Preliminary study comparing smear and standard tuberculosis culture to GeneXpert MTB/Rif Ct categories in pulmonary and extrapulmonary samples in a local laboratory setting

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Introduction and Objectives: Tuberculosis is an airborne infection caused by *Mycobacterium tuberculosis* complex (MTBC) with pulmonary and extra-pulmonary forms. The recommended Xpert MTB/Rif (Cepheid, USA) assay uses four *RpoB* and two *IS6110/IS1081* gene probes to simultaneously detect MTBC and rifampicin resistance. Results are categorised based on *RpoB* cycle threshold (Ct) values: Very Low (Ct > 28.01), Low (Ct 22.01-28), Medium (Ct 16-22), and High (Ct < 16). The relationship between smear, culture with Ct value categories in pulmonary (Pul) and extrapulmonary (EP) samples were analysed to evaluate the standard methods.

Methods: A total of 39 Xpert positive [Pul (n=29), EP (n=10)] samples were collected from the Chest Clinic, Bogambara, from January to March 2024. Decontaminated samples (Pul by modified Petroff's method and highly contaminated samples with 0.5M H2SO4) or sterile samples were directly cultured (Lowenstein Jensen) and examined weekly. Sample smears were stained with Ziehl-Neilsen (ZN) stain. MTBC colonies were confirmed using ZN stain and *Hsp65* and *GyrB* conventional PCR. Positivity of smear/ culture of each Ct category and Spearman correlation of Ct category and week of visible growth was assessed. (GraphPad Prism 10.2.3 Software).

Results: Smear and culture positivity rates were 33.3% overall, (Pul -34.5%, EP -30%). Positivity rates for Pul samples (smear and culture) were, in Ct category 'High'-n=4/7 and 3/7 ; 'Medium'-2/4 and 3/4 ; 'Low'- 3/14 and 4/14; and 'Very low'- 1/4 and 0/4 . Positivity rates for EP samples (smear and culture) were, in Ct category 'High'-n=0/1 and 1/1; 'Medium' -no samples; 'Low'-3/8 and 2/8; and 'Very low' -0/1 and 0/1, respectively. There was no significant correlation between Ct category and week of visible growth, though an increasing trend in smear/ culture positivity rate was seen.

Conclusions: Smear and culture positivity was similar in Pul and EP samples but were variable in Xpert Ct categories with poor positivity rates even in 'High' Ct samples. Improving culture sensitivity, particularly by modifying decontamination methods is required.

Keywords*: Mycobacterium tuberculosis, molecular diagnosis, Xpert MTB/RIF Ultra, ct value, Lowenstein–Jensen medium culture*

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