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SHORT COMMUNICATION

Bridging the gap between PCR detection of *Mycobacterium tuberculosis* complex and tuberculosis diagnosis

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SUMMARY

The growing demand for rapid diagnosis of tuberculosis (TB) has led to the incorporation of nucleic acid amplification (NAA) tests in case definitions. The objective of this study was to evaluate the contribution of a real-time polymerase chain reaction (PCR) assay in providing a result predictive of a confirmed TB case. Respiratory and extra-pulmonary specimens (*n* = 308) were subjected to NAA, culture and smear microscopy. Qualitative PCR assessment, translated by an increase in NAA cycles, disregarding template copy number, resulted in an increase in confirmed cases, helping to bridge the gap between the test's analytical performance and its actual performance in TB diagnosis.

KEY WORDS: *Mycobacterium tuberculosis* complex; real-time PCR; acid-fast smear microscopy; diagnosis; tuberculosis

NUCLEIC ACID AMPLIFICATION (NAA) tests and acid-fast bacilli (AFB) smear microscopy are rapid diagnostic tests for tuberculosis (TB). The low sensitivity of smear microscopy[1,2] is countered by the excellent analytical performance of commercial NAA tests. However, this may not always reflect clinical diagnosis.[2–4] Although NAA tests have been included in TB case definitions, their use as a stand-alone test is not consensual.[1,5,6]

Real-time polymerase chain reaction (PCR) is sensitive and specific for detecting *Mycobacterium tuberculosis* complex (MTC) bacilli directly from clinical specimens.[2,7] However, the choice of parameters, such as the number of amplification cycles relative to diagnostic criteria, remains open. The objective of this study was to evaluate the role of a real-time PCR assay in providing a result predictive of a confirmed TB case.

MATERIALS AND METHODS

The 308 specimens included in the study comprised 75 respiratory and 233 extra-pulmonary specimens (165 cerebrospinal fluid [CSF] alone). Contaminated specimens were processed using *N*-acetyl-L-cysteine-sodium hydroxide. Non-contaminated specimens were concentrated by centrifuging for 10 min at 13200× *g*. DNA was extracted from 100 to 500 μl of processed specimens. Ziehl-Neelsen smear microscopy and Löwenstein-Jensen (LJ) culture (at 37°C for 60 days) were performed for all specimens. AccuProbe (Gen-Probe, San Diego, CA, USA) was used to confirm MTC in NAA-negative, culture-positive specimens. The QIAamp® DNA Mini Kit (Qiagen, Izaza, Portugal) was used for DNA extraction from heat-inactivated specimens.

The artus® *M. tuberculosis* RG PCR Kit (Qiagen, Quilaban, Sintra, Portugal) was used for amplification of a single copy specific genomic region. Positive, negative, extraction and internal controls were used with each 45-cycle run on the Rotor-Gene™ 3000 (Quilaban, Sintra, Portugal). Test results were validated based on expected results for all controls.

For quantitative interpretation of NAA, quantification standards were used to generate a standard curve and calculate the number of copies of the template (directly proportional to the number of MTC bacilli present in the sample). Only copy numbers above the kit's analytical detection limit of 0.9 copies/μl were considered positive.

Qualitative interpretation of test results was based on the visual comparison of readings with those of the positive and negative controls, disregarding the template copy number or the number of cycles to a positive result.

The NAA test was interpreted blinded to the results of both smear microscopy and culture.

The test results were classified as per the European Centre for Disease Prevention and Control and World Health Organization TB case definitions.[1]

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