ESBL Disc Tests

Disc tests for detection of ESBL-producing Enterobacteriaceae.

DESCRIPTION

Extended-spectrum β-lactamases (ESBLs) are enzymes hydrolyzing most penicillins and cephalosporins, including oxyimino-β-lactam compunds but not cephamycins and carbapenems. Most ESBLs belong to the Amber class A of β-lactamases and are inhibited by β-lactamases inhibitors: clavulanic acid, sulbatam and tazobactam. ESBL production has been observed mostly in Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pnuemoniae*, but all other clinically-relevant Enterobacteriaceae species are also common ESBL-producers. In many areas, ESBL detection and characterization is recommended or mandatory for infection control purpose.
ESBL detection involves two important steps. The first is a screening test with an indicator cephalosporin which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harboring ESBLs. The second step is a confirmation test which evaluates the synergy between an oxyimino cephalosporin and clavulanic acid, distinguishing isolates with ESBLs from those that are resistant for other reasons.

METHOD PRINCIPLE

ESBL Disc Screening

Disk-diffusion method for ESBL screening can be performed using **cefpodoxime**, **ceftazidime**, **aztreonam**, **cefotaxime** and **ceftriaxone**, according to EUCAST and/or CLSI guidelines **(table 1)**. Since the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection. However, it is adequate to use the couple cefotaxime (or ceftriaxone) and ceftazidime. If only one drug can be used, cefpodoxime is the most sensitive indicator cephalosporin for detection of ESBL production may be used for screening. However, it is less specific (high number of false positive) than the combination of cefotaxime (or ceftriaxone) and ceftazidime and only the latter compounds are used in the confirmation testing.

ESBL Disc Confirmation

Enterobacteriaceae suspected to be producers of ESBLs enzymes may be submitted to the follow confirmation tests: Combination Disc Test (CDT) and/or Double-Disc Synergy Test (DDST). These tests permit to evaluate the inhibition of ESBL activity by **Clavulanic acid (table 2)**.

Combination Disc Test (CDT)

For each test discs containing cephalosporin alone (cefotaxime, ceftazidime, cefepime) and in combination with clavulanic acid are applied. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared with the zone around the disc with the cephalosporin alone. The test is positive if the inhibition zone diameter is ≥ 5 mm larger with clavulanic acid than without.

Double-Disc Synergy Test (DDST)

Discs containing cephalosporin (cefotaxime or ceftriaxone, ceftazidime, cefepime) are applied next to a disc with clavulanic acid, amoxicillin + clavulanic acid or ticarcillin + clavulanic acid. Positive result is indicated when the inhibition zones around any of the cephalosporin discs are augmented in the direction of the disc containing clavulanic acid. The distance between the discs is critical and 20 mm center-to-centre has been found to be optimal for cephalosporin 30 µg discs; however it may be reduced (15 mm) or expanded (30 mm) for strains with very high or low resistance level, respectively.

GATHERING AND KEEPING SAMPLES

The colonies that are to be subjected to the susceptibility test are taken up by culture media that have been previously swabbed with the sample under examination.

TEST PROCEDURE

1. Using a fresh, pure culture prepare a suspension of the test organism equal to 0.5 McFarland Standard.
2. Using a sterile cotton swab, spread the adjusted suspension over the entire area of a Mueller Hinton agar plate.
3. Apply the discs onto the inoculated plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones.
4. Incubate at 35±2°C for 18-24 hours.

EVALUATING THE RESULTS

At the end of the incubation period, measure the inhibition halos and interpret as indicated in the following tables:

Table 1. ESBL Disc Screening.

EUCAST recommended			CLSI recommended			
Antibiotic Disc		**Conduct ESBL-testing if**	**Antibiotic Disc**			**Conduct ESBL-testing if**
Cefotaxime	CTX 5 µg	Inhibition zone < 21 mm	Cefotaxime	CTX 30 µg	*, **	Inhibition zone ≤ 27 mm
			Ceftriaxone	CRO 30 µg	*	Inhibition zone ≤ 25 mm
Ceftriaxone	CRO 30 µg	Inhibition zone < 23 mm	Ceftazidime	CAZ 30 µg	*, **	Inhibition zone ≤ 22 mm
			Aztreonam	ATM 30 µg	*	Inhibition zone ≤ 27 mm
Ceftazidime	CAZ 10 µg	Inhibition zone < 22 mm	Cefpodoxime	PX 10 µg	**	Inhibition zone ≤ 22 mm
Cefpodoxime	PX 10 µg	Inhibition zone < 21 mm	** Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli* *** Protesu mirabilis*			

Table 2. ESBL Disc Confirmation.

Method	Antibiotic Disc		Confirmation is positive if
Combination Disc Test (CDT)	Cefotaxime alone and with Clavulanic acid *	CTX 30 µg CTL 30+10 µg	≥ 5 mm increase in inhibition zone of cephalosporin with Clavulanic acid
	Ceftazidime alone and with Clavulanic acid *	CAZ 30 µg CAL 30+10 µg	
	Cefepime alone and with Clavulanic acid **	FEP 30 µg FEL 30+10 µg	
** Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella* spp., *Shigella* spp. *** Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, *Providencia* spp., *Hafnia alvei*.			
Double-Disc Synergy Test (DDST)	Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime	CTX 30 µg CRO 30 µg CAZ 30 µg FEP 30 µg	expansion of indicator cephalosporin inhibition zone towards antibiotic with Clavulanic acid
	Amoxicillin + Clavulanic acid	AUG 20+10 µg	
	Ticarcillin + Clavulanic acid	TTC 75+10 µg	

False-positive results

ESBL confirmation test that use Cefotaxime as the indicator cephalosporin may be false-positive for *Klebsiella oxytoca, Proteus vulgaris, Citrobacter koseri, Citrobacter sedlakii, Citrobacter farmeri, Citobacter amalonaticus* and *Kluyvera* spp.

False-negative results

High-level expression of AmpC β-lactamases may mask the presence of ESBLs. Resistance to third generation cephalosporins and also resistance to cephamycins, e.g. cefoxitin MIC > 8 µg/ml, may be indicative of AmpC phenotype. To confirm ESBL phenotype in these isolates it is recommended that an additional ESBL confirmation test is performed with cefepime as the indicator cephalosporin, as cefepime is not hydrolyzed by AmpC β-lactamases. Alternative approach is the use of cloxacillin, a good inhibitor of AmpC enzymes: perform CDT or DDST methods on MH agar plates supplemented with cloxacillin.

QUALITY CONTROL

Appropriate strains for quality control of ESBL detection tests:

Microorganism		ESBL phenotype	AmpC phenotype
Klebsiella pneumoniae	ATCC® 700603	+	–
Enterobacater cloacae	ATCC® BAA-1143	–	+
Escherichia coli	ATCC® 25922	–	–

LIMITS

Diffusion susceptibility tests use an *in vitro* technique and cannot therefore reproduce the extremely complex *in vivo* conditions. Nevertheless, it is a useful and important tool that helps the clinician choose the correct therapy. Many variable factors influence the final result of the diffusion susceptibility test. The main ones are: the culture medium used, impregnation of the discs, inoculation of the medium, temperature, time and incubation atmosphere of the plates, pre-incubation and pre-diffusion conditions, depth of the medium, etc.

PRECAUTIONS

The disc 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 sensitization in sensitive subjects if they come into contact with the skin.
The discs are disposable products. They 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

Store the unopened blister at -20°C to +8°C till the expiry date. Allow unopened cartridge to come to room temperature before removing it from the blister for minimising condensation on the discs. Leftover discs from an opened cartridge should be stored at 2-8°C for no more than 7 days. Return unused discs to the refrigerator as soon as the application of the discs has been completed. Dispose of expire discs.

ELIMINATING USED MATERIAL

After use, the discs and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

REFERENCES

- CLSI M100-S24 - Performance Standards for Antimicrobial Susceptibility Testing, 2014.
- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In Vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution,consult accompanying documents	Do not reuse

LIOFILCHEM® s.r.l.

Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.net liofilchem@liofilchem.net

CE IVD