

## The laboratory as a tool to qualify tuberculosis diagnosis

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### SUMMARY

**OBJECTIVES:** To evaluate the performance of laboratory diagnosis of tuberculosis, clinical samples underwent culture, species identification and drug susceptibility testing (DST).

**METHODS:** A total of 554 samples from 269 patients were tested for smear microscopy using Kinyoun stain. Culture was performed in Ogawa-Kudoh medium and species identification was performed using the IS6110 amplified region. DST for rifampicin, isoniazid (INH) and streptomycin were carried out using the Resazurin assay.

**RESULTS:** Cultures augmented the number of cases diagnosed by 22.1%, IS6110 amplification identified all *Mycobacterium tuberculosis* strains thus isolated and DST detected three strains resistant to INH and one multidrug-resistant strain.

**CONCLUSION:** Simultaneous use of different techniques enhanced culture yield, species identification and detection of drug resistance even in a laboratory with limited facilities.

**KEY WORDS:** tuberculosis; culture; *Mycobacterium tuberculosis*; diagnosis

THE LIMITATIONS of sputum microscopy are well known.<sup>1</sup> Culture as a routine procedure will enhance the number of cases detected and is considered as a gold standard. Moreover, culture allows species identification and determination of drug resistance.<sup>2</sup> Ogawa-Kudoh medium culture has shown comparable results with Löwenstein-Jensen (LJ) and is less expensive and simpler.<sup>3-6</sup> The utilisation of molecular methods to identify species of mycobacteria and colorimetric Resazurin assay for DST provided rapid results.<sup>7,8</sup> The present study is a report on the advantages of combining these methods in the diagnosis of tuberculosis (TB).

### MATERIAL AND METHODS

#### Sample

This is a retrospective study of 554 sputum samples from 269 patients of both sexes attending the phthysiology municipal service in Rio Grande from December 2005 to August 2006. The project was approved by the ethics committee of Fundação Universidade Federal do Rio Grande. Smear microscopy was performed using Kinyoun's cold staining method, examining a minimum of 200 fields. For culture, the sputum samples were kept in 4% sodium hydroxide for 2 min and inoculated onto Ogawa-Kudoh medium.<sup>3-5</sup> The cultures were then incubated at 37°C and examined for up to 8 weeks. Species identification was car-

ried out using IS6110 amplification, as described previously.<sup>9</sup> Drug susceptibility testing (DST) was performed for rifampicin (RMP), isoniazid (INH) and streptomycin (SM) with the Resazurin microplate assay (REMA) using Middlebrook 7H9 medium in 96-well plates, as described previously.<sup>7,8,10</sup>

### RESULTS AND DISCUSSION

Of the 269 patients included in the study, 63 had samples positive to any one of the methods employed. The majority of these cases (87%) were in the economically productive age group 20-59 years. This finding is in accordance with studies conducted in other populations with similar characteristics.<sup>11</sup> Samples from 13 patients were smear-positive for acid-fast bacilli (AFB), but with no culture growth (Table 1). Seven of these patients were undergoing treatment, and one had recently completed treatment. Positive microscopy results and negative cultures may indicate the presence of non-viable bacilli in the sample; this may be observed in patients who are undergoing treatment. In the remaining five samples, this phenomenon could be due to the limitations of Ogawa-Kudoh medium to support the growth of the causative organism. It is estimated that in Southern Brazil, about 3-6% of mycobacterial isolates are bovine TB. *Mycobacterium bovis* normally requires specific media such as Stonebrink for growth.<sup>12</sup>

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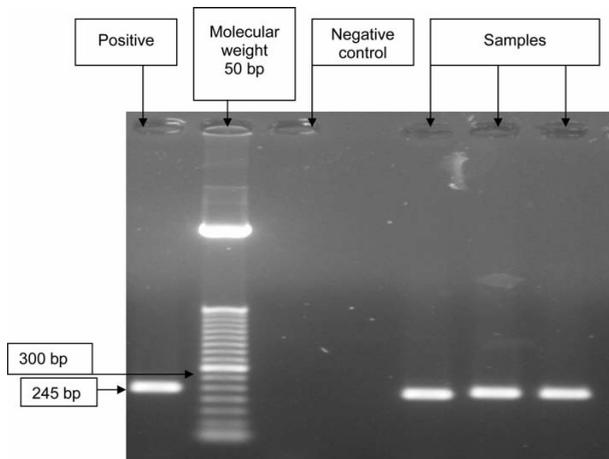
**Table** Positive test results from microscopy and culture

	Positive microscopy n (%)	Positive culture n (%)	Positive microscopy and culture n (%)	Total
Test (diagnostic)	5 (7.35)	15 (22.1)	29 (42.6)	49
Test (control)	8 (11.8)	5 (7.4)	6 (8.8)	19
Total	13 (19.15)	20 (29.5)	35 (51.4)	68

Positive culture and negative microscopy results were observed in 20 samples. Of these, five were from TB suspects who had attended for treatment, and the remaining 15 were processed for diagnostic purposes (Table). Sputum smear microscopy is easy, inexpensive and provides rapid results. For these reasons, its application is recommended in resource-limited countries. However, its limitations include its low sensitivity and the fact that it cannot be used in determining non-viable organisms and species identification.<sup>1</sup>

The Ogawa-Kudoh culture method has several advantages, including low cost and the fact that it does not require a centrifuge. This step minimises aerosol spread and transmission and allows easy performance of culture.<sup>13</sup> In this study, culture gave an additional yield of 22.1%, thereby curtailing transmission. This is sufficient reason for implementing culture in highly endemic resource-limited settings such as Brazil. In addition, the five smear-negative culture-positive results also indicate the importance of culture for diagnosis and for monitoring patients while on treatment.

Employing an in-house polymerase chain reaction (PCR) with IS6110 region amplification allowed rapid molecular identification of species. The presence of 245 bp (base pair) banding (Figure), IS6110 region, was observed in all isolates of *M. tuberculosis* complex strains in this study. As the treatment protocol varies



**Figure** Detection of PCR product (IS6110 region) was analysed by electrophoresis in 1.5% agarosis gel stained with ethidium bromide. bp = base pairs; PCR = polymerase chain reaction.

for different mycobacterial species, rapid identification of species by PCR is a step further in this effort.<sup>14</sup>

Of the 63 cases bacteriologically proven by smear or culture, 50 grew in the Ogawa-Kudoh media. These isolates were evaluated according to their susceptibility profile against RMP, INH and SM. Fourteen (28%) isolates were obtained from patients with no history of previous treatment, nine (18%) from patients with a history of treatment and an additional 14 (28%) were from patients who were on treatment. The situation was unknown for 13 patients (26%). Three INH-resistant strains were from patients with no previous known treatment history, and were thus primary resistant strains. A single isolate of multidrug-resistant tuberculosis (MDR-TB) was from a patient with a prolonged history of previous treatment. He was also diabetic, and this could be one of the contributory reasons for the emergence of this resistant strain.<sup>15</sup>

The REMA method is a good alternative for routine use in small laboratories as it is inexpensive and easy to carry out in settings with minimal laboratory facilities. Besides being rapid, it also compares well with the standard indirect DST methods, including the BACTEC 960 system.<sup>16</sup> Speed and cost wise, this method also compares favourably with the thin-layer culture technique.<sup>17</sup>

The present study therefore shows the usefulness of rapid identification DST methods in combination with the conventional culture system without the use of a centrifuge in a resource-limited setting in Brazil.

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## R É S U M É

**OBJECTIFS :** Pour évaluer les performances du diagnostic de laboratoire de la tuberculose, des échantillons cliniques ont été soumis à la culture, à l'identification de l'espèce et aux tests de sensibilité (DST).

**MÉTHODES :** 554 échantillons provenant de 269 patients ont été testés par examen microscopique des frotis après coloration de Kinyoun. Les cultures ont été faites sur milieu d'Ogawa-Kudoh et l'identification des espèces a utilisé la région amplifiée IS6110. Les tests de sensibilité à la rifampicine, l'isoniazide (INH) et la streptomycine ont été menés par la technique de Resazurine.

**RÉSULTATS :** Les cultures ont augmenté le nombre de cas diagnostiqués de 22,1%, alors que l'amplification d'IS6110 a identifié chaque souche isolée de *Mycobacterium tuberculosis* et que les DST ont détecté trois souches résistantes à INH et une souche multirésistante.

**CONCLUSION :** L'utilisation simultanée de différentes techniques a augmenté le rendement des cultures, l'identification des espèces et la détection de la résistance aux médicaments, même dans un laboratoire ayant des ressources limitées.

## R E S U M E N

**OBJETIVOS :** Con el propósito de evaluar la eficacia del diagnóstico de laboratorio de la tuberculosis, se sometió una serie de muestras clínicas a cultivo, identificación de especie y pruebas de sensibilidad a los medicamentos.

**MÉTODOS :** Se analizaron 554 muestras provenientes de 269 pacientes mediante examen microscópico del esputo con coloración de Kinyoun, cultivos en medio Ogawa-Kudoh e identificación de especies por amplificación de la región IS6110. Las pruebas de sensibilidad a rifampicina, isoniazida (INH) y estreptomycina se realizaron con el ensayo de azul de Alamar (Resazurin).

**RESULTADOS :** Los cultivos aumentaron un 22,1% el número de casos diagnosticados y con la amplificación de la región IS6110 se identificaron todas las cepas de *Mycobacterium tuberculosis* así aisladas ; mediante las pruebas de sensibilidad se detectaron tres cepas resistentes a INH y una cepa multidrogorresistente.

**CONCLUSIÓN :** La aplicación simultánea de diferentes técnicas aumentó la eficacia de los cultivos, la identificación de especies y las pruebas de farmacorresistencia, aún en un laboratorio con medios limitados.