnostics, Basel, Switzerland), one of the most widely utilized molecular assays for *M. tuberculosis* detection. In this prospective study, 320 consecutive respiratory specimens were processed simultaneously using acid-fast bacillus (AFB) staining, mycobacterial cultures with both solid and liquid media, and the Cobas and Xpert assays. The Xpert assay was performed with direct respiratory specimens, while the Cobas assay was done with decontaminated concentrated specimens. Based on the culture as a reference method, the overall sensitivities of the Cobas and Xpert assays were 71.4% and 67.9%, respectively. When AFB smear results were taken into consideration, the sensitivities of the Cobas assay for smear-positive and -negative specimens were 87% and 54%, while those of the Xpert assay were 67% and 69%, respectively. The Cobas assay showed 100% specificity and 100% positive predictive value (PPV) regardless of smear results, while the Xpert assay showed 100% specificity and 100% PPV for smear-positive specimens but 98% specificity and 60% PPV for smear-negative specimens. In conclusion, the Xpert assay showed performance that was slightly inferior to that of the Cobas assay but seems useful for the rapid detection of *M. tuberculosis*, considering that it was performed without laborious and time-consuming decontamination and concentration procedures.

Tuberculosis (TB) is a worldwide public health concern. According to the 2012 World Health Organization (WHO) global tuberculosis report, the tuberculosis incidence rate in South Korea was 100 per 100,000 individuals in 2011, and an estimated 1,800 cases of multidrug-resistant (MDR) TB were reported in 2011 (1). Rapid diagnosis can reduce TB-related morbidity and mortality rates and the risk of person-to-person transmission. Since the introduction of molecular methods for the detection of *Mycobacterium tuberculosis*, the diagnostic time has been reduced to days, whereas diagnosis by conventional culture requires several weeks (2). In addition, several commercial systems using nucleic acid amplification detection methods have been developed.

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA), recently endorsed by the WHO for rapid TB diagnosis, allows fully automated sample preparation, amplification, and simultaneous detection of *M. tuberculosis* and rifampin resistance by real-time PCR with a single-use disposable cartridge (3). This assay can be completed within 2 h. Since the Xpert assay was introduced, it has been compared with other well-established molecular assays for *M. tuberculosis* detection using a standard culture as the reference method (4–6). However, there are few data on direct comparisons with the Cobas TaqMan MTB test (Roche Diagnostics, Basel, Switzerland), one of the most widely utilized molecular tests for *M. tuberculosis* detection.

The aim of this prospective study was to evaluate the performance of the Xpert assay compared to that of the Cobas test for the detection of *M. tuberculosis* in respiratory specimens. In addition, the ability of the Xpert assay to detect rifampin resistance was compared with that of conventional anti-tuberculosis drug susceptibility testing methods.

MATERIALS AND METHODS

Study design. A total of 320 respiratory specimens, including 254 sputum and 66 bronchoalveolar lavage (BAL) fluid samples, were prospectively collected from 311 adult patients with suspected pulmonary TB between 26 May 2011 and 2 December 2011 at a tertiary care hospital in Seoul, South Korea. Clinical data, including the medical history and radiologic and laboratory findings, were collected from each patient. All clinical specimens were examined blindly by fluorescence staining for acid-fast bacilli (AFB), by cultures with both solid and liquid media, and by the Cobas and Xpert assays. Considering that the Xpert assay was developed as an on-demand near-patient technology, we tried to evaluate its performance with unprocessed specimens without laborious and time-consuming decontamination and concentration procedures. The study protocol was approved by the institutional review board.

Processing of specimens. For the Xpert assay, 1 ml of a respiratory specimen without decontamination or concentration was used. The remaining respiratory specimens (~5 ml) were processed with 2% *N*-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH), followed by centrifugation at 3,000 × *g* for 20 min. After resuspension of the sediments in phosphate buffer, smears with fluorescence staining were prepared and examined by an experienced laboratory technologist. The mycobacterial cultures were prepared by inoculation of 500-μl and 300-μl aliquots of the decontaminated samples into a mycobacterial growth indicator tube

Received 23 May 2013 Returned for modification 18 June 2013
Accepted 11 July 2013
Published ahead of print 17 July 2013
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doi:10.1128/JCM.01335-13

TABLE 1 Comparison of the Cobas TaqMan MTB test, Xpert MTB/RIF assay, and culture for *M. tuberculosis* detection from a single sample, according to the AFB smear

AFB smear[a]	Results (n) for detection in culture-positive samples (n = 28) that were:			Results (n) for detection in culture-negative samples (n = 292) that were:			Total (n)
	Cobas$^+$/Xpert$^+$	Cobas$^+$/Xpert$^-$	Cobas$^-$/Xpert$^+$	Cobas$^-$/Xpert$^-$	Cobas$^-$/Xpert$^+$	Cobas$^-$/Xpert$^-$	
Positive	10	3	0	2	0	11	26
Negative (trace)	4	0	1	0	0	7	12
Negative	2	1	2	3	6[b]	268	282
Total	16	4	3	5	6	286	320

[a] Specimens with grades 1 to 4 for AFB smears were regarded as smear positive.
[b] Nontuberculosis mycobacterial growth was observed in 2 cases.

(MGIT 960 system; Becton, Dickinson, Sparks, MD) and 3% Ogawa agar (Shinyang, Seoul, South Korea), respectively.

Mycobacterial stains and cultures. Acid-fast staining of decontaminated respiratory specimens was performed with an auramine-rhodamine fluorescent stain, followed by confirmation with Ziehl-Neelsen staining. The results were graded according to U.S. Centers for Disease Control and Prevention (CDC) recommendations. Specimens with grades 1 to 4 for the AFB smears were defined as smear positive (7). For mycobacterial cultures, both the liquid and the solid media were incubated for 6 weeks. Any positive cultures were subjected to AFB staining to confirm the presence of AFB and to exclude contamination. In addition, positive cultures in liquid media were confirmed by the presence of cord formation and by MPT64 antigen testing (SD Bioline TB Ag MPT64 Rapid; Standard Diagnostics Inc., Yongin-si, Gyeonggi-do, South Korea). If any of these tests gave negative results, an *rpoB* gene-specific PCR test using the MTB-ID V3 kit (YD Diagnostics), which has the ability to differentiate between *M. tuberculosis* and nontuberculous mycobacteria (NTM), was performed. Positive cultures found only in solid media were also confirmed by conventional PCR testing.

Detection of rifampin resistance. Rifampin resistance as detected by the Xpert assay was compared with the results from the MGIT 960 system and absolute concentration (AC) method with Löwenstein-Jensen medium. All *M. tuberculosis* isolates were tested for resistance to rifampin using the MGIT 960 system and were also referred to the Korean Institute of Tuberculosis for conventional drug susceptibility testing (DST) using the AC method (8). Critical concentrations for rifampin resistance were 1.0 μg/ml and 40 μg/ml in the MGIT 960 system and in the AC method, respectively.

Cobas TaqMan MTB and Xpert MTB/RIF assays. The Cobas and Xpert assays were conducted according to the manufacturers' instructions, as described previously (3, 9).

Statistical analysis. The sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated based on the results of concurrently performed cultures.

RESULTS

A total of 320 consecutive respiratory specimens were tested by AFB staining, cultures, and the Cobas and Xpert assays. As shown in Table 1, 26 (8.1%) were smear positive, while 294 were smear negative, including 12 trace and 282 negative results. Twenty-eight out of 320 specimens (8.8%) showed positive cultures for *M. tuberculosis*. The Cobas and Xpert assays yielded concordant results in 302 out of 320 specimens (94.4%) and detected *M. tuberculosis* in 20 (6.3%) and 25 (7.8%) of all specimens, respectively. Neither the Cobas nor the Xpert assay was able to detect *M. tuberculosis* in five culture-positive cases, and there were six positive cases by the Xpert assay but negative by culture and by the Cobas assay.

Based on mycobacterial culture as the reference method, the overall sensitivity, specificity, PPV, and NPV (95% confidence interval [CI]) of the Cobas assay were 71.4% (51.3 to 86.8%), 100% (98.7 to 100%), 100% (83.2 to 100.0%), and 97.3% (94.8 to 98.8%), respectively, while those of the Xpert assay were 67.9% (47.7 to 84.1%), 98.0% (95.6 to 99.2%), 76.0% (54.9 to 90.6%), and 97.7% (94.3 to 98.6%), respectively (Table 2). The Cobas assay gave better results than the Xpert assay for smear-positive specimens, while the Xpert assay yielded better sensitivity than the Cobas assay for smear-negative specimens.

Among the 19 positive cases of *M. tuberculosis* detected by both mycobacterial culture and the Xpert assay, two cases (10.5%) showed rifampin resistance by the Xpert assay, which was concordant with the results by the MGIT 960 system and the AC method. Among 17 cases without rifampin resistance as determined by the Xpert assay, only one case showed a discordant result. This case was susceptible as determined by the MGIT 960 system but was

TABLE 2 Performances of the Cobas TaqMan MTB and Xpert MTB/RIF assays, with *M. tuberculosis* culture results as the gold standard

Assay	Smear result[a]	Performance (% [95% CI])[b]			
		Sensitivity	Specificity	PPV	NPV
Cobas	Positive	86.7 (59.6–98.3)	100 (71.5–100)	100 (75.3–100)	84.6 (54.6–98.1)
	Negative	53.9 (25.1–80.8)	100 (98.7–100)	100 (59.0–100)	97.9 (95.5–99.2)
	All	71.4 (51.3–86.8)	100 (98.7–100)	100 (83.2–100)	97.3 (94.8–98.8)
Xpert	Positive	66.7 (38.4–88.2)	100 (71.5–100)	100 (69.2–100)	68.8 (41.3–89.0)
	Negative	69.2 (38.6–90.9)	97.9 (95.4–99.2)	60.0 (32.3–83.7)	98.6 (96.4–99.6)
	All	67.9 (47.7–84.1)	98.0 (95.6–99.2)	76.0 (54.9–90.6)	97.7 (94.3–98.6)

[a] Specimens with grades 1 to 4 for AFB smear were regarded as smear positive.
[b] CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

resistant according to the AC method. Therefore, the concordance rates of rifampin susceptibility by Xpert assays were 100% with the MGIT 960 system and 94.7% (18/19) with the AC method.

DISCUSSION

Several studies have evaluated the performance of the Xpert assay in comparison with those of other molecular assays for either respiratory or nonrespiratory specimens (4–6, 10–12). In those studies, the Xpert assay showed performance comparable with that of other molecular assays for respiratory specimens with sensitivities reported between 79 and 93%, while those of other assays ranged from 76 to 96.8%. In the present study, the overall sensitivities of the Xpert and Cobas assays were 68% and 71%, respectively, which are lower than those reported in other studies.

Interestingly, the Xpert assay showed better sensitivity than the Cobas assay for smear-negative specimens. In addition, there were six positive cases by the Xpert assay that were negative by both culture and the Cobas assay. These results might be attributable to the decontamination and concentration steps. The Xpert assay was performed with direct specimens, while the Cobas assay used decontaminated and concentrated sediments, which may have resulted in a loss of *M. tuberculosis* during the process. This factor could explain why the sensitivity of the Xpert assay is lower than the sensitivities in the previous reports in which decontaminated samples were used (4, 5, 10, 11). Alternatively, the Cobas assay revealed better sensitivity than that of the Xpert assay in smear-positive specimens. It is not certain why the Xpert assay showed a sensitivity inferior to that of the Cobas assay in these smear-positive specimens, but a possible explanation is the presence of a PCR inhibitor in the direct specimens which could have been removed by the decontamination procedure.

The detection of rifampin resistance by the Xpert assay could not be fully evaluated because there were only three cases with rifampin resistance by any of the three assays (the Xpert assay, the MGIT 960 system, and the AC method). Nevertheless, the Xpert assay showed 100% and 94.7% concordance rates of rifampin susceptibility with the MGIT 960 system and the AC method, respectively.

In comparison to the Cobas assay, which requires laborious decontamination and concentration steps, the fully automated and closed system of the Xpert assay may be advantageous for reducing hands-on time. In conclusion, the performance of the Xpert assay was slightly inferior to that of the Cobas assay but is useful for the rapid detection of *M. tuberculosis*, considering that the Xpert assay may be performed without laborious and time-consuming decontamination and concentration procedures.

ACKNOWLEDGMENT

This study was supported by Hoil Biomed (Seoul, South Korea), which had no involvement in the study design, data interpretation, or writing of the manuscript.

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