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School of Medicine
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21st June 2019

Dear Editor

Re: Tuberculosis Molecular Bacterial Load Assay (TB-MBLA) test

Effective diagnostics are crucial for the END-TB initiative. The gold standard diagnostic for tuberculosis (TB) is culture, which depends on chemical decontamination to remove non-TB microorganisms in order to reduce false positive culture. We have recently shown NALC/NaOH-based decontamination of on the viability of *Mycobacterium tuberculosis (Mtb)* and subsequent time to culture positivity of results (doi:10.1128/jcm.01992-18). As solution we developed the TB-MBLA that uses 16S rRNA as a reference gene to detect and quantify *Mtb* load in sputum samples of TB patients within a matter of hours. Detection of RNA rather than DNA makes it ideal test for viable bacterial load estimation. We recently demonstrated that this assay can be successfully conducted after heat inactivation of sputum thus obviating the need for category 3 laboratory and increasing the possibility of low resource laboratories to conduct the test (doi: 10.1128/JCM.01778-18). In this manuscript we have added more evidence to this fact. We have also demonstrated that fall in bacterial load measured by TB-MBLA correlates strongly with rise liquid culture time to positivity. However, the two assays are technically and operationally different with TB-MBLA giving results in as little as 4 h and is very specific to *Mycobacterium tuberculosis* complex and thus not affected by non-TB contaminating flora. By publishing a visualised protocol with JoVE we believe potential users across the globe will self-train and apply the test. Subsequently we will reduce the carbon footprint by reducing flights taken by trainers while increasing the users of the test.

Looking forward to your consideration of our manuscript,

Sincerely,

Dr. Wilber Sabiiti
Senior Research fellow in Medicine

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