Validation of Bedaquiline Drug-Susceptibility Testing by BACTEC MGIT 960 System for Mycobacterium tuberculosis

Gomathi Sivaramakrishnan1, Balaji Subramanyam1, Michel Prem Kumar2, Radhika Golla1, Srikanth Prasad Tripathy1, Rajesh Mondal1
1Department of Bacteriology, ICMR-National Institute for Research in Tuberculosis, Chennai, Tamil Nadu, India

Abstract

Background: Bedaquiline (BDQ) is a new antituberculosis (TB) drug effectively used for the treatment of multidrug-resistant and extensively drug-resistant TB. However, the reports on drug-susceptibility testing (DST) for BDQ are scarce. The study aimed to validate and standardize BDQ DST by BACTEC MGIT 960 system for Mycobacterium tuberculosis. Methods: A panel of ten M. tuberculosis isolates comprising 8 BDQ sensitive and 2 BDQ resistant strains were used to test accuracy, repeatability, and reproducibility of BDQ DST by MGIT 960. BDQ DST by Middlebrook 7H11 agar method using polystyrene tubes was used as a standard method to calculate the accuracy of the validation. Results: DST by MGIT for BDQ showed 100% accuracy, repeatability, and reproducibility, although variations were observed in the growth units of the “test” MGIT tubes between technologist and drug stocks while testing for reproducibility. Conclusion: BDQ DST by MGIT 960 system is accurate, repeatable, and reproducible and hence can be implemented in certified laboratories routinely performing DST by MGIT 960 system.

Keywords: Bedaquiline, MGIT 960, Mycobacterium tuberculosis

Introduction

Bedaquiline (BDQ) belonging to the group of a diarylquinoline is the first novel anti-tuberculosis (TB) drug approved for human use in more than 40 years.1 The drug has received conditional/accelerated approval for use against drug-resistant TB. Following the approval of BDQ by the US Food and Drug Administration to treat drug-resistant TB, centers for diseases control2 issued federal guidelines on its use for the treatment of TB in children, pregnant women, and individuals with other health complications in addition to multidrug-resistant TB (MDR-TB). The World Health Organization (WHO) issued interim guidelines on the use of BDQ in combination with other TB drugs for MDR-TB and extensively drug-resistant TB (XDR-TB).3 In 2015, BDQ was approved for use in the United States, the EU, South Korea, South Africa, India, the Russian Federation, and Peru.4

In India, BDQ is in use since 2016 for the treatment of MDR-TB and XDR-TB under the conditional access program of Revised National TB Control Program.5 In the current WHO DRTB treatment guidelines,6 the use of BDQ is prioritized over other drugs, especially injectables for the treatment of DRTB. With this, the use of BDQ is likely to be multiple times higher. However, information on existing or emerging resistance to BDQ is currently very limited as a clear guideline on a standard drug-susceptibility testing (DST) method is awaited. Few premier reference laboratories have adopted different methodologies, including the minimum inhibitory concentration (MIC) using solid Middlebrook 7H11 medium or Middlebrook 7H9 liquid broth-based methods.7

DST at a critical concentration of 1 mg/L using the commercial BACTEC MGIT960 liquid culture system has been recently recommended by the WHO.8 DST to BDQ with a single concentration of the drug using MGIT960 system earlier was hampered due to adsorption of the drug to the polypropylene
plastic tubes. With the recent introduction of polystyrene tubes by the manufacturer, this issue has been resolved.

In the present study, validation following the standard protocol described in the current GCLP guidelines for BDQ DST by BACTEC MGIT 960 was carried out.

**METHODS**

**Setting**

The present study was carried out at the NABL accredited ICMR-National Institute for Research in Tuberculosis, Chennai, a Supranational Reference Laboratory under WHO and National Reference Laboratory under the Revised National Tuberculosis Control Program of India. This validation was done as part of continued efforts toward capacity building of the laboratory to be on par with international laboratories to meet crucial demands for TB control.

**Ethical statement**

As the study neither involve human clinical specimens nor clinical isolates, ethical clearance was not necessary.

**Bedaquiline drug**

The pure form of BDQ drug with 100% purity was a kind gift from Cipla Pharmaceuticals, Mumbai, India.

**Mycobacterium tuberculosis strains**

A panel of ten *M. tuberculosis* isolates comprising 8 BDQ susceptible and 2 BDQ-resistant strains were used for the study. All the strains were obtained from the Institute of Tropical Medicine (ITM), Antwerp, Belgium. MIC protocol in Middlebrook 7H11 method using polystyrene tubes approved by EUCAST was used as the gold standard. Only one BDQ-resistant strain was received and the same was duplicated. All isolates were coded for each validation parameter, and the results were compared after decoding.

**Drug stock preparation**

Drug stock was prepared at par with WHO recommended concentration. Accordingly, 1 µg/mL concentration was prepared from the lyophilized BDQ powder using the following formula. Three stocks of 1 ml each were prepared.

\[
\text{Weight (mg)} = \frac{\text{Volume (mL)} \times \text{Concentration (µg/mL)} \times \text{dilution factor/assay potency (µg/mg)}}{}
\]

- **Volume (in milliliters)** is the desired volume of stock solution
- **Concentration (micrograms per milliliter)** is the desired concentration of stock solution
- **Dilution factor** is the number of times the drug added to the tube (100 µl) is getting diluted by the total volume of the medium in the tube (8.3 ml) = 83
- **Assay potency (micrograms per milligram)** is the activity or potency specified by the manufacturer of the reference standard powder. This value usually appears on the label or the certificate of analysis

Accordingly weight (mg) = \(1 \times 1 \times \frac{83}{1000} = 0.083\) mg.

As it was difficult to weigh 0.083 mg, 100 times the weight, i.e., 8.3 mg was weighed and dissolved in 1 ml of dimethyl sulphoxide. The stocks were aliquoted at 120 µl each and stored at −80°C. At the time of MGIT DST, the stock was diluted 1:100 (100 µl of stock + 9.9 ml sterile DW) to get the desired working stock.

**BACTEC MGIT 960**

The isolates were freshly sub-cultured and DST was done at 1 µg/ml using the manufacturer’s protocol for “undefined drug.” The duration of the protocol is 3–13 days.

**Validation parameters**

**Accuracy**

BDQ DST by MGIT 960 was done for the panel of ten cultures was performed by a technologist, and the results were compared with standard method (Middlebrook 7H11 agar tested using polystyrene tubes) to calculate the accuracy of the method.

**Repeatability**

BDQ DST by MGIT 960 for the panel of ten cultures was performed in duplicates by a technologist on the same day (morning and evening) to calculate repeatability of the method.

**Reproducibility**

BDQ DST by MGIT 960 for the panel was performed by two technologists on three different days using three different batches of the drug stocks to calculate reproducibility.

**RESULTS**

**Accuracy**

BDQ DST by MGIT 960 demonstrated 100% accuracy with two known BDQ-resistant strains identified as resistant and all eight BDQ susceptible strains identified as susceptible in comparison with DST on Middlebrook 7H11 agar [Table 1].

**Repeatability**

DST by MGIT960 for the panel of cultures performed by a technologist on the same day in duplicates demonstrated 100% repeatability [Table 2].

**Reproducibility**

The results of BDQ DST by MGIT 960 performed by two technologists on 3 days using 3 different batches of drug stocks demonstrated 100% reproducibility between the users and days and between the batches [Table 3]. However, the growth unit of MGIT showed mild variations but was well within the cutoff.

**DISCUSSION**

Treatment of DRTB till recently comprised less effective but more toxic drugs for prolonged durations. BDQ has been in
Bedaquiline susceptibility testing of M. tuberculosis strains was performed using the BACTEC MGIT960 system. The commercial liquid culture-based system is considered the gold standard for phenotypic DST due to its rapidity, reliability, and reproducibility and can be subjected to quality assurance protocols. There are no conflicts of interest.

**CONCLUSION**

Bedaquiline drug susceptibility testing using BACTEC MGIT960 system has been successfully validated and may be adopted for use with clinical isolates of Mycobacterium tuberculosis.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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