Validation of the Xpert MTB/RIF Assay for Testing Bronchoalveolar Lavage

California Department of Public Health (CDPH)

Marisabel Etter, Shou-Yean Grace Lin, Wendy Cheung, Lourdes Seli, Terry Weber, and Edward Desmond

Microbial Diseases Laboratory, California Department of Public Health, Richmond, CA

ABSTRACT (modified)

Background

The Xpert MTB/RIF (Xpert) assay has FDA market authorization for testing raw or processed (decontaminated and concentrated) sputa. In some clinical situations, testing of bronchoalveolar lavage (BAL) specimens is needed. One application is in tuberculosis screening of deceased organ donors from high risk environments.

Objective

The purpose of this study was to validate the use of the Xpert assay for testing processed BAL specimens.

Materials and Methods

We included 49 **processed BAL specimens** (referred to as **sediments**), which were smear- and culture-negative for *Mycobacterium tuberculosis* complex (MTBC). Because of difficulties to obtain MTBC-positive BAL specimens with diverse *rpoB* mutations, six MTBC strains (one wild-type and 5 strains with different *rpoB* mutations) were used for spiking 40 sediments. MTBC cell suspensions equivalent to a 0.5 McFarland standard (McF) were prepared and further 10-fold dilutions were made for spiking. Fluorescent acid-fast staining and the Xpert assay were performed. For evaluation of specificity, 9 sediments with no MTBC spiked were tested.

Results

The Xpert assay detected MTBC in 7 of 7 (100%) smear-positive sediments where the MTBC concentration was 10^{-3} of a 0.5 McF. MTBC was also detected in 15 of 15 (100%) sediments where the MTBC concentration was 10^{-4} of a 0.5 McF and the smear was negative in 13 of 15 (87%) sediments. In another set of 15 smear-negative sediments where the MTBC concentration was 10^{-5} of a 0.5 McF, MTBC was detected in 11 of 15 (73%) sediments. The final 3 smear-negative sediments where the MTBC concentration was 10^{-6} of a 0.5 McF, MTBC was detected in 1 of 3 (33%) sediments. The limit of detection was determined to be at the MTBC concentration of 10^{-4} of a 0.5 McF, which is similar to that observed in our previous study testing sputum sediments (unpublished data). The Xpert assay also correctly detected the absence or presence of *rpoB* mutations by expected probes in 34 of 34 (100%) sediments where MTBC was detected.

Conclusions

With a limited sample size and using spiked clinical BAL sediments, our results indicate the Xpert can accurately detect MTBC and rpoB mutations. Further studies using clinical BAL sediments from infected patients are recommended.

MATERIALS and METHODS

1. We used 49 smear- and culture- negative BAL sediment specimens for the study; 40 were spiked and 9 were not spiked.
2. Five clinical isolates with various *rpoB* mutations (Table 1) within the 81 bp *rpoB* core region and a wildtype strain, H37Rv (ATCC 27294), were used for spiking BAL sediments.
3. Several 10-fold dilutions of cell suspensions equivalent to McFarland 0.5 from these 6 strains were prepared for spiking. Final dilutions in the spiked solution for testing were 10^{-3} to 10^{-6} of McFarland 0.5. Table 2 shows the estimated CFUs.
4. For each spiked BAL sample, 0.5 mL was used for the Xpert testing and 0.05 mL for making a smear. Fluorescent AFB staining by auramine rhodamine was performed (Table 3).

Table 1. Strains for spiking

Strain	Mutation (codon)	Xpert Probe Detecting Mutation
H37Rv	None (wildtype)	None
Isolate 1	Deletion (508-509)	A
Isolate 2	TTT insertion (after 514)	B
Isolate 3	TCG>ACG (522)	C
Isolate 4	CAC>TAC (526)	D
Isolate 5	TCG>TTG (531)	E

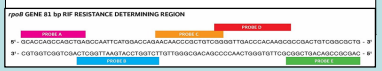

Table 2. Concentrations & CFUs

Final Dilution of 0.5 McFarland	Estimated CFU/mL
Undiluted	5 - 10 x 10^6
10^{-3}	5 - 10 x 10^3
10^{-4}	5 - 10 x 10^2
10^{-5}	50 - 100
10^{-6}	5 - 10

Table 3. Spiking & test volumes

Vol. of BAL Specimen (mL)	Vol. of Cell Suspension for Spiking (mL)	Vol. Run in Xpert (mL)	Vol. for Smear (mL)
0.9	0.1	0.5	0.05

RESULTS

Table 4. Xpert results & MTBC detection rate

Dilution of 0.5 McFarland (Smear)	No. Tested	No. Smear + (%)	No. MTBC Detected by Xpert (%)
10^{-3} (1+)	7	7 (100)	7 (100)
10^{-4} (1+/-)	15	2 (13.3)	15 (100)
10^{-5} (Negative)	15	0 (0)	11 (73.3)
10^{-6} (Negative)	3	0 (0)	1 (33.3)
Non-spiked	9	0 (0)	0 (0)

Table 5. *rpoB* mutations detected in 34 samples

MTBC Strain	Probe	No. Detected by Xpert (dilution)	No. Correctly Identified by Xpert (%)
H37Rv (wt)	None	1 (10^{-3}) 2 (10^{-4})	3 (100)
Isolate 1	A	1 (10^{-3}) 2 (10^{-4}) 1 (10^{-5})	4 (100)
Isolate 2	B	2 (10^{-3}) 3 (10^{-4}) 3 (10^{-5})	8 (100)
Isolate 3	C	2 (10^{-3}) 3 (10^{-4}) 2 (10^{-5})	7 (100)
Isolate 4	D	1 (10^{-3}) 3 (10^{-4}) 2 (10^{-5})	6 (100)
Isolate 5	E	2 (10^{-4}) 3 (10^{-5}) 1 (10^{-6})	6 (100)

1. Sensitivity of detection for MTBC and rpoB mutations was evaluated for various dilutions of MTBC (Table 4).
   - At 10^{-3} (= 5,000-10,000 CFU/mL), all 7 sediments were smear-positive, Xpert had 100% sensitivity.
   - At 10^{-4} (= 500-1,000 CFU/mL), 2/15 sediments (13.3%) were smear-positive, Xpert had 100% sensitivity.
   - At 10^{-5} and 10^{-6} of McF 0.5 (below 100 colonies/mL), all 18 sediments were smear-negative, Xpert had 67% sensitivity. (See discussion)
2. Specificity (Table 4)
   - Nine smear and culture-negative sediments were Xpert-negative for MTBC, demonstrating 100% specificity.

DISCUSSION

1. TB transmission from deceased organ donors has been documented. The need to accurately detect TB in a timely way to prevent such transmission prompted this study. BAL becomes a specimen of choice due to unavailability of sputa. Because of the low incidence of smear-positive BAL in organ donors, we decided to spike BAL sediments using various levels of low MTBC concentrations yielding smear results of ≤ 1+ for the evaluation of Xpert performance.
2. Although Xpert is more sensitive than AFB smear, the sensitivity for testing smear-negative samples is 55-70%[1]. This study indicates the MTBC detection sensitivity is 100% at the first 10-fold dilution of smear 1+ with estimated CFU of 500-1000/mL. Xpert can also detect MTBC in ~70% of sediments with CFU level of100/mL.
3. Limitations of this study: 1) Spiked BAL samples, not samples from infected patients, were used. The variability with the number of MTBC cells for spiking may not be perfectly controlled, which was evidenced by MTBC detection from a specimen with estimated 10 CFU/mL. 2) The total number of specimens studied was small especially when we had to divide them into several groups with different cell concentrations for spiking.

CONCLUSION

With a small sample size and spiked clinical BAL sediments, our results indicate that the Xpert accurately detected MTBC and presence or absence of *rpoB* mutations. Further studies using clinical BAL sediments from infected patients are recommended.

REFERENCE

1. MMWR, 2/27/15. Revised device labeling for Xpert MTB/RIF assay for detecting *Mycobacterium tuberculosis* complex

Contact: grace.lin@cdph.ca.gov