DIAGNOSIS OF PULMONARY TUBERCULOSIS USING COLORIMETRIC METHODS IN HIGH BURDEN RESOURCE LIMITED COUNTRIES.

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BACKGROUND

- The traditional methods for TB diagnostic test sensitivity or require a long time for the detection and identification of M. tuberculosis and are often not suitable to the laboratory conditions of developing countries.
- Colorimetric methods rely on the detection of live bacteria through either enzymatic activity inhibition, or their ability to reduce an oxidation-reduction indicator.
- These methods are fast, simple and easy to implement in a culture laboratory.
- They have been developed and evaluated for detection of MDR-TB but their potential for the diagnosis of TB had not been investigated.

This study evaluated the performance and the feasibility of the Nitrate Reductase Assay (NRA) and Resazurin Tube Assay (RETA) for the detection of M. tuberculosis complex from sputum samples from adult pulmonary TB suspects in a high HIV prevalence setting, using LA and MGIT culture as gold standard. Analysis was stratified by patients' HIV status and sputum microscopy results.

Study site: Mbarara, Uganda.

METHODS

A total of 690 adults patients with cough for more than 2 weeks were enrolled from the OPD and HIV clinic of Mbarara Regional Referral Hospital between the months of April 2010 and June 2011.

- One on spot and one morning specimen were collected. The best quality specimen was used for evaluation of the culture methods.
- MGI (1 tube) and LJ (2 tubes) for the reference standard
- NRA (4 tubes), NRA-TA (RTA), RTA-PNB for the colorimetric methods
- Revelation of the NRA and RTA was performed after 10, 14, 16 and 28 days of incubation.
- If the NRA or RETA tube turned positive, then the NRA-PNB or RETA-PNB tube was revealed, in order to differentiate M. tuberculosis complex (MTBC) and non-tuberculosis mycobacteria (NTM).
- For the reference standard, differentiation between MTBC and NTM from growth on LJ or MGIT media was done using the rapid test iSt Biome MTP164.

Study Profile

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Feasibility of the method

The time for positivity was 10 days (10-18) for both colorimetric methods, 7 (4.5-11) days for MGIT and 25 (21-35) days for LJ.

The quality of the media at the time of identification was good:

- Only 1 invalid result
- 9% and 4% of contaminated tubes in NRA and RETA, respectively

The results were stored at room temperature.

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SUMMARY

- The sensitivity of all colorimetric methods was moderate (83 to 86%) among all TB suspects and low (56 to 64%) among smear-negative TB suspects.
- The RETA method had a good specificity (-99%).
- RETA Sensitivity (84.8% vs 85.8%) and Specificity (98.2% vs 99.8%) were similar to the sensitivity of LJ using MGIT or standard culture.
- The NRA method had low specificity for a culture method (90%), which was only slightly increased by the use of NRA-PNB for the differentiation between MTBC and NTM.
- The turnaround time for a final result was similar for the colorimetric methods and MGIT and lower than for the RTA method.
- Inadequacy for a rapid based culture the contamination rate with RETA was lower in our study setting than the one using LJ.
- The colorimetric methods required more manipulations than the traditional culture methods but they were considered feasible and easy to read.
- Study limitations included the high contamination rate of LJ and MGIT culture media, as well as a lower positive rate than expected, leading to a low number of positive specimens and wide confidence intervals for the stratified analysis.

CONCLUSION

- The NRA and RETA assays: too low specificity to be recommended for the detection of smear-negative TB.
- The RETA assay:
- High specificity and moderate sensitivity; potentially suitable for detection of MTB.
- Sensitivity is similar between the two RTA targets, and can be evaluated as larger scale products.
- Inexpensive, fast but require additional manipulation on positive specimens that need to be confirmed for positivity.
- Although simple, the colorimetric methods remain culture-based methods requiring the same infrastructure and safety conditions than conventional culture methods.
- No advantage compared to Xpert MTB/RIF assay for detection of TB in settings without culture capacity.

Acknowledgements

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