

A Retrospective Analysis on the Use of Algorithm for the Diagnosis of Mycobacterium Tuberculosis

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Abstract

Objective: The aim of this study was to investigate whether an algorithm has been used for the diagnosis of Mycobacterium tuberculosis samples over the last three years.

Material and Methods: A total of 1,036 samples collected between June 2010 and March 2013 were submitted to the laboratory for the diagnosis of Mycobacterium tuberculosis. The samples were studied using the Ehrlich-Ziehl-Neelsen (EZN) staining. Various culture methods such as Löwenstein-Jensen (LJ) medium-BacT/ALERT® 3D and the BD BACTEC™ MGIT™ 320 Mycobacteria Culture System were applied for 508 samples. Real-time Polymerase Chain Reaction (PCR) method was performed in 138 samples. In general, 2 or 3 samples were obtained from each patient for the diagnosis of acid-fast bacilli (AFB), whereas only one sample was obtained for culture.

Results: Of the EZN-positive samples, 30% were PCR-positive and 65% were culture-positive; of the culture-positive samples, 13% were PCR-positive and 54% were EZN-positive; of the PCR-positive samples, 21% were culture-positive and 43% were EZN-positive.

Conclusion: AFB and culture are likely to be inadequate for the determination of Mycobacterium tuberculosis. Therefore, PCR seems to be an essential need. However, the clinical table of the patient should also be considered to determine the need for PCR. Thus, an appropriate algorithm should be suggested for the diagnosis of Mycobacterium tuberculosis.

Key words: Mycobacterium tuberculosis, algorithm, EZN, culture, PCR

Introduction

The classic laboratory approach to the diagnosis of mycobacterial infections involves the phenotypic characterization of colonies growing on Löwenstein-Jensen medium. The diagnostic methods used in the processing of sputum specimens in many laboratories are performed via algorithms. A combination of phenotypic and molecular assays is recommended for the rapid identification of mycobacteria, particularly for the identification of *M. tuberculosis* (1).

One of the common sputum smear microscopy is Ziehl-Neelsen, which is a fast and low-cost technique for detecting tuberculosis (TB) in high-incidence areas but has low sensitivity (2) and high specificity (3). In a study on culture, the concordance rate between the solid and liquid cultures was 92.8%.

No Mycobacterium isolates were detected in 0.4% of the cases in the liquid culture and no Mycobacterium isolates were detected in 6.8% of the cases in the solid culture (4). These results suggest that the Mycobacterium culture method exhibits high growth rates. Used as an advanced technique, PCR appears to be a promising method for the diagnosis of pulmonary tuberculosis, even in paucibacillary specimens. Simultaneous identification and faster results are additional advantages of this method (5).

Conventional bacteriological microscopy and culture are commonly used for the diagnosis of tuberculosis, particularly in developing countries. However, their limited sensitivity, specificity, and delayed results make this provision inadequate.

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Despite the development of quicker and more sensitive novel diagnostic techniques, their complexity and high cost have limited their use in many poor-resource countries. Due to the rapidly growing TB problem in these countries, there is an urgent need to assess promising alternative methodologies in settings with high disease prevalence (6).

In this study, we aimed to examine the relationships between EZN, culture and PCR and to investigate whether an algorithm has been used in the administration of these methods.

Material and Methods

A total of 1,036 samples collected between June 2010 and March 2013 were submitted to the laboratory for the diagnosis of *Mycobacterium tuberculosis*. The samples were studied using the EZN staining. For the diagnosis of AFB, 2 or 3 samples were obtained from each patient, whereas only one sample was obtained for culture. Various culture methods including Löwenstein-Jensen (LJ) medium-BacT/ALERT® 3D and the BD BACTEC™ MGIT™ 320 *Mycobacteria* Culture System were applied for 508 samples. Real-time PCR method (Artus M. tuberculosis PCR Kits, Qiagen) was performed in 138 samples.

Results

In this study, the evaluations were based on the results obtained from 524 EZN, 499 culture, and 138 PCR analyses. Of positive results, 11 samples were positive both on EZN and culture, 4 were positive both on EZN and PCR, 1 was positive both on culture and PCR, and 2 were positive on all tests. Of negative results, 4 samples were negative both on EZN and culture, 1 was negative both on EZN and PCR, 1 was negative both on culture and PCR, and 66 were negative on all tests (Figure 1).

In the 20 AFB-positive samples, 13 were positive on culture and 6 were positive on PCR, 5 were negative on culture and 2 were negative on PCR, and no culture and PCR analyses were request for 2 and 13 samples, respectively (Figure 2).

In the 24 culture-positive samples, 13 were positive on EZN and 3 were positive on PCR, 10 were negative on EZN and 2 were negative on PCR, and no EZN and PCR analyses were request for 1 and 19 samples, respectively (Figure 2).

In the 14 PCR-positive samples, 6 were positive on EZN and 3 were positive on culture, 6 were negative on EZN and 6 were negative on culture, and no EZN and culture analyses were request for 2 and 5 samples, respectively (Figure 2).

In total, of the EZN-positive samples, 30% were PCR-positive and 65% were culture-positive; of the culture-positive samples, 13% were PCR-positive and 54% were EZN-positive; of the PCR-positive samples, 21% were culture-positive and 43% were EZN-positive.

Statistically, all three tests (EZN, PCR, culture) were used in the evaluation of the samples. However, the numbers of the samples evaluated by each test differed from each other. Therefore, no statistical measures such as sensitivity, specificity, positive prediction value, and negative prediction value were used to evaluate the performance of each test. Moreover, considering that the significance of the difference among the rates would provide no valuable information regarding the performance of the tests, no comparison was performed for the resultant rates. On the other hand, since the primary aim of the study was to investigate whether the sequential order of the tests was appropriate and to develop an algorithm for the flow assay, no statistical test or comparison was performed throughout the study. This situation can be regarded as the limitation of the study and thus further studies are recommended to take this situation into account while establishing their research designs.

Discussion

Microscopic examination and culture remain the methods of choice for the diagnosis of TB and the guidance of therapeutic decisions. Nucleic acid amplification and line probe assays speed up the identification and susceptibility testing of mycobacteria in AFB smear positive specimens or in culture (7-9). In our study, PCR was not required for 13 out of the 20 samples positive on AFB and for 19 out of the 24 samples positive on culture (Figure 2). Based on these results, it is obvious that PCR can be ignored for the *Mycobacteria* diagnosed by EZN and culture. Therefore, clinicians are likely to determine the diagnostic tests based on their own needs and thus may prefer not to use a standard algorithm.

The conventional EZN method on direct smears for AFB is widely used and plays a key role in the diagnosis and also in the monitoring of treatment (10). Although easy to perform and specific, it lacks sensitivity, requiring 10,000 bacilli.mL⁻¹ of sputum to become positive (12) with a sensitivity of 22% to 81% (10). Similarly, in a study conducted with EZN, the researchers found that the sensitivity and positive predictive values, in particular, were quite low (11). In our study, considering that culture is the gold standard, we found that almost half (10 out of 24) of the culture-positive samples were negative on EZN (Figure 2).

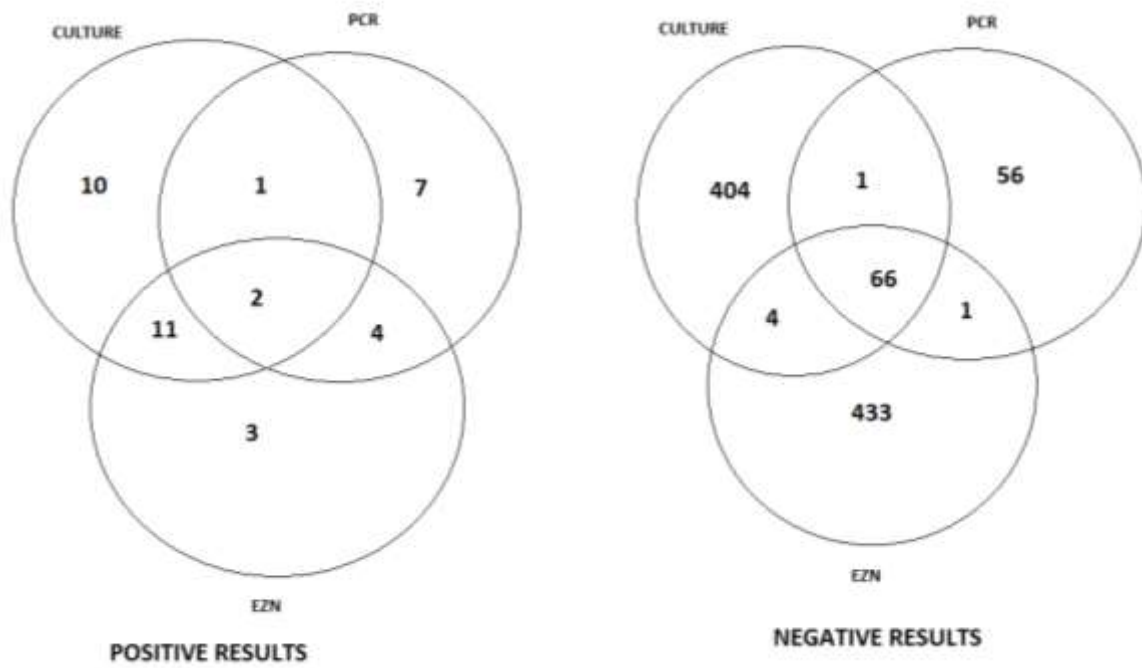


Figure 1: Combination of positive and negative results.

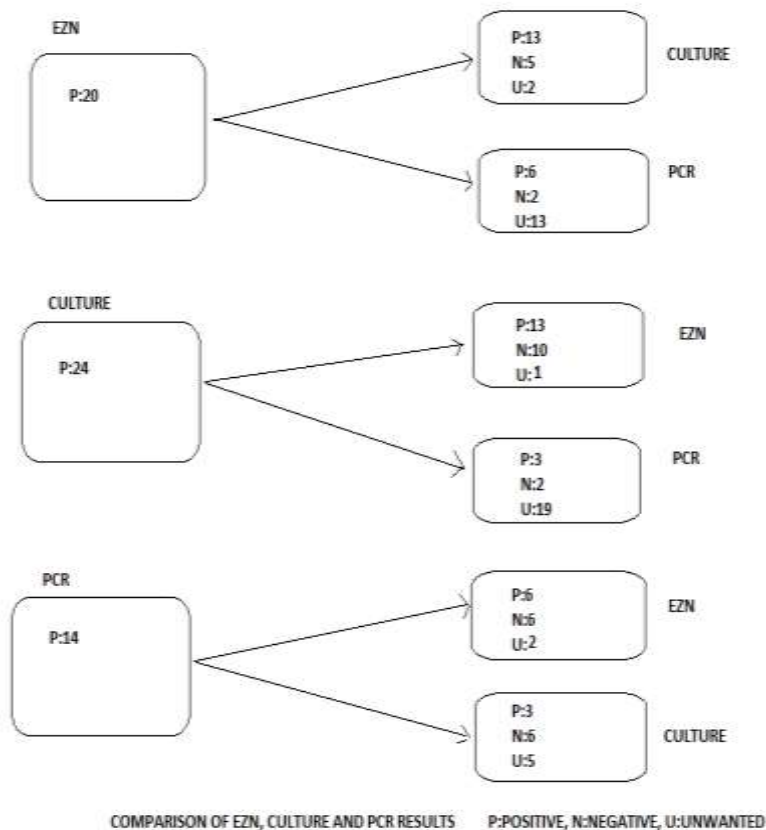


Figure 2: Comparison of EZN, Culture and PCR results [P: Positive, N: Negative, U: Unwanted (no request)]

Besides, Mycobacterial culture is the gold standard method for the detection of tubercle bacilli (70% to 80%), but it is time-consuming and requires specialized safety procedures and must be performed in a biosafety level 3 facility (10,11). It can detect 100 bacilli.mL⁻¹ of sputum in comparison with 5,000–10,000 bacilli.mL⁻¹ needed for microscopy. Following decontamination and liquefaction procedures, sputum samples are inoculated and incubated for morphological growth, which usually occurs after several weeks of incubation (12). As proposed by the algorithm, the samples can be evaluated using a sequential order of EZN, culture, and, if suitable laboratory conditions are available, PCR. In our study, the EZN was followed by culture, and the algorithm was established based on 524 EZN, 499 culture, and 138 analyses (Figure 1). At present, a number of elaborate culture systems are available commercially. Culture methods available today are sufficient to permit laboratories to develop an algorithm that is optimal for patients and administrative needs (13).

In a previous study, the responses obtained from 21 TB reference laboratories were reported. In 17 of the laboratories, the algorithms used to diagnose pediatric and adult TB patients did not differ, whereas four laboratories reported the use of extra tests for children. Most of the primary samples were subjected to smear microscopy, resulting in a 3.3% positivity rate. Culture was more often positive in non-respiratory samples than in respiratory samples. The sensitivity of molecular tests was significantly higher in smear-positive samples than in smear-negative samples (14). In this study, an algorithm was applied for all the samples sent to the laboratory. Respiratory and non-respiratory sensitivity of the culture can vary in the examples. PCR sensitivity of smear positive samples is higher. In our study, the rate of positive samples was higher than the negative samples on EZN. However, since PCR was not required in all positive samples, reliable results were not obtained at the end of the study. In addition, the rate of culture-positive samples among EZN-positive samples is high. Moreover, culture was required in most of the samples that required EZN.

Tuberculosis is globally controlled by low sensitive conventional diagnostic assays. The current gold standard for the diagnosis of tuberculosis is the combination of culture and clinical diagnosis. For rapid diagnosis of Mycobacterium tuberculosis, highly specific and sensitive assays are performed. Nucleic-acid amplification tests (NATs) support rapid diagnosis of TB, particularly in reference laboratories. Therefore, in addition to conventional diagnosis tests, which are time-consuming and labor-intensive and also insufficient for species-level identification, new solutions are required for the problems in routine diagnostic applications for tuberculosis (15).

Conclusion

The results revealed that the samples positive on EZN are not necessarily positive on culture and PCR. Moreover, culture is the gold standard for confirming the diagnosis of Mycobacteria regardless of the positivity on EZN. PCR is a rapid diagnostic test which can be used for identifying the clinical status of the suspected cases. For this reason, the implementation of an algorithm can reduce the duration of diagnosis and also produce more randomized results. On the other hand, ignoring the algorithm and not performing the basic diagnostics steps may lead to serious diagnostic problems as well as loss of time and money.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was completed due to defined rules by the Local Ethics Commission guidelines and audits.

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