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NITRATE REDUCTASE ASSAY USING SODIUM NITRATE FOR RAPID DETECTION OF MULTIDRUG RESISTANT TUBERCULOSIS

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ABSTRACT

We validated the nitrate reductase assay (NRA) for the detection of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) using sodium nitrate ($NaNO_3$) in replacement of potassium nitrate (KNO_3) as nitrate source. $NaNO_3$ is cheaper than KNO_3 and has no restriction on use which facilitates the implementation of NRA to detect MDR-TB.

Key words: nitrate reductase assay; tuberculosis; multidrug resistant, sodium nitrate

Tuberculosis (TB) remains as a serious health problem worldwide. In 2008, 9.4 million new cases of the disease were reported (8). Besides, there were approximately 400,000 new annual cases of TB caused by multidrug resistant (MDR) strains, defined as resistant to at least isoniazid (INH) and rifampicin (RIF) (8). Rapid detection of drug resistance is an urgent priority to identify patients who are not responding to the standard treatment and to avoid the transmission of resistant strains (4). Recently, the WHO endorsed new non-commercial drug susceptibility testing methods for the detection of MDR-TB patients, and among them, the nitrate reductase assay (NRA). NRA is based on the capacity of *M. tuberculosis* to reduce nitrate to nitrite, which is easily detected in a colored reaction (5). NRA was initially standardized using potassium nitrate (KNO_3) (1) as nitrate source, but due to its higher cost and restriction of use in several countries, some authors have used sodium nitrate ($NaNO_3$) as nitrate source (3, 6, 7). However, there is no study comparing the accuracy of NRA using $NaNO_3$ in replacement of KNO_3. In this study, we evaluated the use of the $NaNO_3$ in the NRA test to detect MDR-TB in comparison with KNO_3 and results were compared to those obtained with the conventional proportion method (PM) performed on Löwenstein-Jensen (LJ) medium.

One-hundred and six *M. tuberculosis* strains belonging to the collection of the Institute of Tropical Medicine of Antwerp, Belgium, were studied. The strains were cultured in LJ medium and incubated at 37°C for three weeks. The PM was performed according to Canetti *et al* (2), using critical concentrations of 0.2 µg/mL for INH and 40 µg/mL for RIF. The NRA was performed according to the previously reported methodology

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(1). The NRA was carried out in LJ medium incorporating 1.0 mg/mL of $NaNO_3$ or KNO_3. The critical concentration of INH and RIF were the same used in the PM.

Table 1 shows the sensitivity and specificity obtained with the NRA using $NaNO_3$ or KNO_3 compared to the PM. Drug susceptibility testing for RIF showed a sensitivity of 95% with KNO_3 and 96% with $NaNO_3$. Specificity was 97% for both nitrate sources. For INH the sensitivity was 97% with KNO_3 and 99% with $NaNO_3$ while the specificity was 96% and 93%, respectively.

Out of the 106 strains tested, results were available after 10 days for 101 strains (95.3%) using KNO_3 and for 104 strains (98.1%) using $NaNO_3$. All strains were positive after 14 days with both reagents.

This study showed that the NRA gave similar results using KNO_3 or $NaNO_3$ as nitrate source. NRA using $NaNO_3$ showed high sensitivity and specificity for RIF (96% and 97%, respectively) and INH (99% and 93%, respectively). These results are in agreement with previous studies presented in a meta-analysis that evaluated the accuracy of the NRA for the detection of MDR-TB. According to that meta-analysis most of the studies that applied NRA to test *M. tuberculosis* isolates reported a sensitivity and specificity > 94% for RIF and >92% for INH (5). Another important finding in the present study was that 98% of the strains showed results in 10 days with NRA using $NaNO_3$. This percentage was higher than that obtained using KNO_3 and also in a previous study where 87 % of the strains gave results in 10 days (1).

KNO_3 is considered as a class of "explosive" and consequently difficult to obtain in some countries such as Brazil. Additionally, $NaNO_3$ is cheaper than KNO_3 and has no restriction of use.

Taking into account the high sensitivity and specificity obtained using $NaNO_3$ and the rapid availability of results with 98% of the strains in 10 days, our study validates the use of $NaNO_3$ as the source of nitrate for NRA.

Table 1. Sensitivity and specificity of the NRA using KNO_3 and $NaNO_3$ compared to the PM method.

Drug	PM	NRA-KNO_3				NRA-$NaNO_3$			
		R (n)	S (n)	Sensitivity (%)	Specificity (%)	R (n)	S (n)	Sensitivity (%)	Specificity (%)
RIF	R	72	4	95	97	73	3	96	97
	S	1	29			1	29		
INH	R	76	2	97	96	77	1	99	93
	S	1	27			2	26		

R= resistant; S=susceptible; PM=proportion method

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