BRIEF REPORT

In-house PCR with DNA extracted directly from positive slides to confirm or exclude the diagnosis of tuberculosis: focus on biosafety

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KEYWORDS
M. tuberculosis; Slides; IS6110-PCR; Tuberculosis; Biosafety

Abstract The possibility to obtain DNA from smears is a valuable alternative to remedy the lack of samples when they are totally used for bacilloscopy; this technique solves the biosafety problem related to a possible accident with the transportation of flasks containing potentially transmissible clinical samples. Hence, the purpose of this study was to utilize the insertion sequence IS6110 for amplification of DNA from a smear-positive sample for tuberculosis (TB) diagnosis. Among the 52 positive bacilloscopies, sensitivity, specificity, positive predictive value and negative predictive value were 52.3%, 100%, 100% and 89.7%, respectively whereas accuracy was 90.7%. The IS6110-based PCR for TB diagnosis developed in DNA extracted from a positive smear is a fast, simple, specific, and safe method.

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PALABRAS CLAVE
M. tuberculosis; Láminas; PCR IS6110; Tuberculosis; Bioseguridad

Resumen La posibilidad de obtener ADN a partir de frotis es una valiosa alternativa para remediar la falta de muestras cuando estas son totalmente utilizadas para la baciloscopia; esta opción soluciona, además, el problema de bioseguridad asociado a la posibilidad de accidente al transportar frascos que contienen muestras clínicas potencialmente infectivas. Por lo tanto, el propósito de este estudio fue utilizar para el diagnóstico de la tuberculosis la secuencia de inserción IS6110 para amplificación del ADN a partir de frotis que resultaron positivos por baciloscopia. Del análisis de 52 baciloscopias positivas surge que la sensibilidad, la especificidad,
el valor predictivo positivo y el valor predictivo negativo de esta técnica fueron, respectiva-
mente, del 52,3%, del 100%, del 100% y del 89,7%; y la precisión fue del 90,7%. La PCR IS6110
para el diagnóstico de tuberculosis, desarrollada con ADN extraído de frotis positivos, es un
método rápido, simple, específico y seguro.

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Tuberculosis (TB) continues to be a large global health problem. In 2011, there were 84,137 notified TB cases
in Brazil, 40,289 of which showed positive-bacilloscopy, accounting for 66 % of pulmonary TB; 12,683 new cases
presented negative bacilloscopy findings, and 10,067 were extrapulmonary TB cases. In view of such data, Brazil
currently ranks 15th on the list of 22 countries presenting the highest TB indexes all over the world1-4. Nontubercu-
los Mycobacteria (NTM) also have the capacity to cause the disease, and their importance is progressively growing
with the isolation of different mycobacterial species in the laboratory. The clinical-laboratory correlation is of
crucial importance for the diagnosis and determination of a therapeutic strategy. Bacilloscopy is a simple, fast, and
inexpensive test, being the most commonly used technique for TB diagnosis. The possibility to obtain DNA from
smears is a valuable alternative to remedy the lack of samples when these are totally utilized for bacilloscopy; in addition,
this technique solves the biosafety problem related to the trans-
portation of swabs with clinical samples that are potentially transmissible, in case of an accident5,6,9,10. The amplifica-
tion of nucleic acids by means of PCR directly from smears has been described by some authors as more specific with
respect to bacilloscopy and faster than culture methods2,4,10. Positive bacilloscopy does not differentiate Mycobacterium
tuberculosis complex (MTBC) species from Nontuberculous Mycobacteria (NTM) ones; however, when associated to PCR,
it increases the accuracy of TB diagnosis3-6,8. The goal of the present study is to use the insertion sequence IS6110 for
amplification of DNA from smears for TB diagnosis and to exclude it when the smear is positive, taking biosafety into
account.

Two hundred eighty-seven smears (convenience samples) coming from the Faculty of Medicine/Laboratory of
Research of Mycobacteria/University Hospital – Federal University of Minas Gerais (Faculdade de Medicina/Laboratório
de Pesquisa em Micobacterias/Hospital das Clínicas Universidade Federal de Minas Gerais – FM/LM/HC-UFMG) were
selected and stained by the Ziehl Neelsen and Auramine method, along the period between 2010 January and 2011
December. Only those smears having culture results were included. Of the 287 smears, 26 were excluded because
they did not meet the inclusion criteria; therefore 261 smears remained. Fifty-two (52) of the smears presented
positive bacilloscopies and cultures whereas 35 had negative bacilloscopies and positive cultures and 174 negative
bacilloscopies and cultures. Seventy-two (72) of them were identified as MTBC and 15 as NTM. The culture was
developed in Löwenstein–Jensen medium and the identification test used phenotypic methods. DNA extraction was done
by Chelex-100 + Nonidet P40 (NP40) in smear where a volume of 25 μl of Chelex solution, containing 5 % Chelex-100,
1 % Nonidet P40, 1 % Tween 20, and distilled water was added on the smear. The smear was scraped off with the help of
a tip, up to complete removal; then, it was transferred to an Eppendorf tube containing 75 μl of the same solu-
tion. After stirring, the Eppendorf tube was incubated for 30 min at 100 °C. The samples were centrifuged for 10 min at
13,000 × g; then, the supernatant was transferred to a new sterile Eppendorf tube and 5 μl of the solution was utilized
for PCR1,6.

The PCR was developed under the following conditions: 7.0 μl of buffer (10 ×), 3.0 μl of 50 mM MgCl2, 0.2 μl 25 mM
DNTP, 10 pmol of every oligonucleotide (IS5-5′ CCT GCC AGC GTA GGC GTC GG 3′ and IS-5′ CTC CG! CCG TTC TG
3′), and 0.5 μl of Taq DNA Polymerase (500 U) Invitrogen9, in a final volume of 50 μl. The initial DNA denaturation
occurred at 94 °C for 2 min, and after a total of 40 cycles of 94 °C for 30 s, 68 °C for 2 min, 71 °C for 1 min, and a final
extension at 72 °C for 10 min. The amplicons were revealed in 2 % agarose gel, stained by ethidium bromide. The ampli-
cations were developed blindly. Statistical analysis: the results of sensitivity and specificity, the positive and nega-
tive predictive values, and accuracy were compared with results of culture and phenotypic identification methods1,6.
From the 87 smears with positive culture, 69 were from sputum, seven from bronchoalveolar lavage (BAL), six from
tracheal aspiration, two from axillary abscesses, two from pleural liquids, and one from secretion (abscess); there was
no sensitivity or specificity difference among these samples in the PCR amplification. The sensitivity and specificity
of DNA amplification with IS6110 were 81.5 and 100 %, respectively. The positive predictive value (PPV) was 100 %, the
negative predictive value (NPV) 80.8 %, and accuracy was 82.8 %. Among the 22 negative bacilloscopies with positive
cultures of MTB, four were positive by PCR (18 %). Among the 52 positive bacilloscopies, the sensitivity, specificity,
PPV and NPV were 52.3 %, 100 %, 100 % and 89.7 %, respectively; accuracy was 90.7 %. In Fig. 1, the electrophoresis of
PCR IS6110 amplifications is demonstrated. The possibility of obtaining DNA from smears is a valuable alternative
to solve the difficulties in transporting biological samples (long distances and biosecurity), and excludes the diagnosis
of tuberculosis when the smear is positive due to the high specificity of IS6110, which is not possible when only stained
microscopic preparations are used. The high specificity found in this study is relevant, as it avoids the introduction of unnecessary treatments and con-
ducts in the cases where bacilloscopy is positive and an NTM infection is in course. Therefore, this methodology could be
applied in places where NTM species are often isolated, such as in Brazil, for instance, where during the period between
2000 and 2008, a total of NTM infection cases of 2139 were reported. Most of such cases were notified in the Southwestern
region (1458), which includes the State of Minas Gerais, where this study was developed. The remaining cases out of
Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

References


Further reading