

### Exploring early, culture-negative TB with ultrasensitive nucleic acid tests.

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**Background:** Culture has been the reference standard for TB diagnosis. New highly-sensitive PCR tests such as GeneXpert Ultra have begun to replace culture. Since its WHO endorsement in 2017, there has been increasing observations of Ultra “false-positives” where Ultra is positive, but culture is negative. We hypothesized that a proportion of these symptomatic individuals actually have early or culture-negative TB.

**Methods:** We conducted a longitudinal sub-study to a multicenter diagnostic accuracy evaluation of Ultra carried in Uganda, Kenya and South Africa between July 2018 and May 2019. Adults with signs and symptoms of pulmonary TB, and without recent TB treatment were recruited. Sputum, blood and urine were collected on enrollment and follow up. Based on results of mycobacteriology test participants were classified as “discordants” (Positive-Ultra and negative-culture). A negative control (Negative-Ultra and negative-culture) and positive control group (Positive-Ultra and positive-culture) matched to discordants by age, sex, HIV status, and previous TB history were also enrolled. Participants were followed over 12 months where augmented culture methods, blood collection for TB host signatures, and repeat clinical and microbiologic assessments were performed.

**Results:** We enrolled 1195 participants (94 discordants, 52 positive controls and 29 negative controls). There was no statistical difference of gender, age, enrollment site, HIV status amongst discordants and non-discordants. History of TB was significantly higher on discordants (45% vs 23%,  $p < 0.05$ ). Ultra results of discordants included trace (63%), very low (23%) and low (14%). We found a predictive positive value of 65% to have a discordant result if the Ultra was trace or very low. During the follow up, almost half of discordants (49%) were started on TB treatment. Among the non-treated, one participant grew MTB and 11 had a persistence Ultra result that negativized during the follow up without any intervention.

**Conclusion:** We found microbiologic signals of TB (Persistent Ultra, culture-positive TB during follow up and with augmented culture methods) among Ultra-positive but culture-negative individuals, suggesting that Ultra can also identify some cases of culture-negative TB. Ongoing evaluation of host and bacterial biomarkers from the substudy samples will help us further understand early or culture-negative TB.