Original Article

Gene probes versus classical methods in the identification of mycobacteria*

Estudo comparativo entre um sistema de sonda genética e métodos clássicos na identificação das micobactérias

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Abstract

Objective: The emergence of tuberculosis/HIV co-infection and the increase in the number of cases of infection with nontuberculous mycobacteria (NTM) require rapid laboratory test results in the isolation and identification of mycobacteria. The objective of this study was to evaluate the identification of mycobacteria by means of gene probes in comparison with that obtained using classical biochemical methods. **Methods:** Between 2002 and 2004, 178 mycobacterial cultures, all testing positive for acid-fast bacilli, were analyzed. Samples were obtained from clinical specimens of patients with respiratory symptoms or with clinical suspicion of pulmonary tuberculosis/mycobacteriosis who were treated in the greater metropolitan area of Santos. **Results:** The gene probe identified 137 samples (77%) as *Mycobacterium tuberculosis* complex and 41 (23%) as NTM. Discordant results between the methods (3%) were obtained only in the year of implementation (2002). When comparing the methods, the sensitivity, specificity, positive predictive value and negative predictive value of the gene probe method were 98%, 93%, 98% and 93%, respectively. **Conclusions:** Despite the cost, the identification of mycobacteria using the molecular technique is faster: maximum 3 h *vs.* 28-30 days for classical methods. The use of gene probes is a validated molecular technique. It is fast, easy to use and readily available on the market. It has high specificity and sensitivity, which justifies its implementation and routine use in referral laboratories, since it facilitates the diagnosis providing agile clinical interventions.

Keywords: Mycobacterium tuberculosis; Tuberculosis/diagnosis; Mycobacterium/classification; DNA probes.

Resumo

Objetivo: O aparecimento da co-infecção tuberculose/HIV e o aumento de casos de doenças provocadas por micobactérias não-tuberculosas (MNT) exigem repostas laboratoriais rápidas tanto no isolamento como na identificação das micobactérias. O objetivo deste trabalho foi avaliar a identificação das micobactérias através de sonda genética em comparação com os métodos bioquímicos clássicos. **Métodos:** Entre 2002 e 2004, foram analisadas 178 culturas de micobactérias, confirmadas como bacilos álcool-ácido resistentes e obtidas de isolados clínicos de pacientes sintomáticos respiratórios ou com suspeita clínica de tuberculose pulmonar e/ou micobacterioses, atendidos nas Unidades de Saúde da Baixada Santista. **Resultados:** A sonda genética identificou 137 amostras (77%) como complexo *Mycobacterium tuberculosis* e 41 (23%) como MNT. A discordância observada de 3% entre os métodos ocorreu apenas no ano de implantação (2002). Ao comparar os métodos, a sensibilidade, especificidade, valor preditivo positivo e valor preditivo negativo da sonda genética foram 98%, 93%, 98% e 93%, respectivamente. **Conclusões:** Apesar do custo elevado, a identificação de micobactérias pela técnica molecular é mais rápida: máximo de 3 h *vs.* 28-30 dias para os métodos clássicos. A utilização de sondas genéticas é uma técnica molecular validada, simples e disponível no mercado, com elevada especificidade, sensibilidade e rapidez, o que justifica sua implantação e uso rotineiro em laboratórios de referência, facilitando o diagnóstico e permitindo uma intervenção clínica ágil.

Descritores: Mycobacterium tuberculosis; Tuberculose/diagnóstico; Mycobacterium/classificação; Sondas DNA.

Introduction

Tuberculosis is a globally-distributed, endemic infectious disease caused by *Mycobacterium tuberculosis*.

The emergence of tuberculosis/HIV co-infection, as well as the increase in the number of multidrug-resistant strains of *M. tuberculosis* and in the number of cases of infection with nontuberculous mycobacteria (NTM), has culminated in the development of new diagnostic methods for obtaining faster results in the isolation and identification of agents.

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Due to the greater length of time required to identify mycobacteria using conventional techniques, microbiology laboratories need to be constantly updated in order to be able to identify agents more rapidly, and this has been an ongoing challenge for tuberculosis control programs.

Molecular biology has proven to be a useful tool for diagnosing tuberculosis, since it makes it possible to estimate the number of cases attributed to recent transmission of *M. tuberculosis*,[1] identify risk factors,[2] document exogenous reinfection,[3] study patterns of drug resistance,[4] and detect phenotypic differences—rapidly and with a more accurate yield than that obtained using traditional methods.[5]

Studies have evaluated the use of gene probes for the identification of mycobacteria that grow on or in culture media (solid or liquid). As an alternative to biochemical tests, gene probes have been used in combination with hybridization techniques, which allows the identification of *M. tuberculosis* complex, *M. avium* complex, M. *kansasii*, *M. intracellulare*, and *M. gordonae*.[6,7]

The use of this method in countries with a high prevalence of tuberculosis but with limited financial resources should be evaluated in comparison with the use of conventional techniques and in terms of the cost-benefit ratios involved.[8]

Therefore, as a referral laboratory in the greater metropolitan area of Santos, a region with a high incidence of tuberculosis (104/100,000 inhabitants),[9] as well as an increased incidence of forms of multidrug-resistant tuberculosis and tuberculosis/HIV co-infection,[10] our objective in this study was to evaluate, in terms of sensitivity, specificity, and timing, the identification of *M. tuberculosis* complex by means of gene probes in comparison with that obtained using classical phenotypic methods in our laboratory routine.

Methods

This was a retrospective analysis of manually registered (not computerized) data related to the laboratory routine employed in the analysis of biological samples sent to the *Instituto Adolfo Lutz* (IAL, Adolfo Lutz Institute) Santos Regional Laboratory between 2002 and 2004. The samples were collected from patients with respiratory symptoms or with clinical suspicion of pulmonary tuberculosis/mycobacteriosis who were treated at primary health care clinics in the greater metropolitan area of Santos. All samples were collected using the techniques recommended by the Brazilian National Ministry of Health (NMH).[11]

Table 1 - Distribution of the 178 strains analyzed, by method of identification. Adolfo Lutz Institute Santos Regional Laboratory, 2002-2004.

Technical identification	Gene probes	Classical methods
Mtb complex, n (%)	137 (77)	137 (77)
NTM, n (%)	41 (23)	41 (23)
Total, n (%)	178 (100)	178 (100)

Mtb: *M. tuberculosis*; and NTM: nontuberculous mycobacteria.

All positive culture samples processed in the study period and whose identification test results were obtained using both types of analysis were included.

Those samples presenting contamination of the culture material in laboratory tests, as well as technical problems that made it unfeasible to perform the identification test, were excluded from the study.

The data sources used in the research were the IAL Santos Regional Laboratory, whose sources are the Registry of Sputum Smear Microscopy and Culture for the Diagnosis and Control of Tuberculosis and the patient laboratory chart, as well as the IAL Central Laboratory.

For their grouping and for the comparative analysis of the methods, the data obtained from the laboratory registries were entered into and stored in a specific database, created using Microsoft Excel 2000 for Windows XP.

The laboratory techniques used were those routinely employed in the diagnosis of tuberculosis, standardized in accordance with norms and guidelines described in the Guidebook for Tuberculosis Bacteriology published by the NMH.[11]

Table 2 - Comparative results obtained using gene probes and classical methods in the 178 strains analyzed. Adolfo Lutz Institute Santos Regional Laboratory, 2002-2004.

Identification	Gene probes	Classical methods	
		Mtb complex	NTM
Mtb complex, n (%)	137 (77)	134 (75)	3 (2)
NTM, n (%)	41 (23)	3 (2)	38 (21)
Total, n (%)	178 (100)	137 (77)	41 (23)

Mtb: *M. tuberculosis*; and NTM: nontuberculous mycobacteria.

Sputum smear microscopy was performed using Ziehl-Neelsen staining, after each sample collected was smeared. In positive slides, semiquantitative bacillary counts were performed in accordance with the sputum smear microscopy index recommended by the NMH.[11]

Positive cultures were obtained, in accordance with standard practice in the Santos Regional Laboratory, by isolation of the mycobacterium in Middlebrook 7H9 medium (MB/BacT system™) and on Löwenstein-Jensen medium, after digestion and decontamination using the Petroff method.[12]

Subsequently, the mycobacteria isolated in Middlebrook 7H9 (MB/BacT system™) and on Löwenstein-Jensen media were identified concomitantly using a molecular method and classical biochemical methods.

The use of the gene probe system AccuProbe® (Gen-Probe Inc., San Diego, CA, USA) was performed by the IAL Santos Regional Laboratory. This is a molecular method that uses an acridine ester-labeled single-stranded DNA probe complementary to the rRNA of the target microorganism. After cell lysis, the rRNA is released, and the labeled probe combines with the rRNA, forming a probe+rRNA complex. The complex formed is detected by chemiluminescence using a luminometer, the amount of light produced being proportional to the amount of probe+rRNA complex present in the sample.[6,11] The manufacturer recommendations were strictly followed throughout the procedure.[13]

The classical biochemical methods used in the identification of mycobacteria were performed by the IAL São Paulo Central Laboratory, and are based on the analysis of the biochemical[13] and phenotypic[11] properties of the mycobacteria.

The method under study was statistically evaluated by calculating sensitivity, specificity, positive predictive value, and negative predictive value. Statistical calculations were performed in order to determine inter-method reliability values (kappa). In addition, the time required for the identification and the release of results was evaluated for both types of analysis.

Results

Of the 178 strains analyzed, 134 (75%) tested positive for *M. tuberculosis* complex using the gene probes and the classical methods, 3 (2%) tested positive for *M. tuberculosis* complex using the gene probes and positive for NTM using the classical methods, 3 (2%) tested negative for *M. tuberculosis* complex using the gene probes and positive for *M. tuberculosis* complex using the classical methods, and 38 (21%) tested negative for *M. tuberculosis* complex in both types of analysis (Table 1).

Table 3 - Annual distribution of the 178 strains analyzed, by method of identification. Adolfo Lutz Institute Santos Regional Laboratory, 2002-2004.

Year	Classical methods		Gene probes	
	Mtb complex, n (%)	NTM, n (%)	Mtb complex, n (%)	NTM, n (%)
2002	77 (43)	18 (10)	77 (43)	18 (10)
2003	32 (18)	5 (3)	32 (18)	5 (3)
2004	28 (16)	18 (10)	28 (16)	18 (10)
Total	178 (100)		178 (100)	

Mtb: *M. tuberculosis*; and NTM: nontuberculous mycobacteria.

The statistical calculation of the inter-method reliability values revealed the following: 98% observed reliability; 66% expected reliability; and 94% adjusted reliability (kappa). Gene probes presented a sensitivity of 98%, a specificity of 93%, a positive predictive value of 98%, and a negative predictive value of 93% (Table 2).

In 2002, year of implementation of gene probes, discordant results (3%) were obtained, whereas no discordant results were obtained in the other years—90% adjusted reliability (kappa)—as shown in Table 3.

Discussion

Of the total number of strains analyzed, the prevalence of species belonging to *M. tuberculosis* complex in the study period (2002-2004) was 77%. Therefore, rapid species identification is important in the diagnosis of tuberculosis.

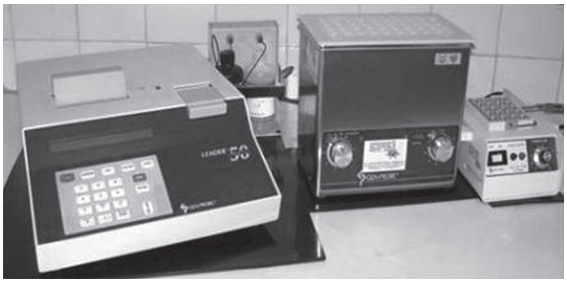

Figure 1 - Gene probe system AccuProbe®. Source: Adolfo Lutz Institute Santos Regional Laboratory.

Sputum smear microscopy is recommended as the principal diagnostic test for tuberculosis, being especially important in the screening of patients with respiratory symptoms.

In large urban areas, such as the greater metropolitan area of Santos, where the incidence and prevalence of tuberculosis is high, there is a need for more reliable and faster laboratory resources that can respond to complications such as tuberculosis/HIV co-infection, drug resistance, and infection with NTM, among other problems.

Currently, there is a tendency to accept the use of modern diagnostic methods in Brazil, and these methods have been rapidly and progressively introduced in universities, referral centers, and private health care facilities. The need for and the possibility of implementing more complex resources in public health care services should be considered.

The tuberculosis referral laboratory in the greater metropolitan area of Santos, the IAL Santos Regional Laboratory, has used gene probes to identify *M. tuberculosis* complex mycobacteria since 2002.

Therefore, in view of the impossibility of conducting a more detailed study on the cost-benefit ratio of this method, since it uses imported equipment and materials, this study evaluated the yield obtained by gene probes in comparison with that obtained by classical methods (tests performed in the IAL Santos Regional Laboratory and in the IAL Central Laboratory, respectively).

Gene probes, despite being more expensive than classical biochemical methods, are faster, yielding results in approximately 3 h. In contrast, classical methods take, on average, 28 to 30 days to yield a result, as well as requiring more staff time, incubators, glassware, and medium preparation. The laboratory protocol for gene probes is very simple, using ready-made reagents and only three pieces of equipment (Figure 1).

The results of the present study confirmed our expectations for sensitivity, specificity, positive predictive value, and negative predictive value, which were satisfactory and very close to those described in the literature.

The analysis of the gene probe performance revealed numbers similar to those described by another group of authors, who, based on 2,727 isolates obtained from cultures using the MB/BacT system™, confirmed that the sensitivity and specificity of the gene probe method was 96.4% and 100%, respectively.[14]

In a national consensus, the II Brazilian Guidelines for Tuberculosis, it was recommended that the use of gene probes be implemented in referral laboratories, since such probes are easy to use and are readily available on the market, together with the fact that it is a validated method.[15]

Based on the positive and negative predictive values found in the present study, we confirmed data in the literature on the benefits of the use of gene probes for the identification of *M. tuberculosis* complex,[16] which justifies its implementation and routine use in referral laboratories, in conjunction with classical methods, and in high-prevalence clinics where complex cases of tuberculosis are treated. The method is highly specific, sensitive, rapid, and efficient. It facilitates diagnosis, allowing early clinical intervention.

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