Evaluation of the BacT/ALERT PZA kit in comparison with the BACTEC 460TB PZA for testing Mycobacterium tuberculosis susceptibility to pyrazinamide

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Objectives: To compare the performance of the BacT/ALERT PZA kit (BioMerieux, Marcy l’Etoile, France) with the radiometric BACTEC 460TB PZA test (Becton–Dickinson method) for testing Mycobacterium tuberculosis susceptibility to pyrazinamide.

Methods: A total of 50 M. tuberculosis strains were tested. Thirty of these strains had been previously considered pyrazinamide-susceptible and 20 pyrazinamide-resistant by BACTEC 460TB.

Results: Final overall agreement was 100%. Time needed for the susceptibility test was 6.69 days for the BacT/ALERT PZA kit versus 8.07 days for the BACTEC 460TB PZA test.

Conclusions: BacT/ALERT PZA test is an excellent alternative to BACTEC 460TB for pyrazinamide susceptibility testing.

Keywords: susceptibility testing, resistance, tuberculosis

Introduction

Pyrazinamide is a nicotinamide analogue that is a frontline drug in tuberculosis treatment. Unlike conventional antibiotics that are active mainly against growing bacteria, pyrazinamide appears to kill at least 95% of the Mycobacterium tuberculosis semi-dormant population that persists in acid pH environments inside macrophages.1 Rapid and reliable susceptibility testing for pyrazinamide is therefore very important. Pyrazinamide susceptibility testing in vitro requires acidic pH for pyrazinamide activity. In many drug susceptibility testing methods problems are faced when testing susceptibility to pyrazinamide. This is mainly because a lower pH reduces the mycobacterial growth. The BACTEC 460TB system (Becton–Dickinson Biosciences, Sparks, MD, USA) has been widely validated for ~20 years in clinical laboratories as a reliable and rapid method for testing of pyrazinamide susceptibility of M. tuberculosis isolates. However, it is semi-automated and entails disposal of a radioactive substance. BacT/ALERT is a non-radiometric antimicrobial susceptibility system for testing M. tuberculosis isolates. Its main advantages are the automated continuous monitoring and non-radiometric detection. It was initially developed to provide susceptibility results for streptomycin, isoniazid, rifampicin and ethambutol, but a new acidified vial for standardized pyrazinamide testing has recently been introduced.

The purpose of this investigation was to compare the performance of the BacT/ALERT PZA kit (BioMerieux, Marcy l’Etoile, France) with the radiometric BACTEC 460TB PZA test (Becton–Dickinson method) for testing M. tuberculosis susceptibility to pyrazinamide.

Materials and methods

A total of 50 M. tuberculosis strains isolated from human samples and selected from a culture collection of the Mycobacteria Study Group of Barcelona were tested. Thirty of these strains had been previously considered pyrazinamide-susceptible and 20 pyrazinamide-resistant by BACTEC 460TB. All resistant strains had mutations in the pncA gene (Table 1). Actively growing cultures were used to conduct the susceptibility test with each system. In BacT/ALERT PZA, each tested strain was seeded in three vials: direct control,
proportional control and pyrazinamide test. The vials, with Middlebrook 7H9 broth, were supplemented with 0.5 mL of reconstitution fluid (oleic acid, glycerol and BSA) and 0.5 mL of acidifying solution. In the pyrazinamide test vial, 0.5 mL of a pyrazinamide solution was added to give a final concentration of 100 μg/mL. An equal amount of sterile distilled water was added to the drug-free control vials. Inoculum was obtained from *M. tuberculosis* positive MP Process Vials kept in the incubator for an additional minimum of 24 and maximum of 48 h. The direct control and the pyrazinamide test vials were seeded with 0.5 mL of the test inoculum and the proportional control was seeded with 0.5 mL of a 10^{-1} dilution. Drug susceptibility testing sets were entered into the BacT/ALERT instrument and continuously monitored. An organism was determined to be susceptible when the antibiotic-containing bottle was not detected as positive or when it had a positive time to detection greater than that of the 10^{-1} proportional control. An organism was determined to be resistant when the antibiotic-containing vial had a positive time to detection that was equal to or less than that of the 10^{-1} proportional control. BACTEC 460TB pyrazinamide-susceptibility tests were performed on *M. tuberculosis* strains from a positive 12B vial—supplemented with reconstitution fluid—with a growth index ranging from 300 to 499. Pyrazinamide test medium was inoculated with 0.1 mL of a pyrazinamide drug solution to give a final concentration of 100 μg/mL. The growth control vial was inoculated with 0.1 mL of reconstitution fluid. The pyrazinamide test medium and the growth control were inoculated with 0.1 mL of the inoculum vial. All vials were incubated at 37°C and tested daily in the BACTEC 460TB instrument. Readings were evaluated according to the established criteria for calculating susceptible, resistant and borderline results.\(^2,3\)

\(M. \) *tuberculosis* H37Rv (ATCC 27294), susceptible to pyrazinamide, and *M. bovis* BCG (ATCC 35745), resistant to pyrazinamide, were included in each run for the two methods as a quality control.

When a strain showed a discrepant result between BacT/ALERT and BACTEC 460TB systems, or if the test validation criteria were not respected, the strain was re-analysed using both systems.

### Results and discussion

The majority (72% to 97%) of pyrazinamide-resistant isolates of *M. tuberculosis* exhibit mutations in their *pncA* gene or upstream area leading to loss of pyrazinamidase activity.\(^1,2\) In the present study, all of the resistant strains had their mutations in the *pncA* gene or upstream region. No technical problems were detected using either BacT/ALERT or BACTEC 460TB. However, it was necessary to re-test three strains, two because there was no growth in BACTEC 460TB and one because validation criteria for the BacT/ALERT PZA test was not met. The final overall agreement between the two systems was 100%. That is, there were no differences in the BacT/ALERT PZA test’s ability to detect the susceptible strains or the strains that were resistant to pyrazinamide. Although previously published papers testing pyrazinamide in BacT/ALERT\(^6,8\) showed similar results, this is the first study with the commercial kit.

Total turnaround time (time need for inoculum preparation and susceptibility test) was 13.87 days for BacT/ALERT PZA test versus 12.39 days for BACTEC 460TB. The susceptibility test mean time for BacT/ALERT PZA was 6.69 days, whereas it was 8.07 days for BACTEC 460TB.

In conclusion, final overall agreement between the two systems was 100%. The main advantages of the BacT/ALERT PZA test were the automated, continuous monitoring and non-radiometric detection, making the system less labour-intensive than the BACTEC 460TB. This system could thus be an excellent alternative to BACTEC 460TB for pyrazinamide susceptibility testing.

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### Transparency declarations

None to declare.

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