ONE MONTH TO ONE DAY? CAN WE REALLY REDUCE THE DETECTION TIME OF TUBERCUSOSIS THAT MUCH?

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Introduction

- *Mycobacterium tuberculosis* (Mtb) remains important to clinicians and patients because it can be highly infectious and challenging to diagnose. The CDC reports that there were 10,528 cases of TB in the U.S. in 2011, and 529 deaths due to TB in 2009 (1). The rapid diagnosis of Mtb is essential for early disease management.
- Equally important for clinicians is the exclusion of Mtb from the differential diagnosis. Per the University of Missouri Infection Control Manual, precautions for a patient with suspected infectious pulmonary tuberculosis include a private room with a closed door, negative air pressure, and a “stop sign alert on the door” (Figure 1). This is inconvenient for patients and care providers and adds expense to the hospital stay.
- Patients suspected of having TB should have 3 sputum samples tested with acid fast bacilli smears and culture, and one sample should undergo nucleic acid amplification testing (2). Unfortunately, standard culture techniques can take a month to detect Mtb. Here we present a commercially available assay validated in our lab that reduces detection time to less than two hours.

Isolation Room

Figure 1: Private room with negative air pressure at the University of Missouri Hospital

Materials & Methods

- The Cepheid GeneXpert MTB/RIF assay detects the target sequences in pulmonary specimens using real-time PCR. Workflow is highly efficient with approximately 2 minutes of hands-on time. The cartridge (figure 2) comes pre-loaded with all necessary reagents, and once the sample is added, the cartridge is simply loaded onto the instrument (figure 3). Probes detect Mtb DNA as well as rifampicin resistance, a marker of multidrug resistance.
- We validated this non FDA-approved PCR test for daily use in our clinical laboratory to offer superior sensitivity and specificity to the currently approved nucleic acid amplified technique.

Figure 2

Figure 3

- 111 patient respiratory specimens were digested, decontaminated, and concentrated by the method of Kent and Kubica (3) and cultured for Mycobacteria. Aliquots of digested specimens were frozen below -60ºC at the Missouri State Health Lab, Jefferson City, MO.
- Additionally 100 liquid mycobacterial growth indicator tubes (MGIT) with various mycobacterial growths were evaluated by PCR after similar digestion at UMHC.

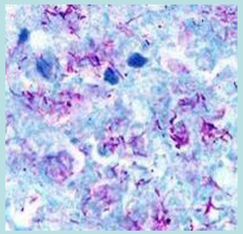
Results

- Respiratory sample results for 37 smear positive, Mtb culture positive samples were PCR positive.
- 29 smear positive samples that grew non-Mtb mycobacteria were PCR negative.
- Two smear negative, Mtb culture positive specimens were PCR positive.
- Only 21of 41 samples from positive Mtb patients receiving antitubercular therapy were PCR positive. 100 Mtb positive and negative MGIT tubes showed 100% correlation with PCR results.

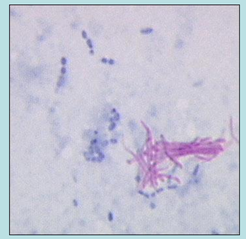
•Of 100 (MGIT) samples tested, 85 PCR negative were found to be no growth or non-Mtb types.

•15 PCR positives were found to be on Mtb known patients.

•Overall 100% correlation!



Smear Positive for AFB, not Mtb



Smear Positive for AFB, Mtb

Discussion and Conclusion

- Excluding patients receiving Mtb antimicrobial therapy, validation results show 100% sensitivity and specificity. Patients who are receiving therapy may continue to harbor tuberculosis DNA even though they are not infected with viable organisms. Thus, a positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB and/or Rifampicin resistance.
- We have now detected two Mtb positives in 441 patient specimens and markedly reduced the detection time.
- There are a number of benefits to the rapid detection of Mtb and rifampicin resistance.

•(1) **Improved patient care**. Patients can be treated quickly with appropriate therapy when the test is positive, and perhaps as importantly, not treated for Mtb when the PCR is negative. Also, when the test is more widely accepted, patients will not need to remain in respiratory isolation when they have negative PCR.

•(2) **Cost savings**. In particular, patients and insurance companies may be spared the extra expense of a private room with negative air pressure.

•(3) **Public safety**. In the case of an epidemic, rapid and accurate detection of Mtb will be invaluable.

References

1. Centers for Disease Control. Trends in Tuberculosis, 2011. http://www.cdc.gov/tb/publications/factsheets/statistics/TBTrends.htm
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3. Kent, P. & Kubica, G. (1985). Public Health Microbiology: a Guide for the Level III Laboratory. Atlanta: Centers for Disease Control.