ENGLISH

Imipenem-relebactam (10+25) I/R 35 µg

INTENDED PURPOSE

Imipenem-relebactam (10+25) I/R 35 µg is an *in vitro* semi-quantitative method for antimicrobial susceptibility of clinical isolates tested on agar media using overnight incubation.

The test is used against the following microorganisms:

Gram-negative bacteria
Enterobacterales
Pseudomonas aeruginosa

DESCRIPTION

Antibiotic Disc are paper discs with special features, that are impregnated with antibiotic and used for the susceptibility test according to the Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing).

The simultaneous growth of the bacteria and diffusion of the antimicrobials compounds forms a zone of inhibition of growth. Zone size observed in a disk diffusion test has no meaning in and of itself. This information is correlated with *in vivo* test able to determinate the resistance and susceptibility to antimicrobials and result in the interpretive standards.

Imipenem-relebactam (10+25) I/R 35 µg is used for determining bacterial antibiotic susceptibility in the treatment of infectious disease.

Imipenem-relebactam disc content is 10 µg of imipenem and 25 µg of relebactam.

KIT CONTENT

Antibiotic Disc is supplied in different packaging options (no additional reagents are included):
Disc in cartridge

- The 50-test box contains 1 cartridge with 50 discs packed in desiccant envelops.
- The 250-test box contains 5 cartridges of 50 discs, each cartridge individually packed in a desiccant envelope.
- Each package also contains a transparent resealable bag.

Discs in canister

- The canister contains 250 discs and a desiccant tablet.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as:

- sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors

- suspension medium - McFarland turbidity standard - agar plate medium (validated by the media manufacturer for use with antimicrobial susceptibility testing, 90- or 150-mm plates)

- forceps - incubator - quality control organisms

PRINCIPLE OF THE METHOD

The discs are applied to the surface of a culture medium inoculated with a pure colony suspension of the microorganism under examination. After incubation, the plates are examined, the inhibition zone diameter around each disc are examined and compared with the standard inhibition zone diameter: in this way the microorganisms are defined as being susceptible, intermediate or resistant to the tested antimicrobial agents.

SPECIMEN COLLECTION AND PREPARATION

Antibiotic Discs are not for use directly with clinical or other specimens. The product is used to indicate appropriate patient treatment against infections caused by microorganisms that can be isolated from clinical samples of adult, juvenile and pediatric patients. There are no different indications for use according to sample source.
The microorganism to be tested must first be isolated on a nonselective culture medium, such as blood agar or tryptic soy agar (TSA). In case of mixed culture, selected colonies should be purified by subculturing. Differential media harboring chromogenic or fluorogenic substrates should not be used for the subculture. It is recommended that cultures be no more than 24 hours old unless additional incubation is required to achieve sufficient growth.

TEST PROCEDURE

Handling

Before using the Antibiotic Disc from an unopened package, visually inspect to ensure the package is intact. Do not use the discs if the package has been damaged. When removed from the refrigerator/freezer, allow the package or storage container to reach room temperature for about 30 minutes. Moisture condensing on the outer surface must evaporate completely before opening the package. Use forceps or a similar device to pick up a disc.

When using Antibiotic Discs from a canister, replace the lid immediately after use and store as outlined under STORAGE.

Inoculum Preparation

Suspend well-isolated colonies from an overnight agar plate into the suspension medium to achieve the recommended McFarland standard. If the inoculum concentration is correct, a confluent lawn of growth will be obtained after incubation. If insufficient growth occurs, the testing should be repeated.
McFarland turbidity standards do not guarantee the correct number of viable cells in the suspension. In order to verify that your procedure gives the correct inoculum density in terms of CFU/mL performing regular colony counts is recommended. An acceptable inoculum should give approximately 1-2 x 10^8 CFU/mL.

Inoculation

Dip a sterile swab in the broth culture or in a diluted form thereof and squeeze it on the wall of the test tube to eliminate excess liquid. Streak the swab over the entire sterile agar surface. Repeat this procedure by streaking 2 more times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. Allow excess moisture to be absorbed so that the surface is completely dry before applying discs.

Use well-defined, high-quality media for AST that supports good growth. The brand chosen should have good batch-to-batch reproducibility to ensure that accurate and reliable zone diameters values are obtained.

The agar medium should have a depth of 4.0 ± 0.5 mm, a pH of 7.3 ± 0.1 and all other quality specifications should be fulfilled. Refer to the media manufacturer's instructions for more information.

Application

Apply the disc to the inoculated agar surface. Pressing it with a sterile forceps on the surface of the agar. Once applied, do not move the disc.

Incubation

Incubate the agar plates in an inverted position at the appropriate temperature, atmosphere and time, according to the methodology followed.

NOTES:

- The medium to be used depends on the organism under investigation and the methodology followed and must be validated by the media manufacturer for antimicrobial susceptibility testing.
- It is recommended to use the inoculum suspension within 15 minutes of preparation, apply discs within 15 minutes of inoculation and incubate plates within 15 minutes of disc application.

For more details, please refer to the current published standards.

READING THE RESULTS

At the end of the incubation period, measure the inhibition zone diameters (mm) with zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.

Measure zone diameters to the nearest millimeter with a ruler or a calliper. If an automated zone reader is used, it must be calibrated to manual reading.

Do not read the plate if the culture appears mixed or if the lawn of growth is too light or too heavy.

For full instructions relating to the interpretation of the results according to CLSI and/or EUCAST methodology please refer to the relevant current standards.

NOTES:

- Excessively wet plates prior to inoculation, insufficient drying before applying discs and/or unevenly streaked surfaces may give non-confluent growth. Repeat the test if the inhibition zone diameter is difficult to read.
- Occasionally, certain antimicrobial agent/microorganism combinations may give unusual results. In these cases, judgment of the inhibition zone diameter may be difficult for the inexperienced personnel.

Procedures specific to Imipenem-relebactam (10+25) I/R 35 µg are summarized in the following table:

Storage	Temperature at –20°C
Organism	Enterobacterales, *Pseudomonas aeruginosa*
Medium	Mueller Hinton II Agar
Inoculum	Suspension in saline (0.85% NaCl) to 0.5 McFarland standard (1 if mucoid)
Incubation	Agar plates in inverted position at 35 ± 2°C for 16-18 h in ambient air (CLSI) and 35 ± 1°C in air for 18 ± 2 h (EUCAST)

INTERPRETATION OF THE RESULTS

To categorize the result, typically as susceptible, intermediate or resistant, refer to current Antibiotic Disc breakpoints (below).

The inhibition zone diameters obtained should be interpreted according to current CLSI and EUCAST interpretive criteria (see below).

Antimicrobial agent	**Organisms**	**CLSI**			**EUCAST**		
		Zone diameter breakpoint (mm)			**Zone diameter breakpoint (mm)**		
		S ≥	**I**	**R ≤**	**S ≥**	**R <**	**ATU**
Imipenem-relebactam	Enterobacterales	25	21–24	20	22	22	20-22
	Pseudomonas aeruginosa	23	20–22	19	22	22	-

Disclaimer: This breakpoint table might be out-of-date and does not replace CLSI and EUCAST published guidelines, which always should be consulted before disc categorization.

NOTES:

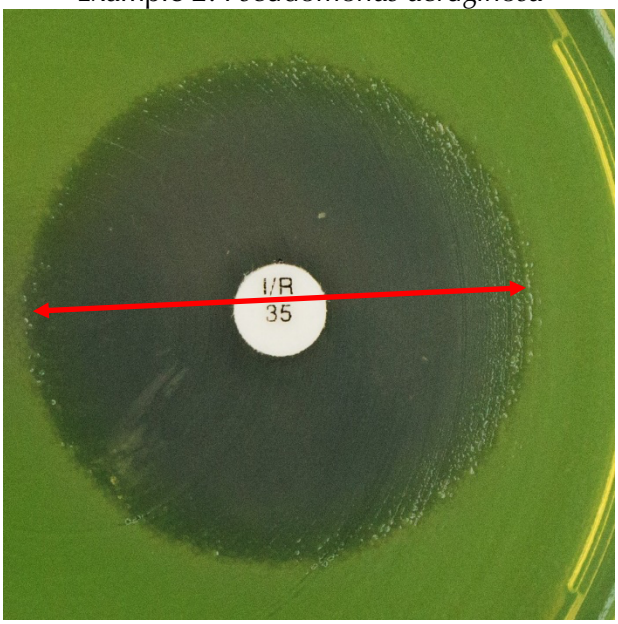
- As with all AST data, discs results are *in vitro* measure only and may provide an indication of the organism's potential *in vivo* susceptibility. The use of results to guide therapy selection must be the sole decision and responsibility of the attending physician. Their judgement should be based on the medical history and knowledge of the patient, pharmacokinetics/pharmacodynamics of the antimicrobial agent, and clinical experience in treating infections caused by the particular microbial pathogen. The drug, dose and dosing regimen must also be considered.
- For details of specific interpretive limitations and/or limitations on the clinical use of an antimicrobial agent in various therapeutic situations, please refer to the tables and footnotes of Antibiotic Disc interpretive standards in the latest CLSI and EUCAST documents.

Imipenem-relebactam (10+25) I/R 35 µg Reading Examples

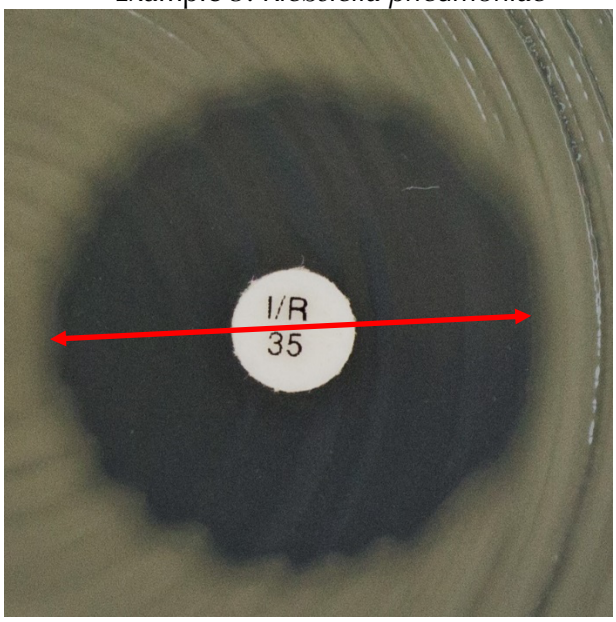
Example 1: *Escherichia coli*

Measure zone diameters to the nearest millimetre with a ruler or a calliper.

Example 2: *Pseudomonas aeruginosa*



Example 3: *Klebsiella pneumoniae*



Inhibition zone diameters marked in red.

USER QUALITY CONTROL

To check the performance of Antibiotic Disc reagents, media and procedure, test the quality control strain(s) as shown below. Results are considered satisfactory if the quality control result(s) fall within the expected range(s). Patient isolate results should not be reported if the quality control results are outside of this stated QC range.

Antimicrobial agent	Control strain		Zone Range (mm)
Imipenem-relebactam I/R	*Escherichia coli*	ATCC® 25922	27–33 [a,b]
	Pseudomonas aeruginosa	ATCC® 27853	26–31 [a,b]
	Klebsiella pneumoniae	ATCC® 700603	26–32 [a]
	Klebsiella pneumoniae	ATCC® BAA-1705	23–29 [a]
	Klebsiella pneumoniae	ATCC® BAA-2814	22–28 [a,b]

a) CLSI M100-Ed34
b) EUCAST Quality Control v.14.0

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of Imipenem-relebactam (10+25) I/R 35 µg was determined by evaluating the agreement of the AST system results with the results generated for the same isolate with the broth microdilution (BMD) reference method. To assess accuracy, Category Agreement (CA) was calculated. CA occurs when the antibiotic disc system results agree with the reference method with respect to the CLSI and EUCAST categorical interpretative criteria.

A total of 300 clinical isolates were tested by three operators. Performance characteristics are summarized below.

CLSI breakpoints

Antimicrobial agent	Organism	N	Accuracy			
			%CA	%mD	%MD	%VMD
Imipenem-relebactam	Enterobacterales	233	98.7	1.30	0.0	0.0
	Pseudomonas aeruginosa	67	94.0	6.00	0.0	0.0
	TOTAL	300	97.7	2.30	0.0	0.0

EUCAST breakpoints

Antimicrobial agent	Organism	N	Accuracy			
			%CA	%mD	%MD	%VMD
Imipenem-relebactam	Enterobacterales	233	98.3	NA	20	0.0
	Pseudomonas aeruginosa	67	100.0	NA	0.0	0.0
	TOTAL	300	98.7	NA	1.6	0.0

N, Number of isolates **mD**, minor discrepancies (NA, not applicable)
MD, major discrepancies
CA, Category Agreement **VMD**, very major discrepancies

Reproducibility

100.0 % of Imipenem-relebactam (10+25) I/R 35 µg results (2 *Klebsiella pneumoniae*, 2 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 1 *Serratia marcescens* and 2 *Enterobacter cloacae* tested in triplicate by 3 operators on 3 days) fell within ± 3 mm of the test mode.

Repeatability

100.0 % of Imipenem-relebactam (10+25) I/R 35 µg results (2 *Klebsiella pneumoniae*, 2 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 1 *Serratia marcescens* and 2 *Enterobacter cloacae* tested in triplicate) fell within ± 3 mm of the test mode.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

WARNINGS AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD) only.**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the kit. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Do not use if material from a packaging or the packaging itself appear to be damaged.
5) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
6) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
7) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
8) Do not mix reagents of different batches. Please use the kit within the validity period.
9) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled
10) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
11) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
12) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

STORAGE

Unopened foil packages and canisters: On receipt, store Antibiotic Disc at −20°C to +8°C until the given expiry date. Some Antibiotic Disc (e.g. carbapenems) should be stored frozen at −20°C. Check the drug label for the specific storage temperature.
Opened canisters: Discs in canister can be used for up to 2 months from first opening (record the date on which the canister was open) and must be stored at the label storage temperature. Before using the remaining discs, check the expiry date indicated on the packaging. Do not store near sources of heat and do not expose to excessive temperature variations.
Protect Antibiotic Disc from moisture, heat and direct exposure to strong light at all times.

DISPOSAL OF USED MATERIAL

After use, the discs and material that has come into contact with the sample must be decontaminated and disposed of in accordance with guidelines used in the laboratory for decontamination and disposal of potentially infected material.

SUGGESTIONS FOR TROUBLESHOOTING

For out-of-range QC, first repeat the test with a pure culture or a freshly subcultured QC strain. If the issue is unresolved, follow this guidance for additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolates results.

Observation	Probable Cause	Comments/Suggested Actions
Inhibition diameter too large	Inoculum too light	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and incubation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 x 10^5 CFU/ml)
Inhibition diameter too small	Inoculum too heavy	
Inhibition diameter too small	Antimicrobial agent is degrading	Use alternative lot. Check STORAGE and package integrity

In case of other malfunctions or defects, contact immediately Liofilchem (*) or the local representative.
In case of incident associated with the device, notify immediately Liofilchem (*) or its local representative and the National Competent Authority.
*Please login to https://www.liofilchemstore.it/login.php (user ID and password required) and click on Complaint.

REFERENCES

1. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2024.
2. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.
3. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org.
4. 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. http://www.eucast.org.
5. ISO 20776-1:2019. Clinical laboratory testing and *in vitro* diagnostic test systems -- Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -- Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious disease.
6. ISO 20776-2:2021. Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -- Part 2: Evaluation of performance of antimicrobial susceptibility test devices.
7. CLSI M02 ED14 QG-2024 — Disk Diffusion Reading Guide, 2nd Edition.
8. Antimicrobial susceptibility testing EUCAST disk diffusion method. Version 12.0 January 2024.
9. EUCAST disk diffusion method for antimicrobial susceptibility testing. Reading guide. Version 10.0 January 2023.

A Summary of Safety and Performance (SSP) will be available on Eudamed (subject to Eudamed availability).
This summary is also available on request at liofilchem@liofilchem.com

Product	µg	Code	Packaging	Ref.
Imipenem-relebactam	(10+25) 35	I/R	5 x 50 1 x 50 1 x 250	9253 9253/1 9253/2

CE 0123 IVD

Table of Symbols

IVD	*In Vitro* Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
	Do not reuse
	Fragile, handle with care
CE 0123	Identification number of notified body
	Manufacturer
	Use by
Σ	Contains sufficient for <n> tests
	Consult instructions for use
	Upper limit of temperature

Revision History

Revision	Release Date	Change Summary
0	02 Nov 2024	Document creation

This document is also available from the online Support Center: **liofilchem.com/ifu-sds**

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