ComASP® Ceftolozane-tazobactam 0.008/4-128/4

ENGLISH

System for ceftolozane-tazobactam susceptibility testing with broth microdilution method, according to ISO 20776-1.

INTENDED PURPOSE

ComASP® Ceftolozane-tazobactam 0.008/4-128/4 is an in vitro diagnostic product for antimicrobial susceptibility testing (AST) of clinical isolates based on growth of the test organism in the presence of various concentrations of an antimicrobial agent.

This ComASP® system is used for quantitative determination of the minimum inhibitory concentration (MIC) of ceftolozane-tazobactam against multidrug-resistant (MDR) Gram-negative bacteria, including *Enterobacterales* and *Pseudomonas aeruginosa*.

DESCRIPTION

ComASP® Ceftolozane-tazobactam 0.008/4-128/4 is a 2-test panel containing the dried-up antibiotic in 15 two-fold dilutions:

Ceftolozane concentration ranges 0.008 to 128 µg/ml, while the concentration of **tazobactam** is fixed at 4 µg/ml.

The ComASP® system is a compact version of the broth microdilution (BMD) reference method allowing to perform the antimicrobial susceptibility testing of Ceftolozane-tazobactam as recommended by international standards (i.e. CLSI, EUCAST, ISO) but in a simpler and less time-consuming way.

KIT CONTENT

- 4 Systems (panels) of ComASP® Ceftolozane-tazobactam 0.008/4-128/8 (panels individually packed in foil with silica gel desiccant)
- 8 Tubes of Mueller Hinton II Broth (3.6 ml)
- Sealing Film
- Instructions Sheet and Test Results Form

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as:

- loops - pipettes - physiological solutions - swabs - culture media - incubator
- test tubes - 0.5 McFarland turbidity standard - quality control organisms

CONFIGURATION

Test	Ceftolozane*-tazobactam concentration (µg/ml)							
A	Growth	0.008	0.016	0.032	0.064	0.125	0.25	0.5
	1	2	4	8	16	32	64	128
B	Growth	0.008	0.016	0.032	0.064	0.125	0.25	0.5
	1	2	4	8	16	32	64	128

*Values on the panel refer to ceftolozane while tazobactam is present at a fixed concentration of 4 µg/ml.

Growth indicates growth control: No antimicrobial agent in the well.

PRINCIPLE OF THE METHOD

Two bacterial isolates may be tested on a single panel.
All wells related to either test A or B are rehydrated with a standardized microbial suspension.
After incubation for 16-20 hours the result is read and interpreted.

SPECIMEN COLLECTION AND PREPARATION

ComASP® Ceftolozane-tazobactam 0.008/4-128/4 is not for use directly with clinical or other specimens. 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

1. Take a panel from its envelope and leave it at room temperature for 10 min,
   DO NOT DISCARD THE ENVELOPE until both tests have been carried out.
2. Prepare a suspension of the test organism using either the direct colony suspension or growth method.
3. Standardize the suspension to the density of a McFarland 0.5 standard.
4. Optimally within 15 min of preparation, dilute the adjusted suspension 1:20 in saline; this will be the **Solution A**.
5. Add 0.4 ml of Solution A to a tube of MH II Broth* provided in the kit to obtain the **Solution B**.
6. Dispense 100 µl of Solution B into each well (test A or B).
7. Cover the panel with the lid provided and incubate at 36 ± 2°C for 16-20 hours in ambient air.

* Mueller Hinton II Broth (g/l): Beef Extract 3.0 g; Acid Hydrolysate of Casein 17.5 g; Starch 1.5 g; Distilled Water 1000 ml; pH 7.3 ± 0.1 (adjusted with appropriate salts to provide 20-25 mg/l of calcium and 10-12.5 mg/l of magnesium).

READING THE RESULTS

At the end of the incubation period observe the growth in the wells and establish the MIC, i.e. the lowest concentration of antibiotic that inhibits visible growth.

The result can be read visually or automatically. Reading by the naked eye can be improved by use of bright indirect lighting against a dark background.

Growth appears as turbidity or as a button at the bottom of the well (compare with the amount of growth in the growth-control well).

The growth-control well should be evaluated first. Make sure it is positive for growth. If not, check the viability of the colonies picked and repeat the test using a new row in the same panel or a new panel and a microbial culture of recent growth.

Note the results on the Test Results Form included in the kit (copy as many form as necessary).

INTERPRETATION OF THE RESULTS

The MIC obtained should be interpreted according to current EUCAST interpretive criteria (see below).

Antimicrobial agent	Organism	MIC Criteria (µg/ml)	
		S≤	R>
Ceftolozane-tazobactam	*Enterobacterales*	2	2
	Pseudomonas aeruginosa	4	4

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

NOTE: Each individual panel is used for performing two tests. If only one test (either A or B) has been performed, use the film provided in the kit to seal the inoculated rows so to prevent any leakage of contaminated fluids. Then, return the panel into its own desiccant envelope and into the refrigerator (see STORAGE).

USER QUALITY CONTROL

QC of ComASP® Ceftolozane-tazobactam 0.008/4-128/4 is performed using the following reference strains:

Control strain	MIC QC range (µg/ml)
Escherichia coli ATCC® 25922	0.12 – 0.5
Pseudomonas aeruginosa ATCC® 27853	0.25 – 1
Escherichia coli ATCC® 35218	0.06 – 0.25
Klebsiella pneumoniae ATCC® 700603	0.5 – 2

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of ComASP® Ceftolozane-tazobactam 0.008/4-128/4 was determined by evaluating the agreement of the AST system result with the result generated for the same isolate with the broth microdilution (BMD) reference method. To assess accuracy, Essential Agreement (EA) and Category Agreement (CA) were calculated. EA occurs when the MIC of the ComASP® system and the reference method agree exactly or is within ± 1 dilution of each other. CA occurs when the ComASP® system results agree with the reference method with respect to the EUCAST categorical interpretative criteria (susceptible, resistant).

A total of 300 clinical isolates were tested by three operators. The following table summarizes performance data from these studies.

Antimicrobial agent	Organism	N	Accuracy				
			%EA	%CA	%mD	%MD	%VMD
Ceftolozane - tazobactam	*Enterobacterales*	180	93.9%	98.3%	NA	2.5%	0.0%
	Pseudomonas aeruginosa	120	94.2%	98.3%	NA	2.2%	0.0%
	TOTAL	300	94.0%	98.3%	NA	2.4%	0.0%

N, Number of isolates
EA, Essential Agreement
CA, Category Agreement
mD, minor discrepancies
MD, major discrepancies
VMD, very major discrepancies
NA, not applicable

Reproducibility
98.1% of ComASP® Ceftolozane -tazobactam 0.008/4-128/4 results (3 *E. coli*, 3 *P. aeruginosa*, 3 *K. pneumoniae,* and 1 *E. cloacae* tested in triplicate by 3 operators on 3 days) were within a doubling dilution of reference microdilution results.

Repeatability
100% of ComASP® Ceftolozane -tazobactam 0.008/4-128/4 results (3 *E. coli*, 3 *P. aeruginosa*, 3 *K. pneumoniae,* and 1 *E. cloacae* tested in triplicate) were within a doubling dilution of reference microdilution results.

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 a panel, tube or packaging appears 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

Store ComASP® Ceftolozane-tazobactam 0.008/4-128/4 at 2-8°C in the original packaging. Once an envelope is opened the panel shall be used within 7 days and stored at 2-8°C. Do not use the panels beyond the expiry date indicated on the label. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, ComASP® Ceftolozane -tazobactam 0.008/4-128/4 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
MIC too low	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)
MIC too high	Inoculum too heavy	
MIC too high	Antimicrobial agent is degrading	Use alternative lot. Check STORAGE and package integrity
Skipped wells	Contamination. Inadequate mixing of inoculum or improper inoculation of panel	Repeat QC test

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. 32nd ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
2. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
3. CLSI. Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed. CLSI guideline M52. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
4. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org.
5. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org.
6. ISO 20776-1:2006. 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 diseases.

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	Packaging	Ref.
ComASP® Ceftolozane -tazobactam 0.008/4-128/4	4x2 tests	75006

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
	Temperature limits

Revision History

Revision	Release Date	Change Summary
1	29 Sep 2023	Updated layout and content in compliance with IVDR 2017/746

Note: Minor typographical, grammar, and formatting changes are not included in revision history.

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

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