Bedaquiline susceptibility testing of *Mycobacterium tuberculosis* in an automated liquid culture system

Gabriela Torrea1*, Nele Coeck1, Christel Desmaretz1, Tim Van De Parre2, Tijs Van Poucke2, Nacer Lounis3, Bouke C. de Jong1,4,5 and Leen Rigouts1,6

1Department of Biomedical Sciences, Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium; 2Quality Department, Institute of Tropical Medicine, Antwerp, Belgium; 3Clinical Virology Department, Janssen Infectious Diseases, Beerse, Belgium; 4Department of Medicine, Division of Infectious Diseases, New York University, New York, NY, USA; 5Vaccinology Department, Medical Research Council Unit, Fajara, The Gambia; 6Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

*Corresponding author. Tel: +32-33455334; Fax: +32-32476333; E-mail: gtorrea@itg.be

Received 11 February 2015; returned 16 March 2015; revised 30 March 2015; accepted 2 April 2015

Objectives: The objective of this study was to evaluate the performance of the BACTEC MGIT960 system to test the susceptibility to bedaquiline for *Mycobacterium tuberculosis* complex.

Methods: We determined the quality control (QC) range of bedaquiline using the *M. tuberculosis* H37Rv reference strain and the epidemiological cut-off (ECOFF) in MGIT960 and on Middlebrook 7H11 agar (M7H11) using 47 strains from bedaquiline treatment-naive patients. The accuracy of MGIT960 was evaluated versus M7H11 using 74 ‘probably susceptible to bedaquiline’ and 18 ‘probably resistant to bedaquiline’ strains. Repeatability and reproducibility of MGIT960 were assessed using five strains showing different resistance levels.

Results: The QC range for the H37Rv strain was between 0.125 and 0.50 mg/L. The WT MIC distribution ranged from ≤0.03 to 1.00 mg/L in MGIT960 and from ≤0.008 to 0.25 mg/L on M7H11 with suggested ECOFFs of 1.00 and 0.25 mg/L, respectively. Applying these ECOFFs, the probably susceptible and probably resistant strains were distinguishable by both methods, albeit with only a 2-fold increased MIC for one of the resistant strains compared with the ECOFF. Intermethod agreement to classify the isolates was excellent (100%). All replicates in the repeatability and reproducibility experiments fell within the normal range.

Conclusions: The MGIT960 system proved to be highly stable, reproducible and accurate relative to the M7H11 agar method for determining the bedaquiline MIC. The small margin between the suggested ECOFF and the lowest MIC for the mutant strains risks making both methods prone to discordant results. Further validation in clinical settings linked to treatment outcome data is needed.

Keywords: multidrug resistance, epidemiological cut-offs, drug susceptibility testing, MGIT960

Introduction

In 2013, 5.3% of global TB cases were estimated to suffer from MDR-TB, of whom 9% had XDR-TB, defined as TB resistant to isoniazid, rifampicin, fluoroquinolones and injectable agents. Treatment for MDR-TB was successful in only 48%, in part due to the high toxicity and long duration of the current regimens and interruptions in drug supplies. To overcome these constraints, new TB drugs have been developed with safety and efficacy being assessed in Phase II and III clinical trials for patients with MDR-TB. One of these novel drugs is bedaquiline (trade name Sirturo, formerly referred to as R207901 or TMC207).

Bedaquiline is a diarylquinoline antimycobacterial compound with a novel mechanism of action, blocking mycobacterial ATP synthesis through the inhibition of ATP synthase, resulting in bactericidal activity. The broad antimycobacterial spectrum of bedaquiline was demonstrated, including against susceptible as well as MDR *Mycobacterium tuberculosis* isolates and a wide range of other non-tuberculosis mycobacterial isolates.

In 2012, the US FDA approved bedaquiline as part of combination therapy to treat adults with MDR pulmonary TB when other alternatives are not available. One year later, in order to avoid uncontrolled use of the drug, WHO published interim policy guidance for the use of bedaquiline in treatment of MDR-TB on the basis of Phase Ib trial data. WHO strongly urged the development of accurate and reproducible drug susceptibility testing (DST) methods for bedaquiline and recommended that ‘in the absence of a specific drug-susceptibility test, resistance to
bedaquiline should be monitored through assessment of minimum inhibitory concentrations (MICs). Previous studies conducted at the Institute of Tropical Medicine (ITM; Antwerp, Belgium), under the auspices of Tibotec Pharmaceuticals (Johnson & Johnson, Beerse, Belgium), suggested that a presumptive MIC for bedaquiline-susceptible M. tuberculosis complex was \(<0.25\) mg/L as determined on Middlebrook 7H11 (M7H11) agar and by resazurin microtitre assay (REMA). The cut-off value \(\leq0.25\) mg/L was approved by EUCAST as a breakpoint for the use of bedaquiline (EUCAST breakpoint). Results of DST for bedaquiline in the automated BACTEC MGIT960 liquid culture system have not been published. The development of a standardized reference method for testing the susceptibility of M. tuberculosis strains to bedaquiline is all the more important given the recent recognition of a cross-resistance mechanism with the old antimycobacterial drug clofazimine. This non-target-based cross-resistance mechanism is based on mutations in the Rv0678 gene, a repressor of the MmpS5-MmpL5 efflux pump. It is to date unknown whether prior treatment with clofazimine affects the efficacy of treatment with bedaquiline.

The main goal of our study was to evaluate the performance of MGIT960 for DST of bedaquiline against the M. tuberculosis complex by determining the quality control (QC) range for the H37Rv strain and assessing its accuracy, reproducibility and repeatability. In an attempt to set the antimicrobial susceptibility testing breakpoint in absence of a previously reported cut-off or critical concentration, we determined the WT distribution of MIC values, or epidemiological cut-off (ECOFF), obtained in MGIT960 and on M7H11.

Materials and methods

Study design and strains

We determined the QC range of bedaquiline MICs to assess the impact of procedural variation on test performance. The pan-susceptible M. tuberculosis strain H37Rv was tested in three different batches of MGIT medium (Lot 1: BD 31550064, Lot 2: BD 3155063 and Lot 3: BD 3144339) twice a week for 5 weeks, resulting in 30 replicates. For each replicate, individually prepared inoculum suspensions were used. Isoniazid was tested as control drug at concentrations of 0.012, 0.025, 0.05 and 0.10 mg/L on only one of the three lots of MGIT medium (Lot 1) for a total of 10 replicates. The mode, geometric mean and standard deviation were calculated and the range of dilutions at which inhibition of the QC strain was observed was noted. The proposed expected QC range was based on the mode \(\pm\) one doubling dilution for a three-dilution range that should include \(\geq95\%\) of the observed MIC values.

We subsequently established the WT (probably susceptible (PS) to bedaquiline) distribution of bedaquiline MICs to determine the ECOFF value in MGIT960 and on M7H11 as a measure of the breakpoint to declare a strain resistant to bedaquiline. To do so, 47 strains isolated from bedaquiline and clofazimine treatment-naive patients from different geographical regions were selected from the collection of the Mycobacteriology Unit at ITM. Among these strains, 22 were fully susceptible to first- and major second-line drugs. The remaining strains included 5 monoresistant and 20 polydrug-resistant strains including 16 MDR strains. The details about the resistance patterns and geographical origin of the strains are shown in Table S1 (available as Supplementary data at JAC Online).

The ECOFF was defined as the upper MIC value of the WT distribution. We subsequently established the WT distribution of bedaquiline MICs to assess the impact of procedural variation on test performance. The pan-susceptible M. tuberculosis H37Rv strain was tested in three different batches of MGIT medium (Lot 1) for a total of 10 replicates. The mode, geometric mean and standard deviation were calculated and the range of dilutions at which inhibition of the QC strain was observed was noted. The proposed expected QC range was based on the mode \(\pm\) one doubling dilution for a three-dilution range that should include \(\geq95\%\) of the observed MIC values.

We then assessed the accuracy of MGIT960 versus M7H11, which was considered the reference method for bedaquiline DST. A second set of 31 ‘PS to bedaquiline’ strains (Table S1) as well as 20 ‘probably resistant (PR) to bedaquiline’ strains (Rv0678 mutants plus one atpE mutant) (Table S2) were tested in parallel. Among the second set of ‘PS to bedaquiline’ strains, 14 were fully susceptible to first-line drugs and the remaining strains were mono- or polyresistant. The Rv0678 mutant strains had been selected on clofazimine-containing M7H11 for which bedaquiline resistance patterns were reported by REMA (N. Coeck, ITM, unpublished results). Additionally, the bedaquiline-resistant reference strain BK12 (ITM no. 121749, available through Belgium Coordinated Collections of Microorganisms (BCCM)/ITM), a known atpE mutant, was tested. The MIC values obtained for the strains used in the second experiment (ECOFF determination) were also included in the analysis. Strains with an MIC value higher than the ECOFF were considered phenotypically resistant to bedaquiline and those with MIC values less than or equal to the ECOFF were considered susceptible. The accuracy was calculated in terms of efficacy as follows: \([\text{true susceptible results in MGIT960} + \text{true resistant results in MGIT960}]/\text{total number of results}\) × 100.

The reproducibility and repeatability were evaluated by repeated testing of five strains (Table S3), including the pan-susceptible M. tuberculosis H37Rv, the in vitro-selected bedaquiline-resistant mutant BK12 and three clinical M. tuberculosis strains with low (0.03 mg/L), medium (0.25 mg/L) and high (1.00 mg/L) MIC values of bedaquiline previously characterized by REMA (data not shown). For evaluating the repeatability (intra-assay precision), the strains were tested in MGIT960 in triplicate, starting from the same bacterial suspension, on the same day by one operator. For evaluating reproducibility (interassay precision), the strains were tested in three different weeks by two operators, each time with a different bedaquiline stock solution and bacterial suspension. Reproducibility and repeatability were analysed as the percentage of MIC values having a maximum one 2-fold dilution difference. More than 95% of replicates having a maximum one 2-fold dilution difference of the MIC values was considered acceptable.

Antimicrobial agents

Pure bedaquiline powder was gifted by Johnson & Johnson (Beerse, Belgium, cat. no. 16175328) and isoniazid powder was obtained from Sigma–Aldrich (I3377). Bedaquiline was dissolved in DMSO (Sigma D5879) and isoniazid in sterile purified water. The stock solutions were made at a concentration of 10,000 mg/mL and stored in small aliquots at below \(-18^\circ\)C for bedaquiline and at 2–8\(^\circ\)C for isoniazid. The drug solutions were aseptically prepared and considered self-sterilizing. The stock solution of bedaquiline was diluted to 1000 mg/mL with DMSO and subsequent dilutions were made in Middlebrook 7H9 (M7H9) for MGIT960 and sterile distilled water for M7H11. The test concentrations used were obtained by serial 2-fold dilutions from 2.00 to 0.03 mg/L for MGIT960 and from 2.00 to 0.008 mg/L for M7H11. Frozen drug solutions were thawed once and discarded afterwards; working solutions were not stored. The stability of bedaquiline stock solutions stored at below \(-18^\circ\)C for up to 3 months had been determined previously by REMA (data not shown).

Test methods and media

MIC determination was performed with MGIT960 growth supplement for DST in the MGIT960 system (Becton Dickinson). The procedure
followed was the standard protocol recommended for DST of first-line drugs (SIRE) using the BACTEC MGIT960 built-in software (version 3.05A). The bacterial suspensions were prepared from subcultures performed on Löwenstein–Jensen medium that were between 3 and 4 weeks old. Briefly, a suspension of 1.0 mg/mL was prepared and allowed to rest for 20 ± 2 min in order for large bacterial clumps to settle. The supernatant was transferred to another sterile tube and allowed to rest for another 15 ± 2 min before transferring the supernatant to another tube and adjusting to 0.5 mg/mL using M7H9. This suspension was diluted 1:5 in sterile physiological water to be used as DST inoculum. The drug-free control was inoculated with 0.5 mL of a 1:100 dilution of inoculum representing 1% of the bacterial population. The algorithm used for interpretation of the MGIT960 results was the same as for the SIRE protocol proposed by the manufacturer. The MIC was determined to be the lowest drug concentration that tested susceptible, i.e., <100 growth units by automated reading when the control vial turned positive (>400 growth units).

The lower-end MIC value of the range was reported as ‘≤’, e.g. all MIC values of <0.03 and those of 0.03 were treated as ≤0.03. If the MIC value was preceded by the ‘>’ sign, the value was reported as ‘>’ the next dilution, e.g. MIC values reported as >2 were treated as ≥4.

MIC determination on M7H11 medium enriched with OAD supplement was performed in polystyrene tubes containing serially diluted drug concentrations and two drug-free tubes as controls to calculate the proportion of inhibition. Bacterial suspensions adjusted to 1 mg/mL with sterile distilled water were allowed to rest for 15 ± 2 min and subsequently diluted to 10−1 and 10−2 with sterile distilled water. The drug-containing tubes and one of both drug-free tubes (controls) were inoculated with 0.1 mL of bacterial suspension 10−1 and the second control tube with bacterial suspension 10−2. All tubes were incubated at 34–38°C with 5%–10% CO2 for 21 days.

Growth in the drug-containing tubes was compared, by visual evaluation by an experienced reader, with the drug-free control tubes inoculated with bacterial suspension 10−3. A result was only considered valid if both controls showed sufficient growth (at least five colonies on the most diluted control tube and ~100 times more growth on the less diluted control tube). The MIC was defined as the lowest concentration that inhibited >99% of growth.

Rv0678 was analysed by Sanger sequencing. A 686 bp fragment was amplified using primers 30F22 (5′-AGCGGAAACTCGTACTCCAC-3′) and 916R20 (5′-GCTGGACAACACGGTCACCT-3′). The PCR was performed with a final volume of 50 μL containing 10 mM Tris-HCl (pH 8.6), 50 mM KCl, 1.65 mM MgCl2, 0.1% Triton x-100, 200 μM of each deoxynucleoside triphosphate, 12.5 pmol of each primer, 1.5 U Taq polymerase (Promega, Madison, WI, USA) and 2 μL of DNA extract. The PCR was performed as follows: the DNA extract was added at 92°C; 5 min at 95°C; 44 cycles of 45 s at 95°C, 1 min 30 s at 72°C, and a final extension of 10 min at 72°C. The amplified product was sequenced in both directions by automated sequencing using the ABI 3730xl Genetic Analyzer. The sequences were aligned with the M. tuberculosis H37Rv sequence (GenBank accession no. NC_000962). QC of MGIT960 broth and the drug-free and bedaquiline-containing M7H11 was assessed by H37Rv as per routine practice. The acceptable bedaquiline MIC range for H37Rv on M7H11 was 0.03–0.12 mg/L based on previous clinical trial (C208) results. For MGIT960, the QC range obtained in the first experiment was used in the remainder of the experiments.

**Results**

The QC range experiment yielded 29 out of 30 MIC values. One replicate was not retained for analysis due to a growth control failure.

**Table 1.** MICs of bedaquiline and isoniazid in MGIT960 for the *M. tuberculosis* H37Rv strain

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>bedaquiline, Lot 1</th>
<th>bedaquiline, Lot 2</th>
<th>bedaquiline, Lot 3</th>
<th>isoniazid, Lot 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1, day 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 1, day 2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 2, day 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 2, day 2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 3, day 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 3, day 2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 4, day 1</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.025</td>
</tr>
<tr>
<td>Week 4, day 2</td>
<td>0.12</td>
<td>0.125</td>
<td>0.125</td>
<td>0.05</td>
</tr>
<tr>
<td>Week 5, day 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.12</td>
<td>0.025</td>
</tr>
<tr>
<td>Week 5, day 2</td>
<td>1.00</td>
<td>0.5</td>
<td>0.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

ND, no data due to failure of growth control.
The distribution of the bedaquiline MIC values for the H37Rv strain revealed a mode of 0.25 mg/L and a geometric mean of 0.17 mg/L (Figure 1). The calculated range was found to be 0.125–0.50 mg/L, with 97% (28/29) of the observations falling within this range, showing an acceptable variability of MGIT960 to test bedaquiline. The range observed for the QC strain should be used to routinely validate the test. This implies that any MIC of bedaquiline obtained in MGIT960 for a clinical isolate is only valid if the MIC for the H37Rv tested on the same batch of MGIT is between 0.125 and 0.50 mg/L. All isoniazid replicates yielded an MIC of 0.025 or 0.05 mg/L (Table 1).

Regarding the ECOFF determination, we obtained interpretable MIC values for 45 out of 47 PS strains in MGIT960 due to insufficient control growth in two tests, whereas all these strains yielded valid results on M7H11. A clear MIC distribution was observed for the two methods (Figure 2). Values were systematically higher in MGIT960 compared with M7H11. The WT MIC in MGIT960 ranged from $\leq 0.03$ to 1.00 mg/L, whereas on M7H11 the value ranged from $\leq 0.008$ to 0.25 mg/L. The suggested ECOFFs are therefore 1.00 mg/L for MGIT960 and 0.25 mg/L for M7H11.

As shown in Figure 3, the distribution of bedaquiline MIC values for both PS and PR (Rv0678 mutant) strains revealed a clear bimodal pattern in MGIT960 and M7H11. MIC values for the Rv0678 mutant strains ranged from 0.50 to $\geq 4.00$ mg/L in MGIT960 and from 0.06 to $\geq 4.00$ mg/L on M7H11 agar (details provided in Table S4). Using the above-suggested ECOFF, one mutant strain was classified as susceptible by both methods. Interestingly, this strain showed a heterogeneous Rv0678 gene (mixture of WT + T276A and WT + T359A). In contrast, three additional strains with a heterogeneous Rv0678 gene were classified as resistant to bedaquiline by MGIT960 and M7H11 (Table S4). We excluded the results obtained for these four strains in the final analysis because we could not definitively classify them as PR. The remaining, homogeneous mutants ($n=16$) were clearly distinguishable from the PS strains by both methods.

One strain (ITM no. 13-0667) from the second set of PS strains was classified as resistant to bedaquiline in MGIT (MIC $\geq 4$ mg/L) and M7H11 (MIC 0.5 mg/L) using the same ECOFF values as cut-offs. Sequencing of the Rv0678 gene revealed an insertion [Tyr92 (TAT)$\rightarrow$92 (ATAT)]. Consequently, this strain was then classified as PR to bedaquiline and included as such in the analysis and Figure 3.

Table 2 shows the correlation between MGIT960 and M7H11 agar. Using the proposed ECOFF values, the percentage of concordance was 100% with a $k$ value of 1.00 showing an excellent strength of agreement.

The efficacy of bedaquiline DST in MGIT960 was 100% when using the 0.25 mg/L ECOFF for M7H11 agar, meeting the predefined acceptance criterion of $\geq 95\%$.

The reproducibility and repeatability experiments yielded interpretable results for 39 out of 40 replicates due to one growth control failure. As shown in Table 3, bedaquiline MIC values in MGIT960 for all replicates differed by a maximum of one dilution in 100% (39/39) of the strains tested, confirming good reproducibility and repeatability.
Our study showed a three-dilution QC range centred on the mode consistent with the CLSI guidelines and the range obtained for the control drug isoniazid was in line with values previously reported. These results allowed us to confirm the reproducibility and repeatability of the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains as susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains as susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.

The concordance between MGIT960 and M7H11 was excellent using the ECOFF values established in this study. Moreover, the MGIT960 system showed an excellent efficacy to distinguish susceptible from resistant strains. However, considering the one-dilution difference between the ECOFF determined in MGIT (1.00 mg/L) and the lowest MIC value for the mutant strains (2.00 mg/L) observed in this study, we caution that this method could misclassify mutant strains susceptible to bedaquiline when tested at the cut-off concentration of 1.00 mg/L due to the accepted methodological variability of maximum error. Nevertheless, as the clinically relevant breakpoint has not been established for bedaquiline, MIC determination rather than testing at a single concentration remains recommended.

A limitation of this study includes the lack of an explanation for an MDR strain from a new patient without overt clofazimine exposure to reveal a resistant pattern by both methods was isolated from a new TB case diagnosed in the context of a drug resistance survey. As it is highly unlikely that the patient had been exposed to bedaquiline or clofazimine in the past, because these drugs were not used for TB treatment at that time, we cannot explain this unexpected finding. The three other heterogeneous isolates tested resistant to bedaquiline by both methods were excluded from analysis because of the unclear impact of the heterogeneous genes on the phenotypic DST results; further evaluation of this phenomenon exceeded the scope of our study.
and 0.25 mg/L for M7H11 as cut-off (breakpoint concentrations), we were able to clearly distinguish PR from PS strains on M7H11 and in MGIT960. However, the MGIT960 and M7H11 ECOFFs being only one 2-fold concentration lower than the lowest MIC value for mutant strains suggests that both methods may risk misclassifying mutant strains as susceptible.

Further studies evaluating the pharmacokinetic/pharmacodynamic and clinical data from a high number of drug-susceptible and MDR-TB patients will be needed to set the definite clinical breakpoint.

Acknowledgements
We thank Janssen Pharmaceutica, Beerse, Belgium for generously providing the bedaquiline powder.

Funding
This work was supported by ‘INTERRUPTB’ (grant number 311725) and ‘Agentschap voor Innovatie door Wetenschap en Technologie’ (‘Innovation by Science and Technology’) (grant number IWT 130308). The consumables were provided by ITM, Belgium.

Transparency declarations
ITM receives financial support from Janssen Pharmaceutica, Beerse, Belgium to test isolates from the clinical trials for bedaquiline and in co-funding of IWT grant 130308. Janssen Pharmaceutica had no influence on the design, execution or analysis of the described experiments. N. L. owns Johnson & Johnson stocks and contributed the bedaquiline powder as well as feedback on the manuscript. All other authors: none to declare.

Supplementary data
Tables S1 to S4 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
11 Salfinger M, Migliori GB. Bedaquiline: 10 years later, the drug susceptibility testing protocol is still pending. Eur Respir J 2015; 45: 317–21.