Fosfomycin MIC Test Strip Technical Sheet

INTRODUCTION

Liofilchem® MIC Test Strip is a quantitative method intended for the *in vitro* determination of antimicrobial susceptibility of non-fastidious Gram negative and Gram positive aerobic bacteria (for example, Enterobacteriaceae, *Pseudomonas*, *Enterococcus* and *Staphylococcus* species) and fastidious bacteria (for example, anaerobes, *Haemophilus* and *Streptococcus* species and *N. gonorrhoeae*). MIC Test Strip consists of specialized paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration (MIC) in µg/mL of antimicrobial agents against bacteria as tested on agar media using overnight incubation and manual reading procedures.

Fosfomycin is a phosphonic acid derivative with *in vitro* activity against a broad range of gram-positive and gram-negative aerobic microorganisms. It is indicated for the treatment of the following infections caused by designated susceptible microorganisms:

- Uncomplicated Urinary Tract Infections as single-dose oral form;
- Severe infections, like bacteremia and pneumonia, as intravenous form usually in combination with other antibiotics.

Fosfomycin MIC Test Strip generates a stable gradient of antibiotic giving an accurate MIC over the ranges (in µg/mL) 0.016-256 and 0.064-1024. These strips contain Glucose-6-Phosphate.

Packages of 10, 30 and 100 tests are available:

- The 10-test box contains 10 strips individually packed in desiccant envelops and an instruction sheet
- The 30-test box contains 30 strips individually packed in desiccant envelops and an instruction sheet
- The 100-test box contains 10 desiccant envelops, each containing 10 strips, and an instruction sheet; this pack contains a storage tube as well.

TEST PROCEDURE

Before using Fosfomycin MIC Test Strip from an unopened package, visually inspect to ensure the package is intact. Do not use the strips if the package has been damaged.
When removed from the refrigerator or 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.

Materials required but not provided:

- Mueller Hinton II Agar, 90 (ref. 10031) or 140 mm (ref. 10231) plates
- Sterile saline (0.85% NaCl) (ref. 20095)
- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- 0.5 McFarland turbidity standard (ref. 80400)
- Incubator (35 ± 2°C)
- Quality control organisms
- Additional technical information from www.liofilchem.net

Inoculum preparation

Suspend well-isolated colonies from an overnight agar plate into saline to achieve a 0.5 McFarland standard turbidity.
A confluent or almost confluent lawn of growth will be obtained after incubation, if the inoculum is correct.
In order to verify that your procedure gives the correct inoculum density in terms of CFU/mL, performing regular colony counts is recommended.

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.
Alternatively, use a rotation plater to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed so that the surface is completely dry before applying MIC Test Strip.

Application

Apply the strip to the agar surface with the scale facing upwards and code of the strip to the outside of the plate, pressing it with a sterile forceps on the surface of the agar and ensure that whole length of the antibiotic gradient is in complete contact with the agar surface. The strip can be repositioned within 3 minutes from its application.

Incubation

Incubate the agar plates in an inverted position at 35 ± 2°C for 16-20 hours in ambient atmosphere. Extend the incubation for up to 48 hours in case of slow growing organisms.

EVALUATING THE RESULTS

Reading

Observe where the relevant inhibition ellipse intersects the strip and read the MIC at 80% inhibition when trailing is seen. Growth along the entire gradient i.e. no inhibition ellipse indicates that the value is greater than or equal to (≥) the highest value on the scale. An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value. **NOTE:** For *Escherichia coli* only, ignore macrocolonies within the ellipse.

Interpretation

Use EUCAST breakpoints shown below. Always round up MIC Test Strip half dilution values to the next upper two-fold value before categorization. For example a *E. coli* fosfomycin MIC of 0.75 µg/mL is reported as 1 µg/mL.

See page 2 for example of results. Also consult the MIC Test Strip Photographic Guide.

QUALITY CONTROL

CLSI-recommended quality control strains are used as outlined under TEST PROCEDURE.

Organism	Breakpoint* (µg/mL)		Quality Control MIC Range (µg/mL)	
	S ≤	R >		
Enterobacteriaceae	32	32	*S. aureus* ATCC® 29213	0.5-4
Staphylococcus spp. (iv)	32	32	*E. faecalis* ATCC® 29212	32-128
			E. coli ATCC® 25922	0.5-2
			P. aeruginosa ATCC® 27853	2-8

* These data have been produced in part under ECDC service contracts and made available by EUCAST at no cost to the user and can be accessed on the EUCAST website www.eucast.org. EUCAST recommendations are frequently updated and the latest versions are available at www.eucast.org.

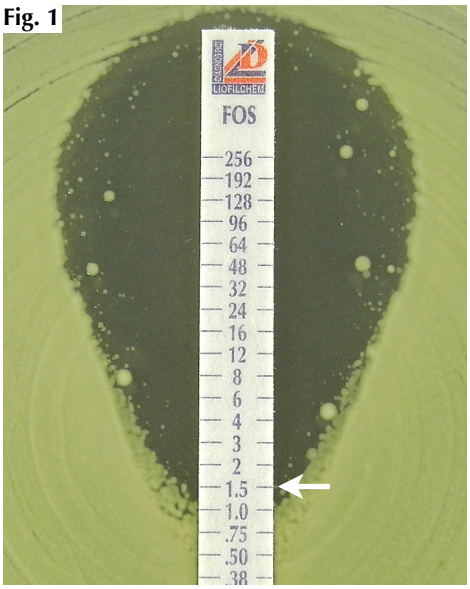
STORAGE

The unopened package of Fosfomycin MIC Test Strip may be stored at temperature equal or below 8°C but is best stored at –20°C until the given expiry date. Leftover MIC Test Strip from an opened package must be stored at 2-8°C in the airtight tube, containing desiccant, provided in the pack for no more than 7 days. Do not store near sources of heat and do not expose to excessive temperature variations.

Fosfomycin MIC Test Strip Reading Guide

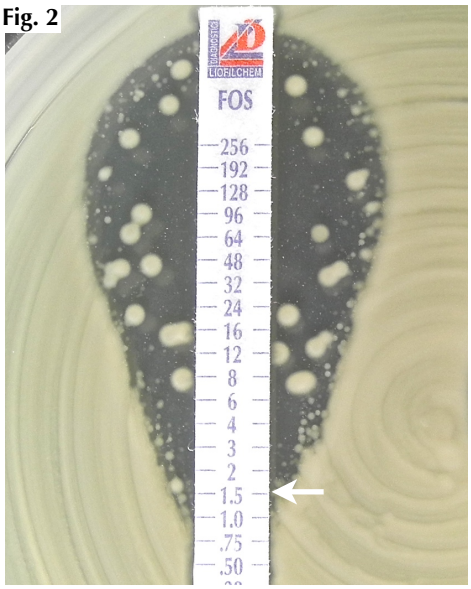
(Examples of inhibition ellipses for *E. coli*)

Fig. 1



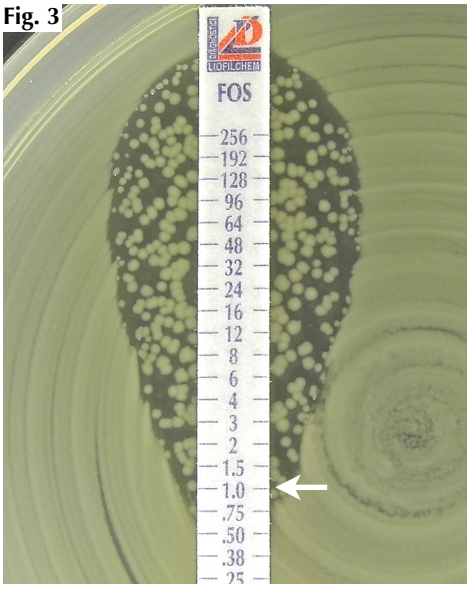
Ignore all colonies:
MIC 1.5 µg/mL, reported as 2 µg/mL

Fig. 2



Ignore all colonies:
MIC 1.5 µg/mL, reported as 2 µg/mL

Fig. 3



Ignore all colonies: MIC 1 µg/mL

Fig. 4

Record as MIC ≥ 256 µg/mL

REFERENCES

- The European Committee on Antimicrobial Susceptibility TestingBreakpoint tables for interpretation of MICs and zone diameters, version 7.1, 2017, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 27th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
- CLSI M07-A10 (2015) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard – 10th Edition.
- http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/050717s005lbl.pdf
- https://www.medicines.org.uk/emc/medicine/28971
- Kaase M et al. (2014) Fosfomycin Susceptibility in Carbapenem-Resistant Enterobacteriaceae from Germany. J Clin Microbiol; 52(6):1893-7

PRESENTATION		µg/mL	Code	Packaging	Ref.
MIC Test Strip	Fosfomycin (Includes Glucose-6-Phosphate)	0.016-256	FOS	10 30 100	920781 92078 920780
MIC Test Strip	Fosfomycin (Includes Glucose-6-Phosphate)	0.064-1024	FOS	10 30 100	920791 92079 920790

MIC Test Strip, International Patent

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