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Short Communication

Evaluation of the Commercial Kit SIRE Nitratase for detecting resistant *Mycobacterium tuberculosis* in Brazil

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Abstract

Introduction: This study aimed to evaluate a new commercial kit, Kit SIRE Nitratase-PlastLabor, for testing the drug susceptibility of clinical *Mycobacterium tuberculosis* isolates. **Methods:** The accuracy of the Kit SIRE Nitratase was evaluated by examining the susceptibility (streptomycin, isoniazid, rifampicin, and ethambutol) of 40 *M. tuberculosis* isolates, using the proportion method with Lowenstein-Jensen medium or the BACTEC MGIT 960 system. **Results:** The detection accuracy for streptomycin, isoniazid, rifampicin, and ethambutol was 95%, 97.5%, 100%, and 80%, respectively. **Conclusions:** The exceptional accuracy demonstrated by Kit SIRE Nitratase for isoniazid and rifampicin makes the kit an attractive option for screening *M. tuberculosis* strain resistance.

Keywords: Tuberculosis. MDR. Nitrate reductase.

Tuberculosis (TB) is one of the most important global public health concerns. An increasing incidence of multidrug resistant-tuberculosis (MDR-TB) cases (simultaneous resistance to isoniazid and rifampicin) has resulted in treatment failure and these drug-resistant strains have been able to spread into the community at large. Recently, extensively drug-resistant strains (additionally resistant to aminoglycosides and quinolones) have also been reported[1]. In terms of public health, the most important measure for controlling these events is the early detection of MDR-TB so that timely and appropriate treatment can be adopted[1]. Two types of methods are commonly used to detect MDR-TB: 1) phenotypic methods, such as proportion method (PM)-based drug susceptibility tests (DST), which measure the 1% proportion of culture growth with solid medium containing antibiotics at a critical concentration[2]; these are laborious and require several weeks to yield results. 2) Molecular-based methods, such as *GeneXpert* MTB/Rif (Cepheid) and *GenoType* MDR *plus* (BioMeriéux) that detect mutations known to be associated with phenotypic resistance to drugs[2]. The commercial automated BACTEC MGIT 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) uses liquid media and is an expensive system requiring specific equipment and technical support[1]. Since 2009, The World Health Organization (WHO) has recommended the use of the noncommercial nitrate reductase assay (NRA) for the rapid screening of patients suspected of having contracted MDR-TB[3]. The NRA uses the detection of nitrate reduction as an indication of growth; therefore, results can be obtained more rapidly than by the visual detection of colonies. Tests results are available in 7 days for most isolates, which is comparable to the time required by the BACTEC liquid medium method and much faster than the solid medium proportion method, which requires 3 to 4 weeks[4].

This method was previously reported as a useful tool for the rapid and accurate detection of drug resistance to first-line antituberculous drugs[2,4]. However, the use of noncommercial tests has been associated with low reproducibility and lack of quality control, as reported for the NRA. Adaptation of the NRA as a commercial test may overcome these limitations; industrial production has been demonstrated to control quality and reproducibility at all the steps of the manufacturing process of an *in-vitro* Diagnostic (IVD) Kit for commercialization[5]. Approximately 30% of DST were performed in Brazil in 2015. Of the 1900 reported cases of MDR-TB, 1197 had a laboratory diagnosis. Reasons for non-performance of DST include difficulties with equipment maintenance and/or the availability of human resources to meet the demand[1,6]. One possible solution

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to this problem is the newly formed partnership between the Brazilian Tuberculosis Research Network (REDE-TB), the Federal University of Minas Gerais (UFMG), and PlastLabor Brazilian Industry, the consortium that originally initiated industrial production of the first Brazilian commercial IVD DST – Kit SIRE Nitratase. Thus, the aim of this study was to assess the accuracy of the Kit SIRE Nitratase.

A total of 40 *Mycobacterium tuberculosis* clinical isolates were selected for this study from the strain collection of each laboratory (the Mycobacteria Research Laboratory of the UFMG Medical School and the Evandro Chagas Institute of the Brazilian Ministry of Health). Susceptibility tests were performed at the Tuberculosis Reference Laboratories of Minas Gerais and Pará, Brazil. DST were performed using the 1% proportion method on Lowenstein-Jensen (LJ) medium[2] (DIFCO; Becton Dickinson Diagnostic Systems) and later adapted to the commercial automated BACTEC MGIT 960 system[7] (Becton Dickinson Diagnostic Systems) with liquid medium; these were considered the *gold standard* methods for comparison with the Kit SIRE Nitratase. The participating laboratories were subjected to external quality control performed by ControLab 2002-2012, Brazil. This company certifies technical competence, credibility, and operational capacity to carry out tests according to ISO 9001 certification.

The commercial Kit SIRE Nitratase was used according to the protocol previously described by Ängeby et al.[4]; the critical concentrations of isoniazid, rifampicin, streptomycin, and ethambutol in the LJ medium were 0.2µg/mL, 40µg/mL, 4µg/mL, and 2µg/mL, respectively. The LJ medium was prepared according to manufacturer's specifications (DIFCO; Becton Dickinson Diagnostic Systems) with the addition of potassium nitrate (final concentration of 1mg/mL). Tubes without drugs were used as the growth control. The company was accredited and validated by the Brazilian National Agency of Sanitary Surveillance [*Agência Nacional de Vigilância Sanitária* (ANVISA)].

Inoculum turbidity was adjusted to McFarland tube nº 1 and diluted 1:10; 0.2mL of the 1:10 dilution was used to inoculate three drug-free tubes and 0.2mL of the undiluted suspension (McFarland tube nº 1) was inoculated into tubes containing drugs. The inoculum originated from the first isolate.

The tubes were incubated at 37 °C for 7 days. The reading included the preparation of a color-developing reagent mixture consisting of one part 50% (vol/vol) concentrated HCl, two parts 0.2% (wt/vol) sulfanilamide, and two parts 0.1% (wt/vol) n-1-naphthylethylenediamine dihydrochloride. These reagents were mixed separately immediately prior to being added to the tubes to detect growth. Following 7 days of incubation, 0.5mL of the reagent mixture was added to one of the three drug-free tubes. If any color change occurred, the corresponding antibiotic-containing tubes were developed by adding the same color-developing reagent mixture. If no color change was observed in the drug-free control tube, this tube was discarded and the other two tubes were reincubated. This procedure was repeated at day 10, using the second growth control tube and, when necessary, at day 14, using the third growth control tube.

A strain was considered resistant if the color change in the tube containing the drug was greater than or equal to the color in the 1:10-diluted growth control tube[4,8]. The *M. tuberculosis* H37Rv reference strain, susceptible to all anti-tuberculosis drugs, as well as *M. tuberculosis* ATCC 35822 (INH-resistant), ATCC 35838 (RIF-resistant), ATCC 35837 (ETM-resistant), and ATCC 35820 (STR-resistant) quality control strains were used as control strains. The ATCC strains were tested in triplicate using the same lot. The specificity, sensitivity, accuracy, and Kappa value were calculated according to Godoy and Braile[9].

The Kit SIRE Nitratase showed 100% accuracy for the ATCC strains (**Table 1**). As shown in **Table 1**, the accuracy for the 40 tested clinical *M. tuberculosis* isolates was high for rifampicin and isoniazid (100% and 97.5%, respectively), but

TABLE 1

The Kit SIRE Nitratase results (n= 40)

Drugs	PM and MGIT 960		Kit SIRE Nitratase		Sensitivity (%)	Specificity (%)	Accuracy (%)
	Drug susceptibility of the strains	Strains (n)	Drug-resistant strains	Drug-susceptible strains			
Streptomycin[a]	Resistant	15	14	1	93.3	96.0	95.0
	Susceptible	25	1	24	95%CI:80.7-100.0	95%CI: 88.3-100.0	95%CI: 88.7-100.0
Isoniazid[b]	Resistant	19	18	1	100.0	95.5	97.5
	Susceptible	21	0	21		95%CI: 86.8-100.0	95%CI: 92.7-100.0
Rifampicin	Resistant	19	19	0	100.0	100.0	100.0
	Susceptible	21	0	21			
Ethambutol[c]	Resistant	13	7	6	77.8	80.6	80.0
	Susceptible	27	2	25	95%CI:50.6-100.0	995%CI: 66.7-94.6	95%CI: 67.6-92.4

SIRE: streptomycin, isoniazid, rifampicin, and ethambutol**; PM:** proportion method; **MGIT 960:** BACTEC MGIT 960 system; **95% CI:** Confidence interval. ***a*:**Kappa = 0.89 (very good). ***b*:**Kappa = 0.95 (very good) . ***c*:**Kappa = 0.50 (moderate).

relatively lower for streptomycin and ethambutol (95% and 80%) compared with the *gold standard* methods (1% proportion method on LJ and the BACTEC MGIT 960 system). The time required for obtaining results ranged from 7 to 10 days. The concordance (Kappa) between the *gold standard* and the Kit SIRE Nitratase was very good for streptomycin, isoniazid, and rifampicin and moderate for ethambutol. These discrepant results for ethambutol have been reported in previous studies[8,10]. The Kit SIRE Nitratase was easy to perform and provided an affordable alternative method for the rapid detection of resistance to first-line antituberculous drugs in the 40 clinical *M. tuberculosis* strains. The accuracy values determined in this study are similar to those reported by other studies using noncommercial NRA[2,4,8,11]. A recent meta-analysis[11] comprised of 38 studies showed that the SIRE Nitratase method exhibits high sensitivity and specificity in identifying isoniazid and rifampicin resistance[10]. Both the sensitivity and specificity values for ethambutol (variation of 55%-75% and 75%, respectively) and streptomycin (variation of 92-100% and 78%-99%, respectively)[11] were lower in the present study. Streptomycin and ethambutol have been established as drugs that are difficult to test by conventional methods[12]. The accuracy for streptomycin reported in previous studies[2,8] is lower than that obtained in this study (85.3% vs. 95%). A number of factors, such as different growth kinetics, cross-contamination, strain growth difficulties, and minimum inhibitory concentrations approaching the critical concentration, may have caused these discrepant results[13]. Nevertheless, the results obtained herein are quite promising, especially in terms of the drugs that need to be assayed by routine DST, isoniazid and rifampicin, which are the two most effective anti-TB drugs currently available for clinical practice and are at the core of the WHO's first-line anti-TB regimen[3].

Additionally, the analytical performance of the Kit SIRE Nitratase demonstrates that it could reduce the turnaround time for drug susceptibility testing if applied directly to sputum smear-positive samples, as previously described[10]. Thus, studies evaluating the direct application of the SIRE Nitratase assay to sputum smear positive samples are necessary and will be performed in the near future. The turnaround time for results in our study (7-10 days) is consistent with the meta-analysis of noncommercial NRA studies conducted by Coban et al.[11], which found that the mean turnaround time for test results ranged from 5 to 14 days.

This study has some limitations. We did not perform the reproducibility test in triplicate on three different days; however, this is a preliminary study and 100% accuracy was obtained using the ATCC strains. Another limitation is that the Kit SIRE Nitratase has a short shelf life (30 days) compared to the BACTEC MGIT 960 system in which the drug is incorporated into the medium at the time of use[7].

In conclusion, the SIRE Nitratase assay constitutes a straightforward and affordable tool for the detection of phenotypic TB drug resistance in low-resource and developing countries with limited laboratory facilities. Its high accuracy, low cost, ease of operation, and lack of requirement for sophisticated equipment make it a sensible option for TB laboratories with limited resources; however, the SIRE Nitratase assay should be carried out at multiple locations. In a study conducted in South Africa, the cost of phenotypic DST using BACTEC MGIT 960 ranged from $20.10-$42.37[14]. These gold-standards methods, especially the automated BACTEC MGIT 960 system recently adapted for the determination of personalized minimum inhibitory concentrations[7], are crucial for national TB reference laboratories to provide reliable information regarding the resistance levels of MDR-TB isolates to first- and second-line drugs; however, a simple and affordable test needs to be implemented in the network of regional and local TB laboratories in Brazil in close connection with the national TB reference laboratories, to increase the national DST coverage. Given the low coverage of DST in Brazil, the incorporation of this test in the public health system in Brazil is predicated to have a positive clinical and economic impact on TB control, especially that focused on phenotypic confirmation/validation of MDR-TB suspicions based on molecular results obtained from the nation-wide implementation of GeneXpert MTB/RIF[15]. The excellent accuracy of the SIRE Nitratase assay for isoniazid and rifampicin makes it an attractive option for screening *M. tuberculosis* strain resistance.

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Conflict of interest

The authors declare that no conflicts of interest exist.

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