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Detection of the Ile491Phe rpoB mutation of Mycobacterium tuberculosis, a rifampicin resistance-conferring mutation undetected by the Xpert MTB/RIF and other commercial assays

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Background: The Xpert MTB/RIF and other commercial assays are not capable of detecting resistance-conferring mutations located outside the 81 bp Rifampicin Resistance Determining Region (RRDR) of the *rpoB* gene. Recently, it has been reported that up to 30% of rifampicin-resistant *Mycobacterium tuberculosis* (MTB) strains in Swaziland harbour the Ile491Phe mutation, which is located outside the RRDR. There is thus a major concern that an important proportion of rifampicin-resistant and multi-drug resistant MTB strains would be undetected by current molecular techniques.

Material/methods: We designed a real-time multiplex allele-specific (MAS) PCR assay which allows to distinguish MTB strains harbouring the *rpoB* Ile491Phe mutation from un-mutated strains. The distinction of amplified PCR products is based on the melt-temperature (MT) of the amplicons. The

test was performed on 78 MTB strains from Swaziland in parallel with conventional sequencing of the *rpoB* gene.

Results: The MAS-PCR results showed 100% similarity with *rpoB* sequencing on a panel of 78 strains from Swaziland among which 39 presented the *rpoB* Ile491Phe mutation and 12 presented at least one other mutation within the *rpoB* gene. Out of the 78 strains 39 showed a MT ranging from 85.21°C to 85.56°C and interpreted as Ile491Phe (mutated). The 39 unmutated strains presented an MT between 90.80°C to 91.06°C and could be clearly distinguished from Ile491-mutated strains (Figure 1).

Conclusions: It has been previously reported that the *rpoB* Ile491Phe mutation should be considered as a public health concern, as it is associated with un-detected MDR-TB. We propose a simple PCR which specifically targets this mutation, and could therefore complement commercial assays for the diagnosis of rifampicin-resistant MTB.

Figure 1: