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/cefotaxime+clavulanic acid (CTX/ CTL), Ceftazidime/ceftazidime+clavulanic acid (CAZ/CAL) and Cefepime/cefepime+clavulanic acid (FEP/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 aztreonam, cefotaxime, ceftazidime, ceftriaxone 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 ß-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 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. The 100-test box also contains a storage tube.

COMPOSITION

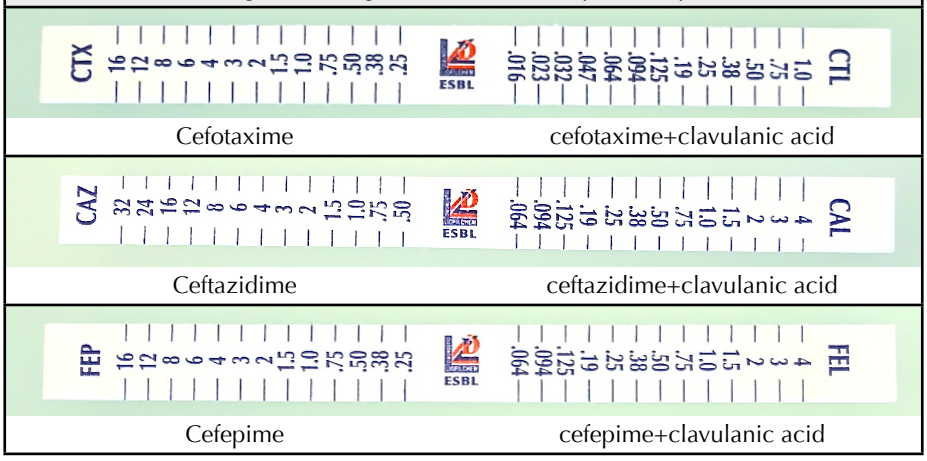
MIC Test Strip ESBL CTX/CTL, CAZ/CAL and FEP/FEL strips (Figure 1) are made of special featured paper carrier.

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.

M.I.C. READING SCALE
Figure 1. Configuration of MIC Test Strip ESBL strips



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 M.I.C. 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 M.I.C. 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 MIC 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 ± 2 °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 M.I.C. values where the respective inhibition ellipses intersect the strips. Growth along the entire gradient (no inhibition ellipse) indicates that the M.I.C. is greater than or equal to (≥) the highest value on the reading scale. An inhibition ellipse below the gradient indicates a M.I.C. inferior to (<) the lowest value on the scale.
In case of mutant colonies found in the inhibition ellipse, read the M.I.C. where these colonies are completely inhibited.
For M.I.C. 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.

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

ESBL	M.I.C. Ratio	Reporting
Positive	M.I.C. CTX ≥ 0.5 and CTX/CTL ratio ≥ 8 or M.I.C. CAZ ≥ 1 and CAZ/CAL ratio ≥ 8 or M.I.C. 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	M.I.C. CTX < 0.5 or CTX/CTL ratio < 8 and M.I.C. 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)	M.I.C. CTX > 16 and M.I.C. CTL > 1 and M.I.C. CAZ > 32 and M.I.C. CAL > 4 and M.I.C. FEP > 16 and M.I.C. 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 M.I.C. 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 = out of range	= ND [3)]
CTX/CTL ESBL negative and CAZ/CAL ND		= ND [4)]

Notes:
1) When M.I.C.s of CTL, CAL or FEL are higher than CTX, CAZ or FEP respectively, it may reflect the induction of ß-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 the M.I.C. 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 M.I.C. 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 an M.I.C. ratio 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 M.I.C. 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 M.I.C. (µg/mL)	Cefotaxime + clavulanic acid M.I.C. (µg/mL)	Ceftazidime M.I.C. (µg/mL)	Ceftazidime + clavulanic acid M.I.C. (µg/mL)	Cefepime M.I.C. (µg/mL)	Cefepime + clavulanic acid M.I.C. (µg/mL)	ESBL Interpretation [1]
E. coli ATCC® 35218	≤0.25 [2]	0.016-0.064					Negative
K. pneumoniae ATCC® 700603	1-4 [3]	0.125-1					Positive
P. mirabilis [4] ATCC® BAA-856	0.5-1	0.032-0.064					Positive
E. coli ATCC® 35218			≤0.5 [2]	≤0.064 [2]			Negative
K. pneumoniae ATCC® 700603			8-≥32	0.125-0.5			Positive
P. aeruginosa ATCC® 27853					0.5-2	1-4	Negative
K. pneumoniae ATCC® 700603					0.25-1 [3]	0.064-0.25	Positive

Notes:
1) See the paragraph EVALUATING THE RESULTS.
2) MIC value below the strip range.
3) MIC 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 M.I.C.s to CTX/TL 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 datasheet 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

All unopened packages and unused MIC Test Strip ESBL strips must be stored at -20 °C or the temperature denoted on the package until the given expiry date. Unused strips must be stored in an airtight storage container with color indicating desiccant. The batch number and expiry date should be clearly marked on the package and/or storage container.
Protect MIC Test Strip ESBL strips from moisture, heat and direct exposure to strong light at all times.
Prevent moisture from penetrating into or forming within the package or storage container. MIC Test Strip ESBL strips must be kept dry.

REFERENCES AND BIBLIOGRAPHY

1. Gales A.C. *et al.* (1997). Antimicrobial susceptibility of *Klebsiella pneumoniae* producing extended spectrum ß- lactamase (ESBL) isolated in hospitals in Brazil. *Brazilian Journal of Infectious Disease*. 1(4): 196-203.
2. Nas Y. *et al.* (1999). Detection of extended spectrum ß- lactamases in *E. coli* and *K. pneumoniae*. JCM. 11(2): 103-106.
3. 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.
4. CLSI M7-A7, January 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*.
5. CLSI M100-S series, latest edition.

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 30 100	921611 92161 921610
MIC Test Strip	CEFOTAXIME / CEFOTAXIME + CLAVULANIC ACID (4 µg/mL)	0.25-16 / 0.016-1	CTX/CTL	10 30 100	921601 92160 921600
MIC Test Strip	CEFTAZIDIME / CEFTAZIDIME + CLAVULANIC ACID (4 µg/mL)	0.5-32 / 0.064-4	CAZ/CAL	10 30 100	921591 92159 921590

TABLE OF SYMBOLS

LOT	Batch code	**IVD**	*In Vitro* 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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Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

MIC Test Strip Technical Sheet **Synergy Testing**

Specimen
Cystic fibrosis, multiple drug-resistant organisms, extreme drug resistant organisms, critical specimens, critical infections, critical patients, limited therapy options.

Procedure
Medium: See specific organism for appropriate agar media (e.g. MHA/aerobes, RPMI/fungi)
Inoculum: Suspension in saline (or broth) to 0.5 McFarland (ref.80400) or 1 McF (ref.80401) depending on bacteria. Inoculate normally by sterile swab.
Incubation: 35 ± 2 °C (or other) / ambient (or other) / 24-48 hours (or other) depending on the specific organism.
Interpretation of results: Bactericidal drugs: interpret the M.I.C. at complete inhibition of growth including microcolonies, hazes and isolated colonies. For bacteriostatic drugs, read at 80% inhibition when trailing is seen. When bactericidal is combined with bacteriostatic, read each agent according to their specific category.

Literature
MTS Pack insert, product labels, MTS Application Guide, MTS Interpretative Criteria and Quality Control , MTS Technical Sheets.

Definitions
MIC_A MIC of drug A alone
MIC_B MIC of drug B alone
MIC_{AB} MIC of drug A in combination with B
MIC_{BA} MIC of drug B in combination with A

Interpretation
Fractional Inhibitory Concentration Index (FIC Index) calculations:

FIC Index (Fractional Inhibitory Concentration Index) calculations:
FIC Index = $MIC_{AB}/ MIC_A + MIC_{BA}/ MIC_B$
MIC_{AB} = MIC of A in the presence of B; MIC_{BA} = MIC of B in the presence of A.

Interpretation	**FIC**
Synergy	≤ 0.5
Additive	> 0.5 and ≤ 1.0
Indifference	> 1 and ≤ 4.0
Antagonism	> 4.0

Results

	Single drug		Combination		FIC index	Interpretation
	MIC_A	MIC_B	MIC_{AB}	MIC_{BA}		
Strain 1						
Strain 2						

MTS Synergy Applicator System		
Product	**REF**	Σ
MTS Synergy Applicator Platform	96860	1
MTS Synergy Delivery Tool	96870	10 Tests

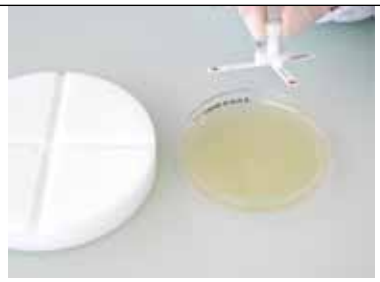
Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

Method

1. Perform standard M.I.C. of drugs A and B prior to synergy set-up.
2. Use the "MTS Synergy Applicator System"* for the synergy testing.
3. Take a MTS (MIC Test Strip) of the first antibiotic (A) with the tweezers and place it on the MTS Synergy Applicator Platform according to position 1.
4. Adjust the MTS (antibiotic A) such that the the MIC value of the first antibiotic (MIC_A) is positioned at the base intersection.

5. Take a MTS of the second antibiotic (B) with tweezers and place it on the base according to position 2.

6. Adjust the second MTS (antibiotic B) such that the MIC value of the second antibiotic (MIC_B) is positioned at the base intersection and intersects MTS-antibiotic A at its MIC value.



7. Use the MTS Synergy Delivery Tool, press hard onto the two carefully positioned MTS (A and B) and move them to the agar plate.

8. Carefully place the MTS Synergy Delivery Tool (with MTS_A and MTS_B) on the agar.
9. Wait until the strips are completely moistened by surface of the agar.

10. Remove the MTS Synergy Delivery Tool from the agar plate leaving MTS_A and MTS_B positioned at 90°. If necessary, use the tweezers to push the strips onto the agar surface.
11. Finally incubate according to the standard MTS procedure for the specific microorganism.

References

- CLSI M100-S22, 2012. Performance Standards for Antimicrobial Susceptibility Testing.
- CLSI M7-A9, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.
- EUCAST. Breakpoint tables for interpretation of MICs and zone diameters Version 2.0, January 2012.

* MTS Synergy Applicator System *PATENT PENDING*: A device for standardising the *in-vitro* synergy testing of two antibiotics through the method of crossing the gradient strips. (Liofilchem, 2012).

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