



MIC Test Strip Technical Sheet ESBL

Cefotaxime/Cefotaxime+Clavulanic acid (CTX/CTL)
 Ceftazidime/Ceftazidime+Clavulanic acid (CAZ/CAL)
 Cefepime/Cefepime+Clavulanic acid (FEP/FEL)
 For in vitro confirmation of ESBL.

INTENDED USE

MIC Test Strip ESBL Cefotaxime (CTX)/Cefotaxime+Clavulanic acid (CTL), Ceftazidime (CAZ)/Ceftazidime+Clavulanic acid (CAL) and Cefepime (FEP)/Cefepime+Clavulanic acid (FEL) strips are intended to confirm the presence of clavulanic acid inhibitable ESBL (Extended Spectrum Beta-Lactamase) enzymes in *Escherichia coli*, *Klebsiella pneumoniae* and *K. oxytoca* and other Enterobacteriaceae species. Both MIC Test Strip CTX/CTL and CAZ/CAL strips can be used to confirm the suspected presence of ESBL in strains with phenotypic susceptibility patterns where M.I.C. values of cefotaxime, ceftriaxone, ceftazidime or cefpodoxime are > 1 µg/mL. MIC Test Strip FEP/FEL with MIC Test Strip CTX/CTL and CAZ/CAL may be used for confirming the MIC Test Strip ESBL when testing organisms where inducible chromosomal AmpC β-lactamases can interfere with the clavulanic acid synergy e. g. *Enterobacter* species., MIC Test Strip FEP/FEL can be used likewise for strains showing non-determinable ESBL results with CTX/CTL and CAZ/CAL.

CONTENTS OF THE PACKAGES

The 10-test box contains 10 strips individually packed in desiccant envelopes and an instruction sheet.
 The 30-test box contains 30 strips individually packed in desiccant envelopes and an instruction sheet.
 The 100-test box contains 10 desiccant envelopes, each containing 10 strips, and an instruction sheet. The 100-test box also contains a storage tube.

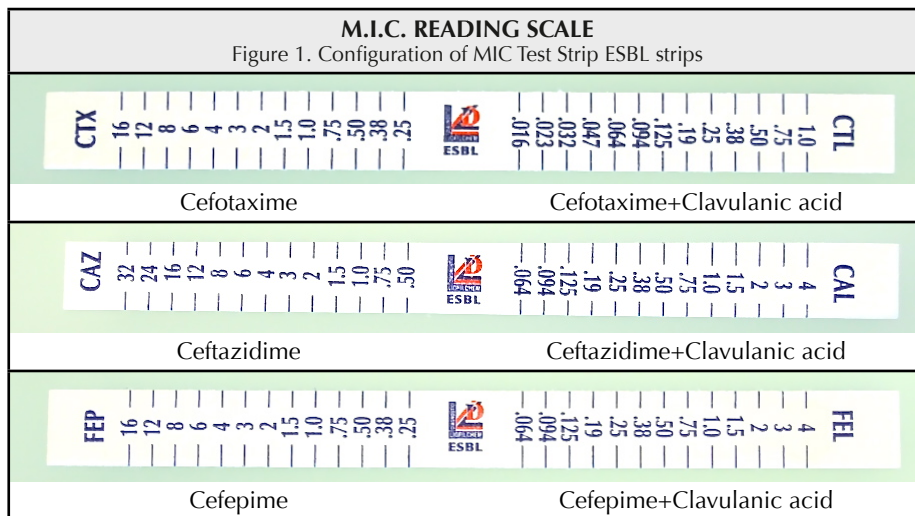
COMPOSITION

MIC Test Strip ESBL CTX/CTL, CAZ/CAL and FEP/FEL strips (Figure 1) are made of special featured paper carrier.

CTX code indicates the cefotaxime (0.25-16 µg/mL) gradient and CTL code indicates the cefotaxime (0.016-1 µg/mL) plus 4 µg/mL clavulanic acid.

CAZ code indicate the ceftazidime (0.5-32 µg/mL) gradient and CAL code indicates the ceftazidime (0.064-4 µg/mL) plus 4 µg/mL clavulanic acid.

FEP code indicate the cefepime (0.25-16 µg/mL) gradient and FEL code indicates the cefepime (0.064-4 µg/mL) plus 4 µg/mL clavulanic acid.



GATHERING AND KEEPING SAMPLES

The colonies that are to test are taken up by culture media that have been previously swabbed with the sample under examination. In the case of mixed colonies the bacterial strains must be purified before inoculation.

TEST PROCEDURE

Before using MIC Test Strip ESBL strips 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 -20 °C 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 plates (ref. 10031)
- Sterile saline (0.9% 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.

Note:

Too heavy inocula may affect the results, since excess enzyme may overcome the clavulanic acid component in the test and consequently reduce the ratio of CTX/CTL, CAZ/CAL or FEP/FEL thus delivering a false negative result.

Analogously, too light inocula may affect results since too little enzyme may provide a lower value for CTX, CAZ or FEP, thus reducing the CTX/CTL, CAZ/ CAL and FEP/FEL ratio.

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 ESBL strips.

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. Once applied, do not move the strip.

Incubation

Incubate the agar plates in an inverted position at $35 \pm 2^\circ\text{C}$ for 16-20 hours in ambient atmosphere.

EVALUATING THE RESULTS**Reading**

When bacterial growth is visible, read the CTX, CTL, CAZ, CAL, FEP and FEL values* where the respective inhibition ellipses intersect the strips. Growth along the entire gradient (no inhibition ellipse) indicates that the value is greater than or equal to (\geq) 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.

In case of mutant colonies found in the inhibition ellipse, read the value where these colonies are completely inhibited.

For CTX, CAZ and FEP values in the high range, inhibition ellipses may be very small or not clearly distinguishable.

A phantom zone (rounded zone) may be seen below the CTL, CAL or FEL gradients and an ellipse can or cannot be visible around the CTX, CAZ or FEP ends.

The CTX, CAZ or FEP inhibition ellipse may also be deformed at the narrow end.

The presence of a phantom zone or ellipse deformation is an advantage of the MIC Test Strip ESBL technique: in these cases the ESBL are clearly detected.

* **Important:** the MIC Test Strip ESBL should be used for confirmation of ESBL production only and is not intended for the determination of the Minimum Inhibitory Concentration.

Interpretation

Table 1: Guidelines for interpretation of MIC Test Strip ESBL.

ESBL	Ratio	Reporting
Positive	CTX ≥ 0.5 and CTX/CTL ratio ≥ 8 or CAZ ≥ 1 and CAZ/CAL ratio ≥ 8 or FEP ≥ 0.25 and FEP/FEL ratio ≥ 8 or "Phantom" zone or deformation of the CTX, CAZ or FEP ellipse.	ESBL producer and resistant to all penicillins, cephalosporins and aztreonam (CLSI M100-S series).
Negative	CTX < 0.5 or CTX/CTL ratio < 8 and CAZ < 1 or CAZ/CAL ratio < 8 .	ESBL non-producer and report actual MICs of relevant drugs as determined by a M.I.C. method.
ND (non determinable)	CTX > 16 and CTL > 1 and CAZ > 32 and CAL > 4 and FEP > 16 and FEL > 4 or when one strip is ESBL negative and the other ND.	ESBL non-determinable and report actual M.I.C.s of relevant drugs as determined by a M.I.C. method. If ESBL is suspected, confirm results with genotyping.

Examples of interpretation of ratios:

CTX/CTL	$8/0.125 = 64$	= ESBL +
CAZ/CAL	$>32/<0.064 = >500$	= ESBL +
FEP/FEL	$1/<0.064 = >15$	= ESBL +
CTX/CTL	$4/>1 = <4$	= ESBL -
CAZ/CAL	$1/0.5 = 2$	= ESBL -
CTX/CTL	$0.25/0.19 = 1.3$ (CTX <0.25)	= ESBL -
CAZ/CAL	$1/41 = 0.25$	= ESBL -
FEP/FEL	$<0.252/0.38 = <0.65$	= ESBL -
CAZ/CAL	$>32/>4 = \text{out of range}$	= ND ³⁾
CTX/CTL ESBL negative and CAZ/CAL ND		= ND ⁴⁾

Notes:

1) When values of CTL, CAL or FEL are higher than CTX, CAZ or FEP respectively, it may reflect the induction of β -lactamase production by clavulanic acid.

2) When FEP < 0.25 , the result for the MIC Test Strip FEP/FEL strip is considered negative.

3) When values are above the test ranges, the result is ND = Non-determinable. When this takes place for both CTX/CTL and CAZ/CAL, further testing with FEP/FEL is recommendable.

4) When one result is ESBL negative and the other ND, the interpretation for the strain should be ND.

QUALITY CONTROL

In order to check the correct performance of the reagents, quality control strains should be tested as described in the TEST PROCEDURE paragraph.

The expected values and interpretation for control strains are provided in Table 2.

ESBL Negative control: *E. coli* ATCC® 35218.

ESBL Positive control: *K. pneumoniae* ATCC® 700603, which delivers a deformed inhibition ellipse or a result according to criteria shown in Table 1. Careful attention should be given to maintenance and storage of *K. pneumoniae* ATCC® 700603 as spontaneous loss of the plasmid encoding the ESBL has been documented and may give QC results outside the acceptable limits.

Either degradation of clavulanic acid or excessively high inoculum cause CTL, CAL and FEL values are higher than specification. Check the storage and handling of strips and repeat the test using the correct inoculum.

Table 2. Quality control specifications for MIC Test Strip ESBL CTX/CTL, CAZ/CAL and FEP/FEL strips.

Strain	Cefotaxime CTX (µg/mL)	Cefotaxime + Clavulanic acid CTL (µg/mL)	Ceftazidime CAZ (µg/mL)	Ceftazidime + Clavulanic acid CAL (µg/mL)	Cefepime FEP (µg/mL)	Cefepime + Clavulanic acid FEL (µg/mL)	ESBL Interpretation ¹⁾
<i>E. coli</i> ATCC® 35218	≤0.25 ²⁾	0.016-0.064					Negative
<i>K. pneumoniae</i> ATCC® 700603	1-4 ³⁾	0.125-1					Positive
<i>P. mirabilis</i> ⁴⁾ ATCC® BAA-856	0.5-1	0.032-0.064					Positive
<i>E. coli</i> ATCC® 35218			≤0.5 ²⁾	≤0.064 ²⁾			Negative
<i>K. pneumoniae</i> ATCC® 700603			8-≥32	0.125-0.5			Positive
<i>P. aeruginosa</i> ATCC® 27853					0.5-2	1-4	Negative
<i>K. pneumoniae</i> ATCC® 700603					0.25-1 ³⁾	0.064-0.25	Positive

Notes:

1) See the paragraph EVALUATING THE RESULTS.

2) Value below the strip range.

3) Ratio may be ≤ 8 but deformation of the CTX or FEP ellipse is indicative of ESBL.

4) Mortensen *et al.* (2005). JCM. 43(5).

LIMITATIONS

1. Inhibitor resistant TEM (IRT) enzymes cannot be detected by MIC Test Strip ESBL strips.

2. An ESBL negative result with elevated values to CTX/CTL and CAZ/CAL may be due to an IRT, AmpC or an ESBL hidden by the concurrent presence of these enzymes and/or other resistance mechanisms. MIC Test Strip FEP/FEL may be additionally tested in these cases.

3. Strains showing non-determinable (ND) results with CTX/CTL and CAZ/CAL strips should be further tested using FEP/FEL strips. If all results are found to be non-determinable, these strains should be further investigated by genotyping.

4. Performance of MIC Test Strip ESBL is based on the use of at least both CAZ/CAL and CTX/CTL strips simultaneously. The use of only one MIC Test Strip ESBL strip to confirm the presence of ESBL is not valid.

PRECAUTIONS

The **MIC Test Strip** cannot be classified as being hazardous according to current legislation but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitisation in sensitive subjects if they come into contact with the skin.

MIC Test Strip are disposable products. **MIC Test Strip** are only for diagnostic *in vitro* use and are intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

STORAGE






The unopened package of MIC Test Strip ESBL should be stored at -20°C until the given expiry date. Leftover strips 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.

REFERENCES AND BIBLIOGRAPHY

- Gales A.C. *et al.* (1997). Antimicrobial susceptibility of *Klebsiella pneumoniae* producing extended spectrum β- lactamase (ESBL) isolated in hospitals in Brazil. *Brazilian Journal of Infectious Disease*. 1(4): 196-203.
- Nas Y. *et al.* (1999). Detection of extended spectrum β- lactamases in *E. coli* and *K. pneumoniae*. JCM. 11(2): 103-106.
- Mortensen J. *et al.* (2005). New quality control strain for use in routine testing for production of extended-spectrum beta- lactamases by Enterobacteriaceae. JCM. 43(5): 2545.
- CLSI M7-A7, January 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*.
- CLSI M100-S series, latest edition.
- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

PRESENTATION					
DESCRIPTION		µg/mL	Code	Packaging	Ref.
MIC Test Strip	Cefepime/Cefepime+Clavulanic acid (4 µg/mL)	0.25-16 / 0.064-4	FEP/FEL	10	921611
				30	92161
				100	921610
MIC Test Strip	Cefotaxime/Cefotaxime+Clavulanic acid (4 µg/mL)	0.25-16 / 0.016-1	CTX/CTL	10	921601
				30	92160
				100	921600
MIC Test Strip	Ceftazidime/Ceftazidime+Clavulanic acid (4 µg/mL)	0.5-32 / 0.064-4	CAZ/CAL	10	921591
				30	92159
				100	921590

TABLE OF SYMBOLS

LOT	Batch code	IVD	<i>In Vitro</i> Diagnostic Medical Device		Manufacturer		Use by
REF	Catalogue number		Temperature limitation		Contains sufficient for <n> tests		Caution, consult accompanying documents

MIC Test Strip, Patent No. 1395483

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