Poster No. P1626

# Evaluation of the Thermo Scientific Oxoid Imipenem/Relebactam (10/25 µg) Antimicrobial Susceptibility Testing (AST) Disc against the predicate AST disc

***J. Bovill[1],** S. Bhalerao[1], A. Lovatt, D. Staats[2], M. Hendrix[2], K. Hajek[2], K. Young[3], M. Motyl[3].

[1]Thermo Fisher Scientific, Basingstoke, UK; [2]Thermo Fisher Scientific, Cleveland, OH, USA; [3] Merck & Co., Inc., Rahway, NJ, USA

## ABSTRACT

### Background

Imipenem/relebactam, a novel β-lactam/β-lactamase inhibitor combination is indicated for the treatment of complicated urinary tract infections (cUTI) and complicated intra-abdominal infections (cIAI) in addition to hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP). The objective of this study was to evaluate the performance and reproducibility of the new Thermo Scientific™ Oxoid™ Imipenem/Relebactam (10/25 µg) (IMR35) Antimicrobial Susceptibility Testing (AST) Disc (Thermo Fisher Scientific, Basingstoke, UK) against a predicate disc imipenem/relebactam 10/25µg MASTDISCS™ (Mast Group Ltd., Bootle Merseyside, UK).

### Methods

The Oxoid IMR35 discs and IMR35 MASTDISCS were tested simultaneously against 350 clinical, 109 challenge isolates and 19 reproducibility isolates including Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus-baumannii complex*. Recommended Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) quality control (QC) organisms were tested simultaneously on each testing day against 2 lots of Oxoid discs and 1 lot of Mast Discs. All isolates were tested in accordance with CLSI M02/M100 and the EUCAST disk diffusion method using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA supplied by Thermo Fisher). All testing was conducted by Thermo Fisher Scientific (Basingstoke, UK).

### Results

Overall, a categorical agreement of 99.44%, 95.56% and 100% was achieved for Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus-baumannii complex*, respectively, when the Oxoid IMR35 disc was compared to the predicate device. All data showed 99.4% reproducibility within-reader and between-reader by calculating as the percent of results which were less than ±3 mm of the modal value. QC results were within the stated limits, 100% of the time for each batch and reader.

### Conclusions

When compared to the Mast Disc, the Oxoid IMR35 disc demonstrated an equivalent level of performance. The high categorical agreement obtained by the Oxoid IMR35 disc indicates that this is an acceptable method for the antimicrobial susceptibility testing of imipenem/relebactam.

## INTRODUCTION

Imipenem (Figure 1) is a novel β-lactam (carbapenem) antibiotic that inhibits cross-linking of peptidoglycan during cell wall synthesis by inactivating penicillin binding proteins, ultimately leading to bacterial cell lysis and death[1].

Relebactam (Figure 2) is a novel β-lactamase inhibitor of class A and C β-lactamases, it can protect imipenem from degradation by Ambler class A and class C β-lactamases and Pseudomonas-derived cephalosporinase (PDC)[1].

Imipenem/cilastatin/relebactam is approved in the USA and EU for the treatment of hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP) in adults and other Gram-negative infections caused by the following susceptible Gram- negative microorganisms: *Enterobacter cloacae, Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Serratia marcescens.* It is also indicated for the treatment of complicated urinary tract infections (cUTIs) [including pyelonephritis] and complicated intra-abdominal infections (cIAIs), in adults with limited or no alternative treatment options[1].

An *in vitro* study was conducted by Thermo Fisher Scientific to evaluate the performance and reproducibility of the new imipenem/relebactam 10/25 µg (IMR35) Oxoid AST disc against a predicate disc.

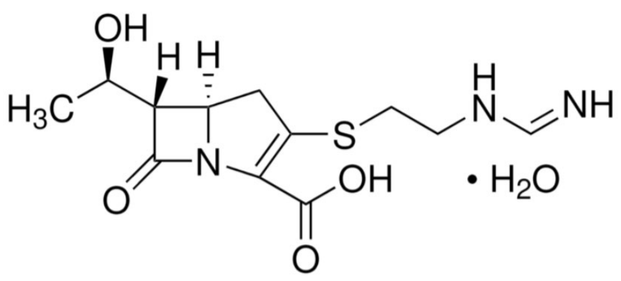


**Figure 1**. The chemical structure of imipenem.

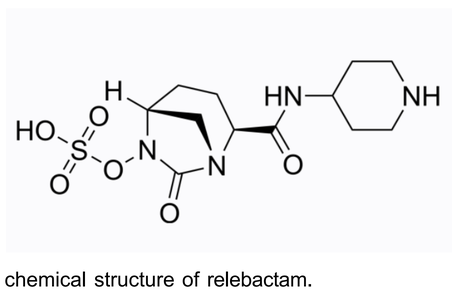


**Figure 2**. The chemical structure of relebactam.

## MATERIALS AND METHODS

All isolates were tested in accordance with CLSI M02[2]/M100[3] and the EUCAST disk diffusion method[4] using FDA-cleared Remel MHA.

Colony counts (CC) were performed as per CLSI M02[2] (13th Ed.) guidelines for at least 10% of clinical and challenge isolates and all QC and reproducibility isolates.

### Quality control

Quality control strains from the American Type Culture Collection (ATCC) were tested daily for both Mast discs and Oxoid discs alongside clinical, challenge and reproducibility isolates to ensure all AST discs were within the QC limits (Table 1). At least 20 replicates of the quality control strains were tested per individual (3 independent individuals) to represent 3 testing sites.

The QC strains must be within specification for at least 95 % of the results during study to meet acceptance criteria[5].

**Table 1.** QC zone size limits for IMR35 AST discs[3,6].

| QC organism | QC limit (mm) | Disc Diffusion method |
|---|---|---|
| ***Escherichia coli*** (ATCC® 25922™) | 27-33 | CLSI, EUCAST |
| ***Pseudomonas aeruginosa*** (ATCC® 27853™) | 26-31 | CLSI, EUCAST |
| ***Klebsiella pneumoniae*** (ATCC® BAA-2814™) | 22-28 | CLSI, EUCAST |
| ***Klebsiella pneumoniae*** (ATCC® BAA-1705™) | 23-29 | CLSI |
| ***Klebsiella pneumoniae*** (ATCC® 700603™) | 26-32 | CLSI |

### Reproducibility

Two lots of Oxoid IMR35 discs were tested and read by three independent individuals against 19 indicated and on-scale reproducibility isolates over a 3-day testing period to generate a total of 343 data points.

≥ 95 % of the results must be within 3 mm of the modal zone diameter to meet acceptance criteria[5].

### Clinical and challenge isolates

One lot of Oxoid IMR35 discs was tested against one lot of IMR35 Mast Discs for a total of 459 clinical and challenge isolates including Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter calcoaceticus-baumannii complex* and *Haemophilus influenzae* (Table 2). All isolates were shared between three independent individuals (approximately 153 isolates each) to represent three testing sites which were then analysed using breakpoints set by EUCAST[7] (Table 3).

Categorical agreement (CA) must be ≥ 90 % when compared to the predicate device and the very major discrepancy and major discrepancy rate must be ≤ 3 % each to meet acceptance criteria[5].

**Table 2.** Number of isolates tested during the study.

| Isolates | Number Tested |
|---|---|
| **Clinical Isolates** | 350 |
| **Challenge Isolates** | 109 |
| **Reproducibility Isolates** | 19 |
| **ATCC Quality Control Strains** | 5 (x 20 repeats) |
| **TOTAL** | 483 |

**Table 3.** Indicated organisms and breakpoints from EUCAST[7]

| Pathogen | Disk Diffusion (zone diameters in mm) | | |
|---|---|---|---|
| | S | R | ATU |
| **Enterbacterales** | ≥22 | <22 | 20-22 |
| ***Pseudomonas aeruginosa*** | ≥22 | <22 | - |

S= Susceptible, R= Resistant, ATU= Area of Technical Uncertainty

## RESULTS

### Quality Control

QC results were within the stated limits for all QC organisms (*Escherichia coli* ATCC® 25922™, *Pseudomonas aeruginosa* ATCC® 27853™, *Klebsiella pneumoniae* ATCC® BAA-2814™, *Klebsiella pneumoniae* ATCC® BAA-1705™ and *Klebsiella pneumoniae* ATCC® 700603™) 100% of the time for each lot of Oxoid IMR35 discs.

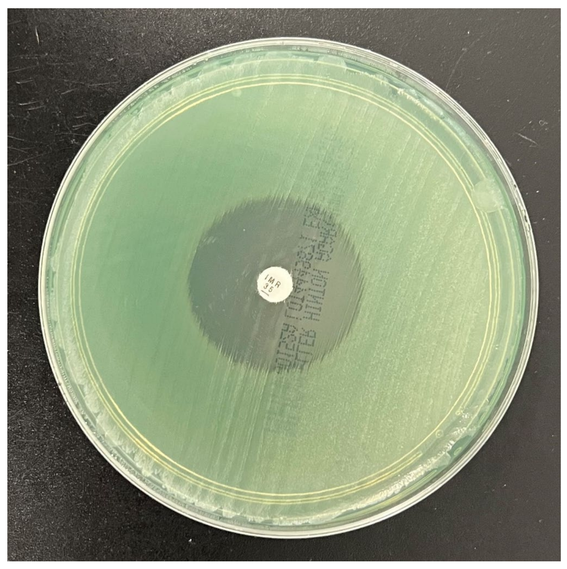

**Figure 3.** Oxoid IMR35 disc zone of inhibition with *Pseudomonas aeruginosa* ATCC® 27853™ on MHA.

### Reproducibility

The reproducibility was calculated as the percent of results which were within 3 mm of the modal value. All results showed reproducibility greater than the acceptance criteria of 95%. The summary is shown in Table 4.

**Table 4.** Summary of the reproducibility of Oxoid IMR35 discs between 2 lots and 3 independent individuals.

| Reproducibility between disc lots | | | Reproducibility between individuals | | | |
|---|---|---|---|---|---|---|
| Lot 1 | Lot 2 | All Lots | Individual 1 | Individual 2 | Individual 3 | All Individuals |
| 98.80% | 98.10% | 99.25% | 100.00% | 96.50% | 99.10% | 99.40% |

### Clinical and challenge isolates

The categorical agreement of the Oxoid IMR35 disc was analysed using charts such as the example shown in Figure 4 and summarised in Table 5.

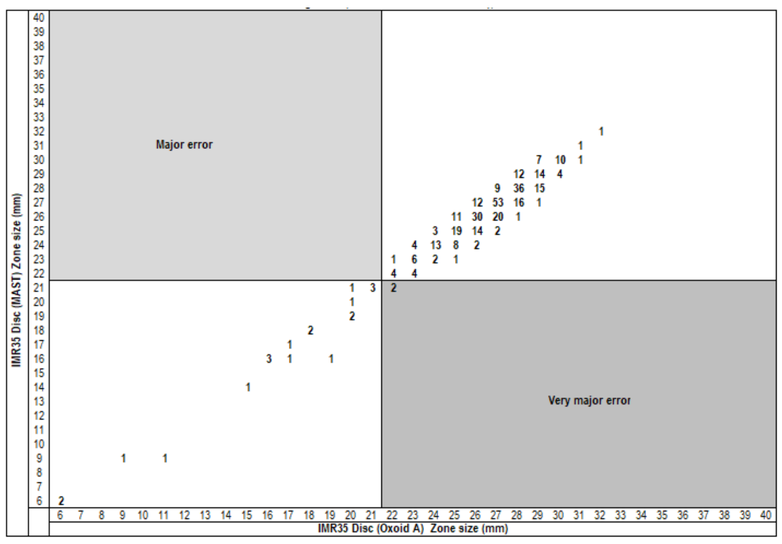


**Figure 4.** Analysis of the IMR35 Oxoid disc against IMR35 Mast discs for Enterobacterales using EUCAST breakpoints.

**Table 5.** Analysis of IMR35 Oxoid disc vs. Mast Disc for indicated species using EUCAST breakpoints.

| Number of isolates | #CA | %CA | #S | #R | MAJ | % MAJ | VMJ | %VMJ |
|---|---|---|---|---|---|---|---|---|
| **Organism** | | | | | | | | |
| **Enterobacterales[a]** | | | | | | | | |
| **Clinical** | 280 | 280 | 100.00% | 270 | 10 | 0 | 0.00% | 0 | 0.00% |
| **Challenge** | 79 | 77 | 97.477% | 67 | 12 | 0 | 0.00% | 2 | 16.67% |
| **Combined** | 359 | 357 | 99.44% | 337 | 22 | 0 | 0.00% | 2 | 9.09% |
| ***Pseudomonas aeruginosa*[b]** | | | | | | | | |
| **Clinical** | 35 | 33 | 94.29% | 31 | 4 | 0 | 0.00% | 2 | 50.00% |
| **Challenge** | 10 | 10 | 100.00% | 1 | 9 | 0 | 0.00% | 0 | 0.00% |
| **Combined** | 45 | 43 | 95.56% | 32 | 13 | 0 | 0.00% | 2 | 15.38% |
| ***Acinetobacter calcoaceticus-baumannii* complex[c]** | | | | | | | | |
| **Clinical** | 20 | 20 | 100.00% | 9 | 11 | 0 | 0.00% | 0 | 0.00% |
| **Challenge** | 10 | 10 | 100.00% | 0 | 10 | 0 | 0.00% | 0 | 0.00% |
| **Combined** | 30 | 30 | 100.00% | 9 | 21 | 0 | 0.00% | 0 | 0.00% |

CA= Categorical Agreement, S= Susceptible, R= Resistant, MAJ= Major errors, VMJ= Very Major Errors

[a] includes *E. coli* (85), *E. cloacae* (64), *K. oxytoca* (55), *K. pneumoniae* (65), *S. marcescenes* (25), *C. freundii* (35) and *K. aerogenes* (30).

[b] *P. aeruginosa* does not have an ATU and therefore cannot have any minor discrepancies.

[C] *Acinetobacter calcoaceticus-baumannii complex* was analysed per EUCAST version 12 (2022) breakpoints (S ≥24). In the latest version the organism has been removed from the breakpoints table.

The overall categorical agreement of 99.44%, 95.56% and 100% was achieved for Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus-baumannii complex*, respectively, when the Oxoid IMR35 disc was compared to the predicate device.

Two very major discrepancies were observed for Enterobacterales *and P. aeruginosa* during the study. Due to the lack of resistant isolates for imipenem/relebactam and considering the VMJ errors had disc zone diameters that were either ≤3 mm (i.e., one MIC doubling dilution equivalent) from the breakpoint or were within (ATU) Area of technical uncertainty, the adjusted VMJ error rate is 0%.

All colony counts were in the region of 1-2 x$10^8$ CFU/mL for all QC and reproducibility isolates and for all the clinical and challenge isolates tested for CC. The average of all the colony counts combined was 1.09 x$10^8$ CFU/mL.

## CONCLUSION

This study validates that the Oxoid IMR35 AST disc has an equivalent level of performance compared to the imipenem/relebactam Mast Disc against EUCAST breakpoints. The high categorical agreement obtained by the Oxoid IMR35 disc indicates that this is an acceptable method for antimicrobial susceptibility testing imipenem/relebactam.

## REFERENCES

[1] Heo YA. Imipenem/Cilastatin/Relebactam: A Review in Gram-Negative Bacterial Infections. Drugs. 2021 Feb;81(3):377-388. doi: 10.1007/s40265-021-01471-8. Epub 2021 Feb 25. PMID: 33630278; PMCID: PMC7905759.

[2] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

[4] The European Committee on Antimicrobial Susceptibility Testing. Disk diffusion method. Version 11.0, 2023

[5] BS EN ISO 20776-2:2007 Clinical laboratory testing and in vitro diagnostic test systems. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Evaluation of performance of antimicrobial susceptibility test devices

[6] The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 14.0, 2024

[7] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024

## TRADEMARKS/LICENSING



**Science at a scan**
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/ast