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Commentary

Culture-free proof of *Mycobacterium tuberculosis* - a new assay for viable bacteria

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The current gold standard for the diagnosis of viable *Mycobacterium tuberculosis* bacteria is culture [1]. Mycobacterial culture is performed in laboratories worldwide providing absolute confirmation of the presence of the pathogen. The culture of mycobacteria has several disadvantages: it requires a high level of technological facilities, and is relatively costly. In addition, time to detection takes up to 2 months, significantly delaying the diagnosis of active tuberculosis in a large number of patients. Although less sensitive, smear microscopy is rapid and widely applied, it is not specific for *Mycobacterium tuberculosis* infection, cannot differentiate between viable and dead bacteria, and is unable to detect drug-resistance. A more rapid assay that can easily detect viable *Mycobacterium tuberculosis* bacteria would be an important advance for clinicians diagnosing and treating patients with tuberculosis.

During the last decade, molecular tests have revolutionized the field of TB diagnostics [2]. Molecular identification of mycobacterial DNA using assays such as the GenXpert MTB/RIF or the line-probe assays (for example, Hain Life Science, Nehren, Germany) allow the pathogen's detection within a few hours [3]. Importantly, these assays simultaneously identify some resistance markers aiding the identification of patients being infected with drug-resistant tuberculosis. However, the long-lasting stability of DNA does not allow one to reliably differentiate between viable and dead bacteria [4].

Published recently in *EBioMedicine*, Wang and colleagues conducted a prospective trial to evaluate a novel culture free assay to detect viable *Mycobacterium tuberculosis* bacteria in sputum samples from Taiwanese tuberculosis patients [5]. By heating the samples to 46 °C, the secretion of a mycobacterial protein (MPT64) is induced. Only viable *Mycobacterium tuberculosis* bacteria are able to respond in this way, hereby allowing the detection of MPT64 by an enzyme-linked immunosorbent assay within five hours. Additionally, as MPT64 is not produced by non-tuberculous mycobacteria, it adds to the assay's specificity. The overall diagnostic performance against culture as gold standard was high with a sensitivity of 86.9% and a specificity of 92.0% corresponding to results from the GenXpert (Cepheid, Sunnyvale, USA). MPT64 concentrations correlated well with mycobacterial viability and load as measured by colony forming units. In a group of treated patients, the specificity, positive and negative predictive values of smear microscopy or GenXpert Ultra fell more than the culture free TB test, suggesting that it is selectively detecting viable organisms opening the possibility of treatment monitoring.

Several molecular methods for treatment monitoring have been reported recently. These depend on various measures of *Mycobacterium tuberculosis* RNA species in sputum samples. These include the tuberculosis molecular bacterial load assay that detects changing concentrations of 16SrRNA [6]. Clinical studies will be needed to see whether this assay described by Wang and colleagues has the capability of delivering treatment monitoring in a clinical situation[2].

This novel culture-free TB test assay represents an attractive alternative to established diagnostics that detect specific molecular targets (cite Wang et al.). Some open questions remain about the implementation of this assay, including whether the heating step and the handling of the sample require specific biosafety considerations, and how robust and reproducible this is in a real-world setting. In contrast to the molecular assays, the MPT64 assay does not allow the detection of drug-resistance, limiting its role as a standalone diagnostic.

There is a pressing need for new diagnostic tools for tuberculosis and the novel MPT64 assay may be useful to detect the presence of viable *Mycobacterium tuberculosis* bacteria rapidly and obviate the need for culture in some circumstances. The possibility of therapeutic monitoring in sequential samples will be of particular interest to clinicians although further studies will be required. This assay cannot fully replace culture as drug susceptibility tests will still be required in the context of drug resistance.

Contributors

JH, SG, and MR all contributed equally in drafting the commentary.

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Declaration of Competing Interests

JH and MR have no conflicts of interest to disclose. SG is working to develop a molecular bacterial load assay for tuberculosis.

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