Rapid Reviews Infectious Diseases

Review 1: "Laboratory Validation of a Simplified DNA Extraction Protocol Followed by a Portable qPCR Detection of M. Tuberculosis DNA Suitable for Point of Care Settings"

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The MIT Press

Published on: May 08, 2024

URL: https://rrid.mitpress.mit.edu/pub/74b5vqb1

RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

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Review: Soares et al have presented the validation a simplified DNA extraction protocol (FTA) followed by a portable qPCR (Q3-Plus) detection of M. tuberculosis DNA suitable for point-of-care settings. There is no doubt having simple and rapid means to isolate nucleic acids will increase the applicability of nucleic acid amplification tests (NAATS) in diverse settings. However, it is critical that the methodology used to validate such novel methods is robust and supports the claims made by the authors. Firstly, for sensitivity and specificity, I note the authors used clinical sputum samples, assessed by sonication, FTA in comparison with Xpert MTB/RIF and culture. The downside is while authors reported quantitative results of sonication and FTA in the form of qPCR CTs, they qualitatively reported Xpert MTB/RIF and culture results. This makes it difficult to assess the sensitivity of the novel tests. It would have made more sense to report Xpert MTB/RIF Cos and culture time-to-positivity results, which are quantitative and can indicate whether the novel extraction method is retrieving a consistent amount of DNA. Furthermore the sample size of 29 is too small to give adequate statistical power to give reliable result on sensitivity and specificity of the novel FTA method. Secondly, limit of detection assessment using H37rv pure culture was not well designed. They started off with McFarland 1 which is conventionally known to contain 1x10^8 CFU/ml. However, in the results the 1/10 dilution was countless, 1/100 was 175 CFU/ml, then 35.5 CFU/ml for 1/1000 etc. This is strange because according to McFarland, a dilution of 1/100 should be equivalent to 1x10^6 CFU/ml, implying that if losses in dilution and poor growth recovery on solid culture are accounted for, the colony count should not fall below 1x10^4 CFU/ml. An effective design should have been an H37rv at McFarland 1 serially diluted 10-fold nine times. Each dilution should be divided into 4 fractions, one fraction for sonication, the other for FTA, then Xpert MTB/RIF and culture (preferably MGIT liquid culture). This design will give the most accurate assessment of FTA's sensitivity and LoD relation to the most sensitive standard-of-care tests.