# Evaluation of the MGIT 960/EpiCenter TB eXiST system for drug susceptibility testing for *Mycobacterium abscessus* group

**Running title: MGIT 960/EpiCenter TB eXiST system used as drug susceptibility testing for *Mycobacterium abscessus* group**

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## ABSTRACT

**Background:** Drug susceptibility test (DST) of the *Mycobacterium abscessus* group (MAG) and other rapidly growing nontuberculous mycobacteria by conventional microplate techniques is complicated due to inducible resistance to clarithromycin and other technical factors. This study evaluated the application of the BACTEC MGIT 960/Epicenter TB eXiST for DST of MAG clinical isolates.

**Methods:** *M. abscessus* ATCC19977 was used as the reference strain for the standardizing the DST by MGIT 960 and as the internal control for testing of 31 clinical isolates tests submitted to a reference laboratory for DST and confirmed as MAG. Clarithromycin genotyping was performed for the loci in the *rrl* and *erm*(41) genes known to impact resistance phenotype.

**Results:** The 31 MAG isolates included 14 *M. abscessus*, 8 *M. massiliense*, and 9 *M. bolletii*. Using conventional microplate technique according to CLSI guideline, the isolates had a high percentage of resistance for cefoxitin (93.5%) and imipenem (100%), and sensitivity for amikacin (96.7%). Comparing microplate and MGIT 960 results across those 93 pairs of results (31 isolates x 3 antibiotics), 73 (80.6%) were concordant and the remaining 18 (19.4%) represented minor errors; there were no major or very major errors. Concordance was 100% for amikacin, 84% for imipenem and 58% for cefoxitin. Clarithromycin DST by microplate identified 14 isolates as susceptible (all susceptible by MGIT 960), 3 isolates as resistant after 3 days incubation, and 14 isolates demonstrating inducible resistance from Day 5 through 14. Among the latter isolates, MGIT 960 reported 8 as resistant and 6 as intermediate, without modifications to the protocol developed. For all isolates, the observed clarithromycin susceptibility phenotypes were consistent with the genotypes.

**Conclusion:** The present study is the first description of a DST protocol for MAG isolates using the MGIT 960/Epicenter TB eXiST system. The protocol developed provided highly reliable results based on direct comparison with the conventional microplate method, including, without further modification, detection of isolates with inducible-resistance to clarithromycin. Laboratories using MGIT 960 for DST of other mycobacteria may find benefit to incorporating MAG into their routine.

Key-words: *Mycobacterium abscessus* group, Drug susceptibility testing, REMA, BACTEC MGIT 960, TB eXiST system

## 1. Introduction

Isolates of the *Mycobacterium abscessus* group (MAG) represent the most frequent species of rapidly growing mycobacteria (RGM) causing clinically significant infection, accounting for 80% of lung disease caused by RGM[1-4]. MAG isolates are widely distributed in the environment and have been associated with both nosocomial and community-acquired opportunistic infections in compromised hosts and in persons with underlying chronic lung disease, including patients with cystic fibrosis (CF)[1-4]. With advances in diagnostic methods, there has been a worldwide increase in the reported incidence of MAG infections[5].

Treatment of these infections is substantially complicated by the high frequency of resistance to many antimicrobials. Consequently, effective management requires drug susceptibility testing (DST) of each clinical isolate[5,6].

The DST method recommended by the Clinical and Laboratory Standards Institute (CLSI) is the microwell technique for determining the minimal inhibitory concentration (MIC), which is defined as the lowest concentration of the drug that inhibits the growth of the microorganism. Although this approach is well-established, there are technical challenges in applying the method to RGM.

Carvalho et al. (2016)[7] reported a standardized method incorporating resazurin, an oxidation-reduction indicator useful for demonstrating bacterial growth, in conventional microwell MIC testing of MAG isolates, with particular attention to detection of induced-resistance to clarithromycin (CLR) with extended incubation. The addition of the dye made visual readings of the microwell cultures easier and facilitated consistency among different observers.

The fully-automated Bactec Mycobacterium Growth Indicator Tube 960 system (MGIT 960) with Epicenter TB eXiST software (Becton Dickinson-BD, v.6.20) allows implementation of different protocols, thus enabling the system to test various drugs and concentrations. Based on World Health Organization (WHO) recommendations that liquid media are preferred for both culture[8] and susceptibility testing[9] of *M. tuberculosis* (MTB), the MGIT 960 system has been established in the network of public and private laboratories in the state of São Paulo, Brazil[10].The TB eXiST software also offers the potential to adapt the system to mycobacteria with different growth rates. Recent publications have validated protocols for susceptibility testing of slow-growing nontuberculous mycobacteria[11,12]. This report now extends the

BACTEC MGIT 960 system with TB eXiST software to rapidly-growing mycobacteria, which, as expected, require a different protocol. Results for DST of MAG isolates obtained using the MGIT 960 are compared with MICs determined by the microplate method using resazurin staining (REMA).

## 2. Material and Methods

### 2.1 Bacterial strains

We selected 31 clinical isolates of MAG submitted by outside clinical laboratories for species confirmation and DST to the Tuberculosis and Mycobacteriosis Laboratory at the Adolfo Lutz Institute, Brazil. Subspecies identification was performed by PRA-*hsp*65 and confirmed by *rpo*B gene sequencing[13,14]. *M. abscessus* ATCC19977$^T$ reference strain was used for assay development and as the control strain during subsequent studies.

### 2.2 Genotyping for Clarithromycin Resistance

Mutations in *erm*(41) and *rrl* genes associated with resistance and susceptibility to CLR were identified using the protocol of Carvalho et al. (2018)[15]. The sequences were analyzed using BioNumerics version 7.1 (Applied Maths, TX, USA). The reference sequence for the *erm*(41) gene was that of *M. abscessus* MAB 30 (Genbank number EU590129), and for *rrl* gene, *M. abscessus* ATCC 19977$^T$ (Genbank number NC010397.1).

### 2.3 Minimal Inhibitory Concentration Determination by microplate method (REMA)

The MIC protocol was performed as recommended by CLSI (2018)[6], modified by addition of the vital stain resazurin as color indicator to facilitate visual growth detection (Carvalho et al. 2016)[7]. The drugs tested were CLR, amikacin (AMK), cefoxitin (FOX) and imipenem (IPM), which represent agents recommended for the treatment of *M. abscessus* infections by the American Thoracic Society (ATS)[15]. The test was done in 96-well microplates using cation-adjusted Mueller-Hinton broth (CAMHB) without Oleic Albumin Dextrose Catalase (OADC) growth supplement. Two types of plates were prepared, one with only CLR and the other with the three remaining antibiotics tested. This design was chosen to permit assessment of clarithromycin-induced resistance, which requires an extended incubation period of 14 days[15]. The MIC was defined as the lowest drug concentration that prevented growth; susceptible, intermediate, and resistant were defined as recommended by CLSI (2018). For CLR, isolates that met criteria for

susceptibility on Day 3, but were classified as resistant at subsequent reading up to Day 14, were reported as “inducible resistance” (IR). Susceptibility results based on *in vitro* growth inhibition cannot be assumed to predict the clinical outcome of infections treated with the drug.

### 2.4 Inoculum standardization for MGIT 960/EpiCenter TB eXiST susceptibility testing

Per the manufacturer recommendations, the standard inoculum for the media only growth control is determined empirically as that which generates a positive growth signal after 72 hours incubation. To evaluate different inocula, a bacterial suspension of 0.5 McFarland of the ATCC 19977$^{T}$ was prepared in sterile distilled water. This suspension was used to prepare dilutions ranging from $10^{-1}$ to $10^{-8}$. Subsequently, 0.5 mL aliquots of each dilution were added to MGIT tubes containing 0.8 mL of media with or without OADC supplementation, as detailed in Results.

Established protocols applying MGIT 960 for DST of *M. tuberculosis* have been designed to be consistent with the traditional Proportion Method in which resistance is defined by as outgrowth by 1% or more of the inoculum at the critical drug concentration. In the MGIT 960 system, that required the inoculum of the concurrent growth controls be 100-fold lower than the inoculum used in the presence of drugs. The appropriate dilution for DST of MAG by BACTEC MGIT 960 was determined by evaluating a range of dilutions prepared as described above.

### 2.5 Evaluation of the BACTEC MGIT 960/EpiCenter TB eXiST susceptibility testing

Each isolate was tested in BACTEC MGIT 960/TB eXiST system at multiple concentrations for each drug and designated susceptible, intermediate, or resistant as specified by CLSI (Table 1)[6].

### 2.6 Data analysis

Results obtained by MGIT 960 system were compared with those by microplate MIC method detailed above and classified as follows: concordant, the same result was obtained by both methods; minor error, an intermediate result by one method with a susceptible or resistant result by the other; major error, MGIT 960 incorrectly reported a susceptible isolate as resistant; or very major, MGIT incorrectly reported a resistant isolate as susceptible[18].

## 3. Results

### 3.1 Bacterial strains identification

Among the 31 MAG isolates in the study set, all 11 isolates designated *M. abscessus* type 1 by PRA-*hsp*65 typing were identified as *M. abscessus* subsp. *abscessus* by *rpoB* sequencing. Of the 20 *M. abscessus* type 2 isolates, eight were identified *as M. abscessus* subsp. *massiliense*, nine as *M. abscessus* subsp. *bolletii,* and three as *M. abscessus* subsp. *abscessus.*

### 3.2 Minimal inhibitory concentration determination with REMA

ATCC 19977$^T$ demonstrated inducible resistance to CLR, intermediate resistance to FOX, susceptibility to AMK, and resistance to IPM. Among the 31 clinical isolates the distribution of susceptibility results by microplate testing differed across the antibiotics evaluated and the species of the isolates (Table 2). For clarithromycin, overall 45% of isolates tested were susceptible, with 100% among *M. abscessus* subsp. *massiliense* and none among *M. abscessus* subsp. *bolletii*; 45% presented induced resistance and 10% were resistant. In contrast, all isolates were susceptible to amikacin, but none were fully susceptible to imipenem. For cefoxitin, 90.5% of isolates were intermediate susceptible.

### 3.3 Association of *erm*(41) and *rrl* genotypes and clarithromycin susceptibility phenotypes

For all 31 isolates, the genotypes at *erm*(41) and *rrl* were compared with the results of CLR microplate susceptibility testing using the Day 14 reading and the subspecies identification (Table 3).

PCR analysis indicated the presence of deletions in *erm*(41) among 11 of the 14 clarithromycin-susceptible isolates, including all 8 *M. abscessus* subsp. *massiliense*. Of the three susceptible isolates without deletions, all were *M. abscessus* subsp. *abscessus* that carried the *erm*(41) T28C point mutation. Among the 17 resistant isolates, PCR indicated that all had an intact WT *erm*(41). Sequencing of *rrl* identified only a single isolate with the A2058G mutation; that isolate also had intact *erm*(41) and was resistant to clarithromycin. Thus, across all 31 MAG isolates CLR susceptibility by microplate testing was consistent with *erm*(41) and *rrl* genotypes.

### 3.4 Inoculum preparation for MGIT 960/TB eXiST susceptibility testing

Initially, growth of ATCC 19977$^T$ was assessed in CAMHB supplemented with OADC using dilutions of $10^{-1}$, $10^{-2}$, $2x10^{-4}$, $10^{-4}$, $2x10^{-5}$ and $10^{-8}$. The BACTEC MGIT 960 system requires that the proportional growth control to reach 400 growth units (GU) after 3.0 days. Growth of ATCC 19977$^T$ in MGIT tube with OADC was detected after one day of incubation for all dilutions up to $10^{-4}$, and after two and three days of incubation for the $2x10^{-5}$ and $10^{-8}$ dilutions, respectively. Subsequently, the $10^{-4}$ dilution was evaluated with and without OADC enrichment and bacterial growth was detected after two and four days of incubation, respectively. Similar results were obtained with a subset of the clinical isolates. Thus, for MAG isolates, which have a faster intrinsic growth rate than MTB or slow-growing nontuberculous mycobacteria (NTM), OADC enrichment resulted in accelerated growth rates inconsistent with the timeframes and endpoints specified for the growth control (GC) in the MGIT 960 system. Consequently, in all subsequent work OADC was omitted from both growth control and drug testing tubes.

The appropriate dilution for DST of MAG by MGIT 960 was determined using three isolates with different susceptibility profiles for CLR by REMA. The three isolates – ATCC 19977$^T$, which demonstrates inducible resistance, plus two clinical isolates, one fully susceptible and one strictly resistant – were evaluated using $10^{-2}$ and $10^{-3}$ dilutions prepared as described. Only the $10^{-2}$ dilution consistently provided the expected susceptibility profiles for all three CLR phenotypes (data not shown) and was subsequently confirmed as satisfactory for the other antibiotics.

### 3.5 Demonstration of the BACTEC MGIT 960/TB eXiST susceptibility test

The TB eXiST software monitors the growth of the organisms over time under different culture conditions and plots the results on a graph with vertical axis representing growth units and the horizontal axis, the number of days of incubation. Figure 1 displays the results for an assay of ATCC 19977$^T$ with CLR. The solid blue line is the growth control (the $10^{-4}$ dilution), which reached 400 GU at 3.5 days. The dotted and dashed blue lines represent $10^{-3}$ and $10^{-2}$ dilutions, respectively, which reached 400 GU in <72 hours and, as noted above, would not be valid controls. The other colored lines represent the growth curves in the presence of different concentrations of CLR. The black vertical line (a mix dots and dashes) to the far right marks the endpoint of the assay, which is prespecified in the TB eXiST software as seven days after

the GC tube reaches 400 GU; in the example shown, GC reached 400 GU at 3.5 days, and so the assay endpoint is 10.5 days.

The interpretation of the MGIT 960 system is based on the incubation time at which growth at the breakpoint (also referred to as the critical concentration) for the antibiotic being tested (e.g., 2 mg/L for CLA) reaches 100 GU relative to the time the GC reaches 400 GU and the time of the assay endpoint. Specifically, the isolate is considered resistant if growth in the presence of antibiotic achieves 100 GU *before* the GC reaches 400 GU; intermediate, if it reaches 100 GU *after* the GC gets to 400 GU, but *before* assay endpoint; and susceptible, if it fails to grow at all or reaches 100 GU only *after* the assay endpoint. In the assay shown in Figure 1, ATCC 19977$^T$ in the presence of CLR 2 mg/L (green line) achieved 100 GU just after 5.5 days incubation, and the isolate is therefore assessed as intermediate susceptibility for CLR. Of note, the organism met criteria for intermediate susceptibility in the presence of CLR across a wide range of concentrations from 0.5 through 8 mg/L, consistent with the inducible resistance phenotype demonstrated in conventional DST using REMA.

The results of CLR susceptibility testing for *M. abscessus* subsp. *abscessus* isolates 1656 and 2566 using the MGIT 960 system are shown in Figures 2 and 3, respectively. At the breakpoint, isolate 1656 achieved 100 GU at 5.25 days, at least 1 full day before the GC curve reached 400 GU, and thus meets criteria for resistant. Isolate 2566 demonstrates susceptibility to CLR, with no growth observed in the presence of any drug concentration tested. In media alone isolate 2566 achieved 400 GU at 4.8 days indicating a valid assay. For both isolates, the MGIT 960 results were concordant with MIC testing.

Using the MGIT 960 system, ATCC 19977$^T$ was assessed as susceptible to AMK, intermediate resistant to FOX, and resistant to IPM (curves not shown). All three results were concordant with MIC testing.

### 3.6 Application of BACTEC MGIT 960/TB eXiST susceptibility test

Using the procedure developed as detailed above, the 31 study isolates were tested for susceptibility to CLR, AMK, FOX, and IPM by BACTEC MGIT 960/TB eXiST system (Table 4) and the results compared to those obtained with MIC susceptibility testing using the REMA protocol (Table 5).

For amikacin, there was 100% concordance with 30 isolates susceptible and one resistant in both systems. For imipenem, 26 isolates were resistant by both systems. The remaining five isolates were also resistant by MIC testing, but were intermediate by MGIT 960, and thus represented minor errors.

For cefoxitin, both methods gave the same results for 18 isolates, which included one susceptible, two resistant, and 15 intermediate. All the discrepancies were minor errors, including 12 isolates assessed as intermediate by REMA, but resistant by MGIT 960 and one isolate that was susceptible by REMA, but intermediate by MGIT.

Comparison of the two methods for CLR requires consideration of the phenomenon of inducible resistance, which, using the REMA method, could only be identified by extending the duration of incubation from 3 days to 14 days. By that technique, all 14 isolates assessed as susceptible (that is, no growth thru Day 14), were also susceptible by MGIT 960; among the three isolates that were resistant (growth at ≥8 mg/L at Day 3), MGIT 960 assessed two as resistant and one as intermediate. The remaining 14 isolates first demonstrated resistance by REMA during the extended incubation period (Day 5 to 14), thereby meeting criteria for inducible resistance. Among those 14 isolates, the MGIT 960 protocol, applied as described without modification, reported 10 isolates as resistant and 7 as intermediate. Accepting the results for the inducible resistant isolates as concordant, then the overall concordance rate for CLR was 97% (30/31). The specific observations in both methods for the 14 inducible resistant isolates are detailed in Table 6. There was no apparent correlation between the duration of incubation required to detect inducible resistance by REMA and whether the isolate was reported as intermediate or resistant by MGIT 960.

## 4. Discussion

Drug susceptibility testing of MAG and other RGM has become of greater clinical importance with the increasing frequency, diversity, and morbidity of infections due to these organisms. The MIC procedures recommended by CLSI represent the current state of the art, but the methodology is technically demanding. Further, because CLR is one of the few agents for which susceptibility by *in vitro* testing correlates with improved clinical outcomes, the accurate detection of inducible resistance is critical and requires the laborious extension of incubation and monitoring from 3 days to 14 days. In core laboratories

providing DST of NTM to a network of clinical sites, a reliable, automated system would be highly desirable.

The BACTEC MGIT 960 system is an automated system originally applied to drug susceptibility testing of MTB, is recommended by the WHO for that purpose, and is currently in wide use. Recently, the system has been enhanced by release of TB eXiST software that supports the use of multiple protocols, including those developed by end users. Lucke et al. (2012)[12] applied the MGIT960/TB eXiST to slowly growing NTM. This report describes the development of a protocol for using this technology to perform DST of RGM.

We used ATCC 19977$^T$ as the primary control strain for developing the methodology and then analyzed the DST results obtained by both the REMA method and the MGIT 960 system for four clinically relevant antibiotics (CLR, FOX, IPM, and AMK) against 31 clinical MAG isolates. The interpretations were compared at the breakpoints specified by CLSI, using the MIC results as the current standard. Across the 124 pairs of results there were no instances of major or very major errors, i.e. no MGIT assays reporting susceptible or resistant where the MIC method reported the opposite. Across all four agents, the MGIT 960 results were strictly concordant in 44 (98%) of 45 instances where MIC testing indicated susceptibility to the antibiotic. The sole exception was a cefoxitin-sensitive isolate reported as intermediate by MGIT 960. Among all the remaining comparisons both systems reported either resistant or intermediate (or in the case of CLR inducible-resistant). Thus, overall, the MGIT 960 system correctly provided the clinically relevant information – antibiotic susceptible or non-susceptible – for 123 (99.2%) of the 124 combinations of agents and organisms tested, with the sole exception being in the direction of a conservative discrepancy.

The analysis of the CLR results is modestly complicated by the phenomenon of inducible resistance (IR). In conventional 3-day MIC testing, all isolates with IR would be falsely reported as susceptible. Detecting IR requires a modified procedure in which the plates are read on multiple occasions over an extended 14-day incubation. Although this procedure is effort intensive, it is reliable, with genotyping indicating that all isolates assessed as either R or IR had intact *erm*(41) and conversely, in all isolates assessed as S that locus was either mutated or absent. This is consistent with studies indicating that the presence of

clarithromycin promotes the activation of the *erm*(41) gene, leading to methylation of the drug binding site and rendering the drug ineffective.

There is no analogous protocol modification appropriate to the MGIT 960 system. However, all isolates that were R or IR by the REMA method were reported as R or I by the MGIT 960 system, and all S isolates gave concordant results with both systems. Thus, applying the standardized workflow described for the MGIT 960 system provided the clinically relevant result. Consequently, we suggest that, to provide the appropriate therapeutic guidance, isolates assessed as clarithromycin intermediate by the MGIT 960 system should be interpreted as resistant.

## 5. Conclusion

Although larger scale studies are required to confirm the robustness of the methodology described here, this report clearly supports the expectation that the BACTEC MGIT 960/TB eXiST, already validated for *M. tuberculosis*[7] and previously demonstrated applicable to slow-growing mycobacteria,[10] can also be used for DST of MAG isolates and, presumably, other rapidly-growing mycobacteria. Thus, for central laboratories that have invested in the MGIT 960/TB for DST of MTB and are also responsible for testing NTM , the system has the potential to offer a single methodology that has multiple advantages, including less manipulation, lower risk of technical errors, a uniform, automated, interpretative algorithm, and explicit documentation of the assay result.

## 6. Acknowledgements

We thank Dr. Miguel Viveiros and Diana Machado, from Universidade Nova de Lisboa, for critical reading of the manuscript.

## 7. Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grant number 2014/50094-4). Technical support was offered by BD for the use of MGIT960/EpiCenter TB eXiST.

## 8. Transparency declarations

None to declare.

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**Table 1.** Drugs concentrations used for susceptibility testing in the BACTEC MGIT960 system and the breakpoints of each drug.

| Drugs | Concentrations used in the MGIT960 system (mg/L) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** |
| **Clarithromycin (CLR)** | X | X | BP | I | R | | | | |
| **Imipenem (IPM)** | | | | BP | I | I | R | | |
| **Amikacin (AMK)** | | | | | | BP | I | R | |
| **Cefoxitin (FOX)** | | | | | | BP | I | I | R |

BP, breakpoint for susceptibility, the MIC that defines a susceptible isolate (CLSI, 2018).
I, intermediate; R, resistant; X, additional concentrations tested;

**Table 2.** Drug susceptibility profiles by microplate method for MAG isolates by species.

| Species | CLR | | | FOX | | | IPM | | | AMK | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **S (N=14)** | **IR (N=14)** | **R (N=3)** | **S (N=2)** | **I (N=27)** | **R (N=2)** | **S (N=0)** | **I (N=0)** | **R (N=31)** | **S (N=30)** | **I (N=0)** | **R (N=1)** |
| *M. abscessus* | 6 | 6 | 2 | 1 | 12 | 1 | 0 | 0 | 14 | 13 | 0 | 1 |
| *M. bolletii* | 0 | 8 | 1 | 0 | 8 | 1 | 0 | 0 | 9 | 9 | 0 | 0 |
| *M. massiliense* | 8 | 0 | 0 | 1 | 7 | 0 | 0 | 0 | 8 | 8 | 0 | 0 |

S: susceptible, I: intermediate, IR: induced resistant, R: resistant. CLR: clarithromycin; FOX: cefoxitin; IPM: imipenem; AMK: amikacin.

**Table 3.** Clarithromycin susceptibility by microplate method after 14 days incubation and genotype profile of the *erm*(41) and *rrl* genes.

| Genotype | | | Susceptibility to CLR[a] | | | Subspecies within *M. abscessus* group | | |
|---|---|---|---|---|---|---|---|---|
| ***rrl*** | ***erm*(41)** | **N** | **S (N=14)** | **IR (N=14)** | **R (N=3)** | ***M. abscessus* (N=14)** | ***M. bolletii* (N=9)** | ***M. massiliense* (N=8)** |
| WT | WT | 16 | – | 14 | 2 | 7 | 9 | – |
| A2058G | WT | 1 | – | – | 1 | 1 | – | – |
| WT | Deletions[b] | 11 | 11 | – | – | 3 | – | 8 |
| WT | T28C | 3 | 3 | – | – | 3 | – | – |
| A2058G | T28C | – | – | – | – | – | – | – |

a. CLR, clarithromycin; S, susceptible after 14-days incubation; R, resistant after 3-days incubation; IR: inducible resistance demonstrated during extended 14-day incubation. See text for details.

b. Two deletions in *erm*(41) of 2 bp (nucleotides 64–65) and 274 bp (nucleotides 159–432)

**Table 4**. Drug susceptibility results obtained by the BACTEC MGIT 960/EpiCenter TB eXiST method for MAG isolates by species.

| Subspecies | CLR | | | FOX | | | IPM | | | AMK | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R | S | I | R |
| *M. abscessus* | 6 | 5 | 3 | 1 | 9 | 4 | 0 | 2 | 12 | 13 | 0 | 1 |
| *M. bolletii* | 0 | 2 | 7 | 0 | 4 | 5 | 0 | 3 | 6 | 9 | 0 | 0 |
| *M. massiliense* | 8 | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 8 | 8 | 0 | 0 |

S: susceptible, I: intermediate, R: resistant.

**Table 5**. Comparison of the drugs susceptibility profiles of 31 MAG isolates for amikacin, imipenem, cefoxitin, and clarithromycin obtained by the microplate MIC and MGIT 960 methods.

| Antibiotic | Crit. Conc. | MGIT 960 | REMA | | |
|---|---|---|---|---|---|
| **AMK** | **16 mg/L** | | S | I | R |
| | | S | 30 | – | – |
| | | I | – | – | – |
| | | R | – | – | 1 |
| **IPM** | **4 mg/L** | | S | I | R |
| | | S | – | – | – |
| | | I | – | – | 5 |
| | | R | – | – | 26 |
| **FOX** | **16 mg/L** | | S | I | R |
| | | S | 1 | – | – |
| | | I | 1 | 15 | – |
| | | R | – | 12 | 2 |
| **CLR** | **2 mg/L** | | S | IR | R |
| | | S | 14 | – | – |
| | | I | – | 6 | 1 |
| | | R | – | 8 | 2 |

Antibiotics: CLR, clarithromycin; FOX, cefoxitin; IPM, imipenem; AMK, amikacin.

Susceptibility: S, susceptible; IR, inducible resistance (applicable only to CLA by microplate MIC with extended 14-day incubation); I, intermediate; R, resistant.

**Table 6**. Comparison between the time of detection of inducible resistance to clarithromycin by microplate MIC compared with results of DST using the MGIT 960 system.

| Isolate | REMA | | | | | | | MGIT 960 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC (mg/L) | | | | | Interpretation | | Day of growth | | Cut-off Day | Interp |
| | Day 3 | Day 5 | Day 7 | Day 10 | Day 14 | Day 3 | Day 14 | Growth Control | 2.0 mg/L | | |
| 3288 | <0.5 | **8** | >64 | >64 | >64 | S | IR | 5 | 6 | 11 | I |
| 5127 | <0.5 | **8** | >64 | >64 | >64 | S | IR | 5 | 6 | 13 | I |
| 2334 | <0.5 | **>64** | >64 | >64 | >64 | S | IR | 5 | 4 | 11 | R |
| 3696 | <0.5 | **>64** | >64 | >64 | >64 | S | IR | 5 | 3 | 13 | R |
| 1477 | <0.5 | 4 | **32** | 64 | >64 | S | IR | 7 | 9 | 14 | I |
| 2526 B | <0.5 | <0.5 | 4 | **64** | 64 | S | IR | 6 | 5 | 13 | R |
| 3988 | <0.5 | <0.5 | 4 | **>64** | >64 | S | IR | 6 | 6 | 13 | R |
| 2878 | <0.5 | <0.5 | 4 | **8** | >64 | S | IR | 6 | 6 | 13 | R |
| 818 | <0.5 | <0.5 | <0.5 | **8** | 16 | S | IR | 7 | 12 | 14 | I |
| 2754 | <0.5 | 1 | 2 | **8** | >64 | S | IR | 5 | 9 | 12 | I |
| 307 | <0.5 | 1 | 2 | **32** | >64 | S | IR | 7 | 6 | 14 | R |
| 3872 | <0.5 | <0.5 | <0.5 | **32** | 32 | S | IR | 8 | 7 | 14 | R |
| 5720 | <0.5 | 2 | 2 | **>64** | >64 | S | IR | 7 | 6 | 14 | R |
| 2335 | <0.5 | <0.5 | <0.5 | 1 | **>64** | S | IR | 7 | 9 | 14 | I |

S: susceptible, I: intermediate, IR: induced resistant, R: resistant. For REMA, on the day inducible resistance was detected (MIC ≥8 mg/L), the observed MIC is shown in **bold**.

## Figures and Legends

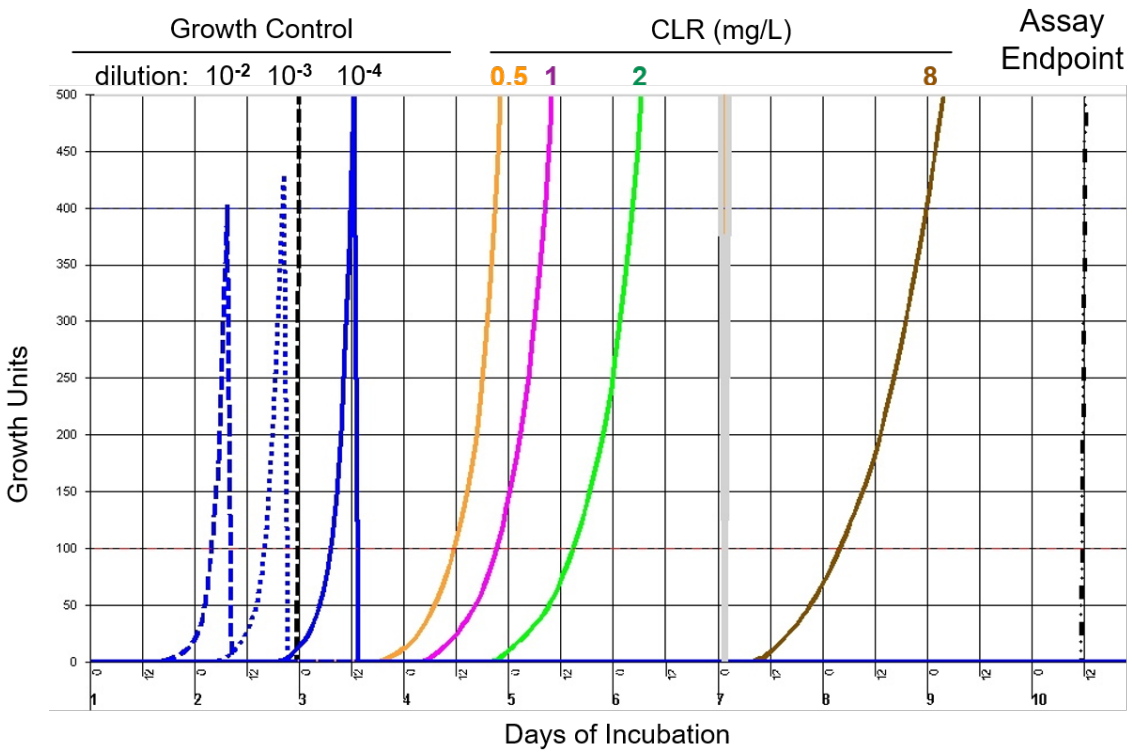


**Figure 1.** Growth curves of *M. abscessus* ATCC 19977$^{T}$ in MGIT 960 system. Growth control tubes (without drug) were inoculated with dilutions of $10^{-2}$ (dashed blue), $10^{-3}$ (dotted blue) and $10^{-4}$ (solid blue). Only the $10^{-4}$ dilution achieved 400 Growth Units (GU) after 3.0 days of incubation. The other dilutions reached that growth level too quickly to be valid controls. All tubes with CLR were inoculated with the 10^-4 dilution. At the breakpoint for CLR (2 mg/L), the culture achieved 100 GU at 5.6 days, after the growth control (3.5 days) and before the prespecified assay endpoint (10.5 days, i.e., 7 days after the growth control; dashed-dotted vertical black line). Consequently, ATCC 19977$^{T}$ was classified as intermediate (I) susceptibility.

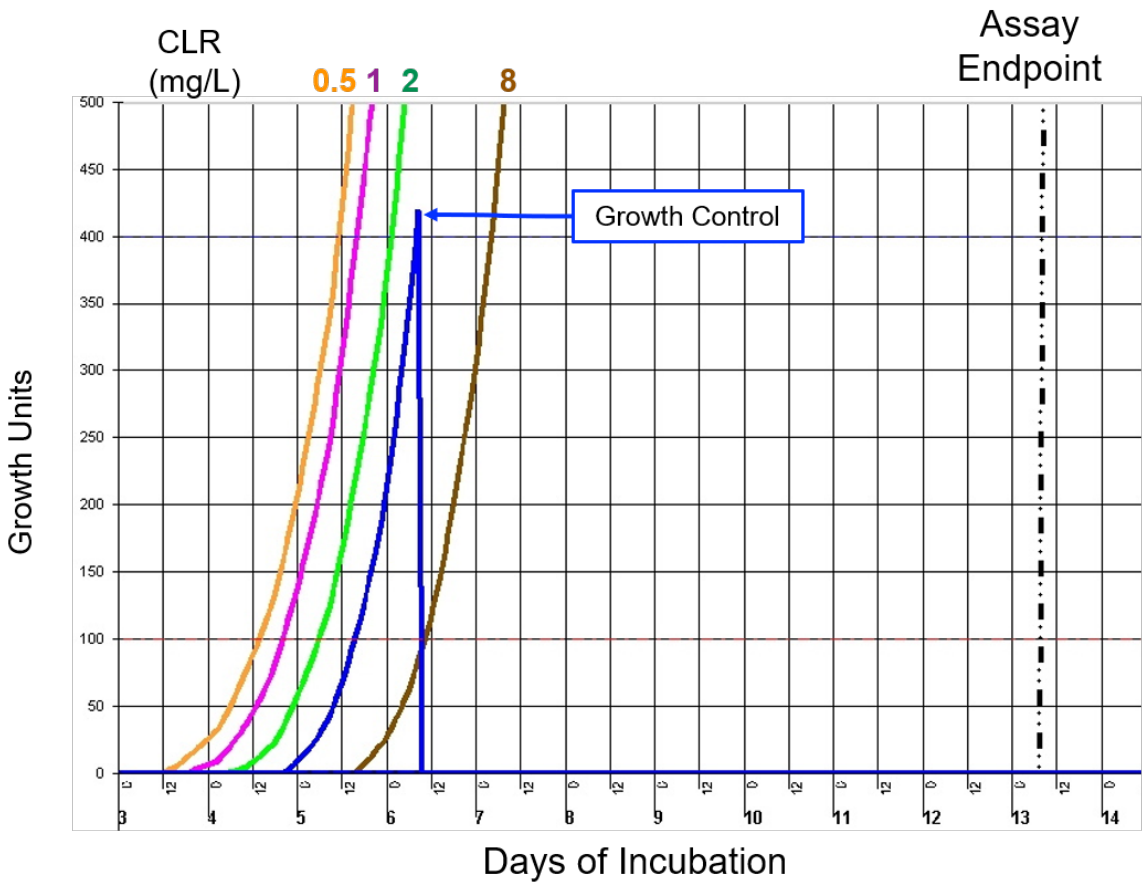


**Figure 2.** Growth curves of *M. abscessus* subsp. *abscessus* isolate #1656 in MGIT 960 system, including Growth Control (without drug - in blue) and with CLR at 0.5 (orange), 1 (pink), 2 (green), and 8 mg/L (brown). At the susceptibility breakpoint (2 mg/L), the organism achieved 100 GU at 5.25 days, at least 1 full day before the Growth Control reached 400 GU, and, therefore, the isolate was assessed as resistant (R).

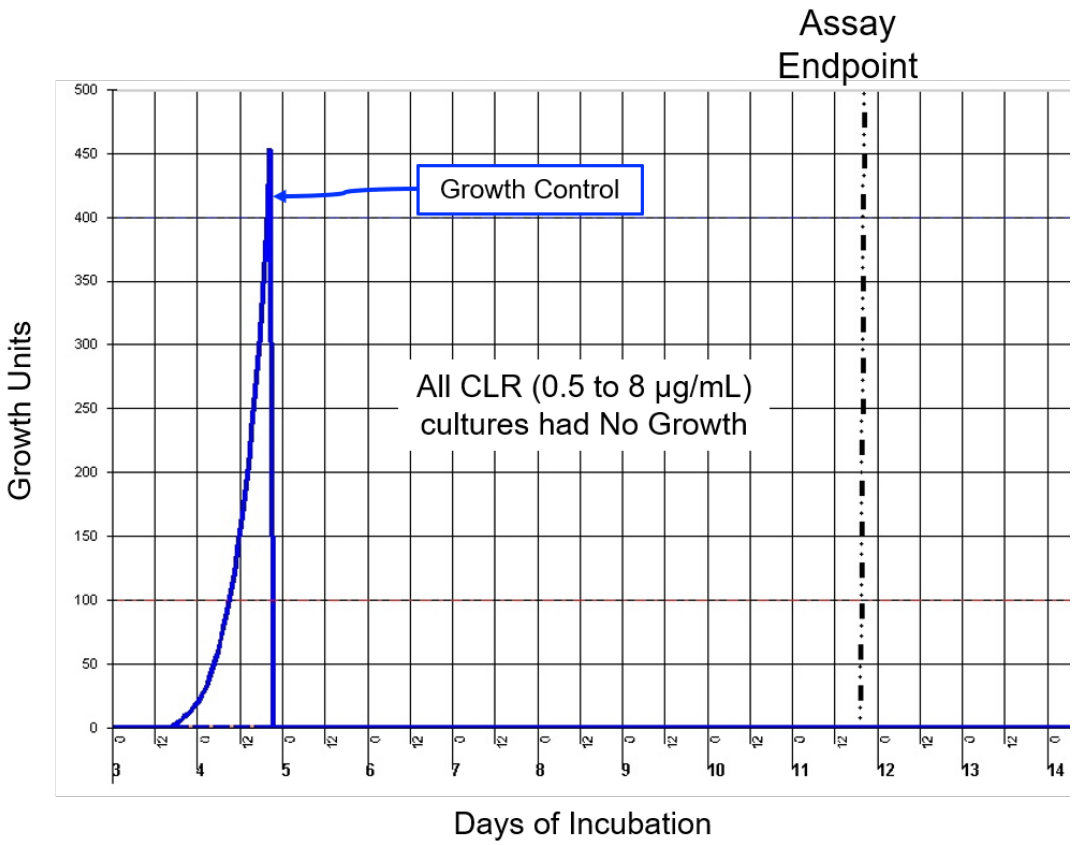


**Figure 3.** Growth curves of *M. abscessus* subsp. *massiliense* isolate #2566 in MGIT 960 system, including Growth Control (without drug). In parallel, the same inoculum was incubated in cultures with CLR at 0.5, 1, 2, and 8 mg/L and no growth was detected at any time. Therefore, the isolate was assessed as susceptible (S).

**List of abbreviations**

MAG - *Mycobacterium abscessus* group

RGM - Rapidly growing mycobacteria

CF - Cystic fibrosis

DST - Drug susceptibility testing

CLSI – Clinical and Laboratory Standards Institute

MIC – Minimal inhibitory concentration

CLR – Clarithromycin

MGIT 960 – Mycobacterium Growth Indicator Tube 960

WHO – World Health Organization

MTB - *Mycobacterium tuberculosis*

REMA – resazurin staining

AMK – Amikacin

FOX - Cefoxitin

IPM – Imipenem

ATS - American Thoracic Society

CAMHB – Cation-adjusted Mueller-Hinton broth

OADC – Oleic Albumin Dextrose Catalase

IR – Inducible resistance

GU - Growth units

GC – Growth control

NTM – Nontuberculous mycobacteria