ComASP® Benzylpenicillin 0.002-32

ENGLISH

System for benzylpenicillin susceptibility testing with broth microdilution method according to ISO 20776-1.

INTENDED PURPOSE

ComASP® Benzylpenicillin 0.002-32 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 benzylpenicillin against *Streptococcus pneumoniae*.

DESCRIPTION

Streptococcus pneumoniae is a common cause of serious infections such as pneumonia and bacteremia. Fatalities often occur and are associated with failing antimicrobial therapy due to antimicrobial resistance.
Benzylpenicillin is highly active against *Streptococcus pneumoniae*, therefore it is important to evaluate the resistance of *Streptococcus pneumoniae* to penicillin by determination of the minimum inhibitory concentration (MIC).

ComASP® Benzylpenicillin 0.002-32 is a 2-test panel containing the dried-up antibiotic in 15 two-fold dilutions:

Benzylpenicillin concentration range 0.002 to 32 µg/ml.

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

KIT CONTENT

- 6 Systems (panels) of ComASP® Benzylpenicillin 0.002-32
  (panels individually packed in foil with silica gel desiccant)
- Sealing Film
- Instructions Sheet (includes Test Results Form)

MATERIALS REQUIRED BUT NOT PROVIDED

- Mueller Hinton Fastidious Broth (Horse blood 5% + 20 mg/L beta-NAD)

- Mueller Hinton II Broth

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	Benzylpenicillin concentration (µg/ml)							
1	Growth	0.002	0.004	0.008	0.016	0.032	0.064	0.125
	0.25	0.5	1	2	4	8	16	32
2	Growth	0.002	0.004	0.008	0.016	0.032	0.064	0.125
	0.25	0.5	1	2	4	8	16	32

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 1 or 2 are rehydrated with a standardized microbial suspension.
After incubation for 16-20 hours the result is read and interpreted.

SPECIMEN COLLECTION AND PREPARATION

ComASP® Benzylpenicillin 0.002-32 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. 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 (a McFarland 1 standard for mucoid isolates).
4. Optimally within 15 min of preparation, dilute the adjusted suspension 1:20 in Muller Hinton II Broth*; this will be the **Solution A**.
5. Add 0.4 ml of Solution A to a tube containing 3.6 ml of Mueller Hinton Fastidious Broth (cation-adjusted MH broth with lysed horse blood and beta-NAD)** to obtain the **Solution B**.
6. Dispense 100 µl of Solution B into each well (test 1 or 2).
7. Cover the panel with the lid provided and incubate at 35 ± 1°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)

** Mueller Hinton Fastidious Broth: Beef Extract 3.0 g; Acid Hydrolysate of Casein 17.5 g; Starch 1.5 g; ß-NAD 20 mg; Lysed Horse Blood 50 ml; Distilled Water 1000 ml; pH 7.3 ± 0.1

READING THE RESULTS

At the end of the incubation period observe the growth in the wells and read the MIC as the lowest concentration of antibiotic that completely inhibits organism growth as detected by the unaided eye.

Haemolysis with turbidity or a deposit of growth (pellet) should be regarded as growth when determining endpoints (see figure 1).

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 (copy as many form as necessary).

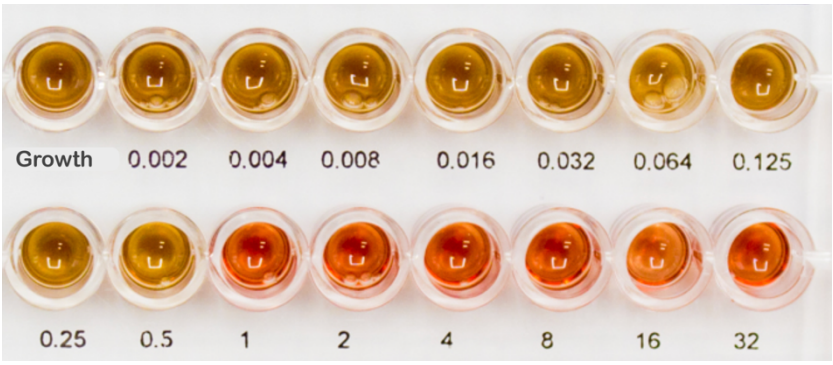

Figure 1. Example showing MIC End Point. The MIC is 1 µg/ml.

INTERPRETATION OF THE RESULTS

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

Antimicrobial agent	Organism	MIC Breakpoints (µg/mL)	
		EUCAST criteria	
		S≤	R>
Benzylpenicillin	*Streptococcus pneumoniae* (for indications other than meningitis)	0.06	2

Disclaimer: This breakpoint table might be out-of-date and does not replace the CLSI and 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 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

Quality control of ComASP® Benzylpenicillin 0.002-32 is performed using the following reference strains:

Strain	MIC QC range (µg/mL)
Streptococcus pneumoniae ATCC® 49619	0.25-1

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of ComASP® Benzylpenicillin 0.002-32 was determined by evaluating the following two metrics: Essential Agreement (EA) and Bias. EA provides a measure of the agreement between the AST system and the reference method, while Bias analysis allows to determine whether the results that differ from the reference method are significantly skewed or predominantly in one direction.

EA occurs when the MIC of the ComASP® system and the broth microdilution (BMD) reference method agree exactly or is within ± 1 dilution of each other. To evaluate bias, the objective is to compare the percentage of test results greater than the reference and the percentage of test results less than the reference.

A total of 63 clinical isolates were tested by a single operator. The following table summarizes performance data from these studies.

Antimicrobial agent	Organism	N	%EA	Bias
Benzylpenicillin	*Streptococcus pneumoniae*	63	92.1	11.1%

N, Number of isolates **EA**, Essential Agreement

According to ISO 20776-2:2021, MIC AST devices should always have both an overall EA of ≥ 90% when compared to the reference method result(s) and less than ±30% bias.

Reproducibility
95% of ComASP® Benzylpenicillin 0.002-32 results (10 *Streptococcus pneumoniae* tested in triplicate by 3 operators on 3 days) were within a doubling dilution of reference microdilution results.

Repeatability
98% of ComASP® Benzylpenicillin 0.002-32 results (10 *Streptococcus pneumoniae* tested 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® Benzylpenicillin 0.002-32 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® Benzylpenicillin 0.002-32 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. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org.
2. 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.
3. EUCAST reading guide for broth microdilution. Version 4.0, 2022. http://www.eucast.org.
4. 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 against reference broth micro-dilution
5. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 6.0, 2020. http://www.eucast.org.
6. 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: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

Product	Packaging	Ref.
ComASP® Benzylpenicillin 0.002-32	6x2 tests	75011

CE IVD

Revision History

Revision	Release Date	Change Summary
0	15 Apr 2022	Not applicable (Initial release)

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

Table of Symbols

IVD	*In Vitro* Diagnostic Medical Device
REF	Catalogue number
LOT	Batch code
	Do not reuse
	Fragile, handle with care
	Manufacturer
	Use by
Σ	Contains sufficient for <n> tests
	Consult instructions for use
	Temperature limits

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Liofilchem®
Microbiology products since 1983

TEST RESULTS FORM

ComASP Benzylpenicillin 0.002-32

Patient data
Name
Surname
Age
Gender
Specimen
Notes

Indicate with the sign **+** the microbial growth.

Indicate with the sign **−** the absence of microbial growth.

Read the MIC and interpret the result according to the current EUCAST interpretative criteria.

TEST	**Benzylpenicillin concentration (µg/ml)**								**MIC VALUE AND INTERPRETATION**
1	Growth	0.002	0.004	0.008	0.016	0.032	0.064	0.125	.....
									.....
	0.25	0.5	1	2	4	8	16	32	.....
2	Growth	0.002	0.004	0.008	0.016	0.032	0.064	0.125	.....
									.....
	0.25	0.5	1	2	4	8	16	32	.....

Use a distinct test results form for each sample under investigation. A horizontal line should be drawn on the empty spaces related to an unused test, if any.

Date of Test	**Operator**