Reviewer's report

Title: Utility of the REBA MTB-Rifa(R) Assay for early detection of rifampicin resistant Mycobacterium tuberculosis and correlation with multi-drug resistance

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Reviewer: Miguel Viveiros

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Major Compulsory Revisions:

Line 73-74 – Background - the sentence “XDR_TB…resistance to a fluoroquinolone and any injectable except streptomycin …” should be revised according to the consensus definition for XDRTB from WHO, ATS, IUTALD..etc.

Line 74-75 – Background - the sentence “Treating patients infected with MDR-TB requires” should be revised. MDR_TB is a clinical condition of the patient/disease and not an infectious agent.

Line 87 - Background - the sentence “…of isolates can be correctly identified as resistant or sensitive” should be revised. Sensitive should be replaced by susceptible a more appropriate designation in laboratory medicine.

Line 103 - Background - the sentence “TB therapy for drug sensitive disease” should be revised. Sensitive should be replaced by susceptible a more appropriate designation in medicine and chemotherapeutics.


Line 108 – Background - The citation 12 and 13 are appropriate but the difficulties and less accuracy involved in the molecular detection of INH resistance are better detailed in the review work of “Alcaide F, Coll P. Advances in rapid diagnosis of tuberculosis disease and anti-tuberculous drug resistance. Enferm Infecc Microbiol Clin. 2011 Mar;29 Suppl 1:34-40.”
Line 115 – Background - the sentence “In contrast, LPAs are extremely cost-effective and redesign to accommodate new mutations is straightforward” should be re-written since LPAs are also expensive if we consider the price of the positive and negative control in each assay plus equipment and trained personnel needed. Not as expensive as gene-expert but expensive as well and it’s not extremely cost-effective compared with Gene-Expert. Both are cost-effective if we include the cost of management of a non-early-detected MDRTB patient but that applies to all molecular techniques for direct detection of MDRTB. The redesign of LPAs to accommodate new mutations it is not straightforward.

Line 139-146 – Methods - Clinical isolates and drug susceptibility testing – the description of the DST protocol is missing as well as the standard references for this procedure.

Line 190 - Methods - REBA MTB-Rifa® assay – “Specific oligonucleotides are immobilized at known locations on a membrane strip” please describe which ones are immobilized and which sequences they detect since these are the targets that allow the completion of the proposed objective of this paper “improved reverse blot assay (REBA MTB-Rifa®) for early detection of RIF resistance and assessed its ability to predict RIF resistance for MDR-TB in South Korea”

Line 208 – Methods - PCR amplification and sequencing of the products – analysis of the sequenced products is missing – How it was done?

Line 220 - Results - Drug susceptibility patterns of MTB isolates –This paragraph and these results are better presented in a table and the results of the drug susceptibility patterns of MTB isolates of such an extensive work (492 M. tuberculosis isolates between 2005 and 2008 ) deserve a better presentation and analysis. The high percentage of XDR-Tb should be addressed and discussed. It’s a very important finding.

Line 230 - Results - Detection of Rif resistance using the REBA MTB-Rifa® assay on DNA from cultured samples – “Alterations in the RRDR of rpoB were detected in 211 of the 492 samples analyzed. As expected, the majority of the mutations (162, 77%) detected involved three codons; 516, 531 and 533 with codon alone contributing more than half (121, 57.3%) (Table 2) of the mutations.” This sentence is very confusing for the reviewer that is used to work and analyse LPAs. Following the REBA MTB-Rifa® assay package insert and protocol the technical procedure can only validate well defined polymorphisms in codons 516, 526 and 531 plus eventually a new mutant probe (533CTG-CCG mutant probe referred in line 279) – These are the only 3-4 mutation probes that are apparently included in the improved version of the REBA MTB-Rifa® assay and the results is valid only when hybridization with the mutat probe is present simultaneously with absence of hybridization with the respective wild-type probe. If this assumption is correct it’s difficult to understand how the REBA MTB-Rifa® assay could detect all the mutations presented in table 2. The only explanation for this
analysis of the results implies that the authors have merged the genomic data from the MTB-Rifa® assay with the sequencing data. This merge biases the results presented in table 1 as well as the performance and accuracy of the assays for the goals presented in the introduction of this paper. The authors are invited to analyse separately the results obtained from the application of the assay from those obtained by sequencing of rpoB gene.

Line 245 - Results - Detection of rifampicin resistance with the REBA MTB-Rifa® directly on smear positive sputum samples - Contrary to many published studies on direct detection of mutation for RIF resistance on smear positive samples the authors had no false negatives (amplification negative with culture positive) by lack of amplification, especially using a simple boiling protocol for extracting MTB DNA from the smear positive samples. This result is also very puzzling.

Line 268 -277 - Discussion – “It is worth noting that the 98.14% detection rate of RIF resistance reported in this study constituted all the M.tuberculosis isolates that harbored mutations in the RRDR of the rpoB gene, the target upon which the assay is based. Unless the new version/improved version of the REBA MTB-Rifa® assay includes all the mutations detected in this study, and this should be clearly detailed in the materials and methods section, this conclusion cannot be taken from the results presented. If the post-assay sequencing analysis of the RRDR of the rpoB gene was included, then the conclusion is obvious and reflect the molecular/mutational epidemiology data of the sample analyzed. This is exactly what the authors do from lines 282 to 297 – analysis of the mutational profile of the samples studied.

Line 336 – “…source of the samples (NMH) being the biggest TB national reference hospital, and it does not negate the fact that the REBA MTB-Rifa® assay has been demonstrated to be as good as or better than other LPAs in detecting RIF resistance “. This conclusion cannot be taken form the data presented. The REBA MTB-Rifa® assay was not compared against any other LPA assay in this study.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

No to all