The Cepheid Xpert® MTB/RIF assay is a rapid Nucleic Acid Amplification Test (NAAT) that will detect Mycobacterium tuberculosis (MTB) and detect of Rifampin (Rif) resistance (indicative of Mycobacterium resistant to Rifampin or MDR tuberculosis). Rapid tuberculosis (TB) diagnosis can facilitate initiation of TB therapy, minimize unnecessary use of antibiotics, assess need for airborne isolation (A.I.), and prioritize contact investigations. Routinely, respiratory specimens are digested and NAAT is performed the following day on the concentrate.

The Xpert MTB/RIF package insert for the FDA-approved assay describes performance on direct and concentrated specimens. However, most published studies are based on concentrated specimens.

Methods

The Orange County Public Health Laboratory (OCPL) and University of California Irvine Medical Center (UCI) performed NAAT on direct and concentrated sputum specimens from patients using the Xpert MTB/RIF assay: 

1. Specimen: Routine sputum specimens were tested from OCPL (1/2018-12/2019) and from UCI (11/2017-3/2018).

2. Inclusion Criteria:
   - Suspect TB patient, age 18+, minimum 7 ml volume collected, AFB smear, NAAT, and final culture results available.
   - Specimen were excluded from analysis if patient had been on TB treatment for 23 days prior to collection.

3. Procedures:
   - An aliquot (minimum 1ml) of each specimen was transferred to a separate tube for direct NAAT and direct AFB smear (unprocessed).
   - Routine AFB culture workup was performed as follows: Sputum specimens were processed using the NALC NaOH method (CDC) and inoculated to Löwenstein-Jensen slants (LJ) and BACTEC 1230 (BD). NAAT was performed using the Xpert MTB/RIF assay. AFB smears were prepared using the Fluorochrome stain. MTB was identified from culture by Positive and culture resistance was determined by phenotypic drug susceptibility testing (DST) using BACTEC MGIT 960 liquid media (BD) on MTB culture. DST was performed on a different isolate for some patients, as necessary.
   - NAAT (Xpert MTB/RIF) was performed on both direct and concentrated specimens according to the manufacturer's instructions (Cepheid). NAAT results from direct specimens were not reported to patient charts since in-house validation had not been completed.
   - Sensitivity: NAAT (direct and concentrated) was performed on all patients for whom airborne isolation was recommended based on initial direct specimens.
   - For specimens with negative NAAT that grew MTB (19% of concentrated specimens), one aliquot was transferred to a separate tube for direct NAAT and direct AFB smear (unprocessed).
   - One patient converted from Rif susceptible to Rif resistant after 3-4 months, the Xpert MTB/RIF and DST Rif results were both Rif susceptible, initially however, after 3-4 months both converted.
   - For specimens with negative NAAT that grew MTB (19% of concentrated and 26% of direct tests), all had TTD >9 days (mean: 29-31 days) indicating low transmissibility.

Of 375 specimens collected, there were 362 eligible specimens (331 from OCPL and 31 from UCI). Eighteen total number of specimens enrolled was limited by ability to get adequate volume of sputum to meet inclusion criteria.

- Exclusions - 13 ineligible specimens from OCPL:
  - 7 had overgrown cultures - all of these were NAAT and NAAT positive specimens, were digested and NAAT performed on the concentrate.
  - 1 had an instrument error on the direct NAAT.
  - 5 had TB treatment prior to collection - Class B immigrant with recent TB history and treatment per chart review. All of these were culture-negative.

- Forty-two cultures were positive for MTB (12%). No Rif-resistant specimens were encountered during the per-protocol testing.

- OCPL had 38 MTB culture-positive specimens
  - 35 were MTB culture-positive
  - One patient converted from Rif susceptible to Rif resistant after 3-4 months, the Xpert MTB/RIF and DST Rif results were both Rif susceptible initially however, after 3-4 months both converted.

- TAT for NAAT on direct specimens was approx. 1 day. 2 days for routine concentrations.

- TAT for NAAT on concentrated specimens was 1 day sooner for specimens tested at UCI Medical Center than those tested at OCPL. This was likely due to the fact that OCPL does not process on the weekends or holidays as well as specimen transport time.

- For specimens with positive NAAT that grow MTB (19% of concentrated and 26% of direct tests), all had TTD >9 days (mean: 29-31 days) indicating low transmissibility.

Results

Table 1. NAAT results and AFB grade for MTB culture-positive specimens

<table>
<thead>
<tr>
<th>NAAT Type</th>
<th>NAAT Result</th>
<th>Specimens (n=31)</th>
<th>Rifresistant (n=2)</th>
<th>Rifsensitive (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (NAAT)</td>
<td>11 (100)</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Positive</td>
<td>20 (185)</td>
<td>1.0%</td>
<td>1.0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Positive (Rif+)</td>
<td>10 (91)</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 2. NAAT result and AFB grade for MTB culture-positive specimens

<table>
<thead>
<tr>
<th>NAAT Result</th>
<th>AFB Grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT Negative</td>
<td>AFB Negative</td>
<td>11 (100)</td>
</tr>
<tr>
<td>NAAT Positive</td>
<td>AFB Positive</td>
<td>10 (91)</td>
</tr>
<tr>
<td>NAAT Negative</td>
<td>AFB Positive</td>
<td>20 (185)</td>
</tr>
<tr>
<td>NAAT Positive</td>
<td>AFB Negative</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 3. Turnaround time (TAT) calculated from date received in the laboratory

<table>
<thead>
<tr>
<th>NAAT Type</th>
<th>TAT (ds)</th>
<th>Mean</th>
<th>Median</th>
<th>Average** (days)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT Direct</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>0.0</td>
</tr>
<tr>
<td>NAAT Concentrated</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NAAT Positive</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NAAT Negative</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Year</th>
<th>2015-17</th>
<th>No. of Patients</th>
<th>Total Isolation Days</th>
<th>Mean (SD) (days)</th>
<th>A.I. Days (SD) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT Direct</td>
<td>621</td>
<td>23</td>
<td>350</td>
<td>549</td>
<td>360</td>
</tr>
<tr>
<td>NAAT Concentrated</td>
<td>82</td>
<td>25</td>
<td>350</td>
<td>549</td>
<td>360</td>
</tr>
<tr>
<td>NAAT Positive</td>
<td>111</td>
<td>11</td>
<td>14</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>NAAT Negative</td>
<td>111</td>
<td>2</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Discussion

In the US, the Cepheid Xpert MTB/RIF NAAT assay is a valuable tool for rapid diagnosis of pulmonary tuberculosis in the United States. The Cepheid Xpert MTB/RIF assay is used for airborne isolation. However, most published studies are based on concentrated specimens.

- Direct specimens have not been studied in parallel with concentrated specimens.
- The sensitivity of the NAAT varies among studies, especially for AFB-negative specimens.
- The finding of potential transmission from AFB-negative cases is of concern (5).

Our study addresses these issues and includes time-to-detection data (4, 5) and sensitivity for AFB-negative specimens of NAAT on direct (16/27 (99%)) and concentrated (7/11 (64%)) specimens. All such specimens needed 2 days to grow, implying low sensitivity of the test. Our data thus provides support for the policy of allowing discontinuation of airborne isolation for APPROPRIATE patients (Fig. 3) after a negative AFB and NAAT on the first good quality specimen. Our current study showed no overall statistical difference between the sensitivity of the NAAT on direct vs. concentrated specimens. Thus, real-time application of the direct NAAT would have saved an estimated 200 to 600 airborne isolation bed-days per year.

We do not have an explanation for the lower sensitivity of the NAAT compared to Cepheid data, especially for AFB-negative specimens (1). Nevertheless, our data supports our clinical policy protocol for direct as well as concentrated specimens. The NTCA guidelines (8) require a negative NAAT test before stopping airborne isolation would increase lab and bed day costs.

Conclusions

1. There was no significant difference between NAAT sensitivity on direct specimens vs. routine concentrates (74% vs. 81%, respectively, p=0.25).
2. For AFB-negative, NAAT-negative (direct or concentrated) specimens that grew MTB, the TTD was 0, indicating low likelihood of transmission.
3. One negative direct NAAT result can facilitate safely discontinuing A.I. for appropriate hospitalized patients, saving an estimated 200-600 A.I. days per year.

References


Acknowledgements

We would like to acknowledge the staff at Orange County Public Health Laboratory Department, UCI Medical Center, Orange County Health Agency Tuberculosis Control, and the Administration of Orange County Health Care Agency, Orange, California.