



ANTIMICROBIAL SUSCEPTIBILITY
SYSTEMS

Technical Information

Kit I for ESBL Identification, Cefotaxime

Product Code: SD1238

Application: Kit I for ESBL Identification, Cefotaxime is used for phenotypic detection of ESBL producers.

Composition**

*Ingredients	Concentration
Each Kit Contains 3 cartridges of Cefotaxime	30 mcg/disc
Cefotaxime/Clavulanic acid	30/10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (DM1173/DM2084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (DM1011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW1096/PW1297)

Principle

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., Ceftazidime, Cefotaxime, and Ceftriaxone) and monobactams (e.g., Aztreonam) but do not affect cephamycins (e.g., Cephoxitin and Cefotetan) or carbapenems (e.g., Meropenem or Imipenem). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. If an ESBL is detected, all penicillins, cephalosporins, and Aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility. CLSI has developed disk diffusion screening tests using selected antimicrobial agents (1). Strains of *Klebsiella* spp. and *Escherichia coli* that produce ESBLs may be clinically resistant to therapy with penicillins, cephalosporins, or Aztreonam, despite apparent in vitro susceptibility to some of these agents. Some of these strains will show zone of inhibition below the normal susceptible population, but above the standard breakpoints for certain extended spectrum cephalosporins or Aztreonam. Such isolates should be considered as potential ESBL-producer if the initial screen tests results are as follows:

Zones for Cefpodoxime(10µg) < 17 mm,
Ceftazidime(30µg) < 22 mm,
Aztreonam (30µg) < 27 mm,
Cefotaxime (30µg) < 27 mm,
Ceftriaxone (30µg) < 25 mm.

Proteus mirabilis isolate should be considered a potential ESBL-producer if the initial screen tests results are as follows:

Zones for Cefpodoxime(10µg) < 22 mm,
Ceftazidime(30µg) < 22 mm,
Cefotaxime (30µg) < 27 mm



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The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one antimicrobial agent suggested for screening will improve the sensitivity of detection. Cefpodoxime and Ceftazidime show the highest sensitivity for ESBL detection.

CLSI recommends performing phenotypic confirmation of potential ESBL-producing isolates of *K. pneumoniae*, *K. oxytoca*, or *E. coli* by testing both Cefotaxime and Ceftazidime, alone and in combination with Clavulanic acid (1). Testing can be performed by the broth micro dilution method or by disk diffusion. For disk diffusion testing, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 should be used for quality control of ESBL tests (1).

Following are quality control recommendations for combination discs used in screening and confirmatory tests for ESBLs. For *E. coli* ATCC 25922, the control limits is as per the standard discs; with combination discs a ≤ 2 mm increase in zone diameter for antimicrobial agent tested alone versus its zone when tested in combination with Clavulanic acid. For the ESBL-producing *K. pneumoniae* ATCC 700603 tested with: Cefotaxime, 17-25mm; with the combination disc, a ≥ 3 mm increase in Cefotaxime/Clavulanic acid zone diameter.

However, the phenotypic confirmatory test does not detect all ESBLs. Some organisms with ESBLs contain other β -lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. Currently, detection of organisms with multiple β -lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing. If an isolate is confirmed as an ESBL-producer by the CLSI-recommended phenotypic confirmatory test procedure, all penicillins, cephalosporins, and Aztreonam should be reported as resistant. This list does not include the cephamycins (cefotetan and Cephoxitin), which should be reported according to their routine test results. If an isolate is not confirmed as an ESBL-producer, current recommendations suggest reporting results as for routine testing. Do not change interpretations of penicillins, cephalosporins, and Aztreonam for isolates not confirmed as ESBLs.

Other isolates of Enterobacteriaceae, such as *Salmonella* species and *P. mirabilis*, and isolates of *P. aeruginosa* produce ESBLs. Though screening of *P. mirabilis* for ESBL production is recommended only when it is deemed clinically relevant (e.g. bacteremic isolate). The decision to perform ESBL screening tests to all urine isolates should be made on an institutional basis, considering prevalence, therapy, and infection control issues

Quality Control

Appearance: Each kit contain 6 cartridges: 3 of Cefotaxime 30mcg (SD1040) & 3 of Cefotaxime/ Clavulanic acid and 30/10 mcg (SD1724).

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar (DM1173) after 18 hrs incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
<i>E. coli</i> (25922) w/SD040	29-35*
<i>E. coli</i> (25922) w/SD724	30-37
<i>K.pneumoniae</i> (700603) w/SD040	17-25*
<i>K.pneumoniae</i> (700603) w/SD724	≥ 28

* = QC ranges as per CLSI standards.

Further Reading

1. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 29th Ed., CLSI Vol. 39 No.1, Jan 2019.
2. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493

Note:

1. Use following media to carry out susceptibility test
2. For rapidly growing aerobic organisms : Mueller Hinton Agar (DM1173/DM2084)
3. For *Haemophilus* spps : Haemophilus Test Agar (DM2259 + MS2117)
4. For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
5. For *Neisseria* spps : G.C.Agar +1% defined growth supplement (DM1434 + MS2025)

*** Not for Medicinal Use**

Storage and Shelf Life

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.



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