

**Title: Preliminary evaluation of the nitrate reductase assay for the fast detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* at the National Reference Laboratory for Tuberculosis**

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**Abstract:**

Tuberculosis (TB) remains as one of the infectious diseases with the biggest burden on mankind, being considered as a worldwide public health concern. Susceptibility testing is an important part of TB control measures, and is used to detect resistance of *M. tuberculosis* isolates to the drugs commonly used during treatment. The nitrate reduction assay (NRA) is widely used to distinguish *M. tuberculosis* from nontuberculous mycobacteria. Recently, the use of NRA on Lowenstein-Jensen medium for susceptibility testing was also described. The method relies on the ability of *M. tuberculosis* to reduce nitrate to nitrite through the action of the enzyme nitrate reductase. The goal of our study was to quickly identify *M. tuberculosis* strains resistant to isoniazid (INH) and rifampicin (RMP) through the NRA. Such assay would be extremely valuable for the control of TB, given that treatment could be initiated sooner and the transmission chain of the organism would be interrupted. Thus, we used 83 isolates from the strain collection of the National Reference Laboratory for Tuberculosis to evaluate the sensitivity and specificity of the NRA when compared to the gold standard, the method of proportion (MP), and the automated MGIT 960 system. Our results showed that, compared to the MP, the NRA presented a sensitivity of 93,2% and specificity of 87,5% for the detection of INH resistance. For RMP resistance, the NRA showed a sensitivity of 83,0% and specificity of 91,7%. For the detection of multidrug resistance (simultaneous resistance to both INH and RMP), the NRA showed 84,8% e 94,0% of sensitivity and specificity, respectively. Compared to MGIT 960, the NRA showed 93,3% of sensitivity and 100% of specificity for the detection of INH resistance. For the detection of RMP resistance, the NRA showed 88,6% of sensitivity and 94,9% of specificity. For the detection of multidrug resistance, the NRA displayed sensitivity and specificity of 93,5% and 94,2%, respectively. In conclusion, the NRA can be used as a valuable alternative to MP and MGIT 960 for the determination of drug susceptibility, due to its speed, accuracy and low cost. The reduction in time to diagnosis achieved by this method may become an important tool in the control of tuberculosis in high-burden countries.

**Keywords:** Tuberculosis, Drug resistance, Diagnosis, Nitrate reductase

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