Adaptation of the use of the GeneXpert Assay for the detection of Rifampin-resistant *Mycobacterium tuberculosis* Complex in culture

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Objective

To detect Rifampin-resistant *Mycobacterium tuberculosis* in culture using the Cepheid GeneXpert assay. This approach provides a rapid and cost-effective means for the diagnosis and treatment of patients.

Methods

1) - Sample Preparation: A multi-step sample preparation was developed to ensure that no cultures were viable when tested using the GeneXpert assay. Cultures were dispersed in TE buffer and heated for 30 minutes at 100°C and then quickly frozen at -20°C for 30 minutes. Finally, 2ML of the sample reagent provided in the GeneXpert assay kit was added to 20ul from the specimen and incubated at room temperature for 15 minutes. To test if any organisms remained viable following the sample preparation, 200ul of each sample was inoculated on solid media and monitored for growth for 6 weeks. None of the cultures grew (see Table 1).

2) - GeneXpert Assay: A total of 60 cultures were prepared as described above and analyzed using the GeneXpert assay following methods described by the manufacturer. (See Table 1) The cultures were previously tested by conventional methods which verified that 26 were rifampin-sensitive *Mycobacterium tuberculosis*, 5 were rifampin-resistant *Mycobacterium tuberculosis*, and 29 were non-mycobacterium (NTM) cultures.

Results

A total of 31 *Mycobacterium tuberculosis* identified using conventional means were correctly identified using the GeneXpert assay. Of these cultures, 26 were rifampin-sensitive and 5 were rifampin-resistant. The GeneXpert assay correctly identified all of these cultures. Finally, the GeneXpert assay failed to detect *Mycobacterium tuberculosis* in all (29/29) of the NTM cultures.

Table 1: Identification of Mycobacterium cultures using the Cepheid GeneXpert assay:

Known Cultures Tested	# of cultures	# of cultures correctly identified by the GeneXpert	# of viable cultures after sample preparation
M. tuberculosis rifampin-sensitive	26	26/26	0/26
M. tuberculosis rifampin-resistant	5	5/5	0/5
NTM	29	29/29	0/29

Conclusions

The results of this study show that we have successfully adapted the use of the Cepheid GeneXpert assay for the detection of rifampin-sensitive and rifampin-resistant *Mycobacterium tuberculosis* in culture. This test algorithm reduces the time for culture identification from 48 hours to 3 hours and saves money that would otherwise be used for test employing additional assays and the cost to maintain other instrumentation other than the GeneXpert system. Also, this approach reduces staff time and it does not require molecular expertise for the detection of rifampin resistance. An additional benefit from testing isolates on solid culture media is that you can compare colony morphology with the GeneXpert results.

References

1) - GenXpert System Implementation Guide. 301-0018. Rev A.1. January 2012.
2) - Suffys PN, Rocha A, Oliveira M, et al. Rapid identification of Mycobacteria to the species level using the INNO-LiPA Mycobacteria, a reverse hybridization assay. J. Clin.Micro. 39:4477-4482, 2001.