

Insert for Kit 98019

Total ESBL + AmpC Confirm Kit

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LANGUAGE: English

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for beta-lactamase identification

MANUFACTURE: ROSCO, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark.

INTENDED USE: Tablets are used for qualitative *in vitro* identification of microbial resistance mechanisms by the agar tablet/disc diffusion method, in order to confirm the mechanism by which the organism has gained resistance to specific antimicrobial agents.

INTENDED USERS: To be used only by professionals, qualified laboratory personnel and people trained to work with microbes and disc diffusion testing.

TEST PRINCIPLE: Six cartridges of tablets containing:
Cefotaxime + Cloxacillin, Ceftazidime + Cloxacillin, Cefotaxime + Clavulanate, Ceftazidime + Clavulanate and the 2 triple combinations: Cefotaxime + Cloxacillin + Clavulanate and Ceftazidime + Cloxacillin + Clavulanate.
The advantage of the kit is that it can be used to detect ESBLs in Enterobacteriaceae producing inducible AmpC (Enterobacter, Serratia, C. freundii, Morganella etc.) as well as in Enterobacteriaceae with little or no chromosomal beta-lactamase activity (E. coli, Klebsiella spp, P. mirabilis).
The test is performed on Mueller Hinton plain. It is not necessary to use Mueller Hinton with cloxacillin added.

DETAILED INSTRUCTIONS: ROSCO's detailed *Instruction for Use for Detection of resistance mechanisms* should be available in laboratories working with ROSCO's Diagnostic products. Latest version of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk
User's Guide can be obtained free of charge from your local distributor on request, or from ROSCO:
E-mail: info@rosco.dk
Phone: +45 43 99 33 77

CONTENT AND FORMULATION: 6 cartridges of tablets, formulated for maximum stability, each containing 50 tablets: Cefotaxime + Cloxacillin, coded CTX CX

- Cefotaxime + Clavulanate, coded CTX+C
- Ceftazidime + Cloxacillin, coded CAZCX Ceftazidime + Clavulanate, coded CAZ+C
- Cefotaxime + Cloxacillin + Clavulanate, coded CTXCC
- Ceftazidime + Cloxacillin + Clavulanate, coded CAZCC

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| STORAGE/HANDLING: | <p>Store at 2-8 °C until the expiration date shown on the product label. Cartridges should be closed during storage. Always seal the cartridges with the original green lid and never place the dispenser in the refrigerator.</p> <p>Allow the cartridges to acclimatize at room temperature (30-60 min) before removing the lid. Cartridges may open and close several times during use, without affecting tablets' shelf-life. The long shelf-life is due to the use of crystalline substances.</p> |
| PRECAUTIONS: | <p>For <i>in vitro</i> diagnostic use only. Safety precautions should be taken and aseptic techniques should be used when working with potential biohazards. To be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal. Refer to Product Safety Data Sheet.</p> |
| REQUIRED BUT NOT PROVIDED MATERIALS: | <p>Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.</p> |
| PROCEDURE: | <p>Using a fresh, pure culture prepare a suspension of the organism to be tested equivalent to McFarland 0.5.</p> <p>Using a sterile swap or Drigalski spatula spread the suspension uniformly over the entire area of a Mueller Hinton susceptibility agar plate.</p> <p>Using a single tablet or a dispenser, place one of each tablet on the inoculated agar plate, ensuring sufficient space between individual tablets to allow for proper measurement of inhibition zones. Notice that more than one Confirm ID Kit can be tested on the same plate.</p> <p>Incubate at 35±1°C for 18±2 hours (overnight).</p> <p>Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone.</p> |
| INTERPRETATION OF RESULTS: | <p>The results are interpreted by comparing the inhibition zones of the different tablets.</p> <ol style="list-style-type: none"> 1. Compare the zone of inhibition around CTXCC tablet to the zone of inhibition of Cefotaxime + Cloxa. If it is ≥ 5 mm larger, the isolate possess ESBL 2. Compare the zone of inhibition of CAZCC tablet to the zone around Ceftazidime + Cloxacillin. If it is ≥ 5 mm larger, the isolate possess ESBL. 3. Compare the zone of inhibition of CTXCC tablet to the zone of inhibition around Cefotaxime + Clavulanate. If it is ≥ 5 mm larger, the isolate is AmpC positive. 4. Compare the zone of inhibition around CAZCC tablet, to the zone around Ceftazidime + Clavulanate. If it is ≥ 5 mm larger, the isolate is AmpC positive. 5. Compare the zone of inhibition of CTXCC against the zones around Cefotaxime + Cloxa and Cefotaxime + Clavulanate. If the zone around the triple tablet is ≥ 5 mm larger than the zones around both double combinations the isolate produces ESBL + AmpC. 6. Compare the zone of inhibition of CAZCC against the zones around Ceftazidime + Cloxacillin and Ceftazidime + Clavulanate. If the zone around the triple tablet is ≥ 5 mm larger than the zones around both double combinations, the isolate produces ESBL + AmpC. <p>Ulstad et al (1) using ROSCO's Total ESBL + AmpC Confirm kit (98019) found that Norway as expected, is a low incidence country for fecal carriage of resistant bacteria among healthy humans (4.9 % ESBLs and 3.2 % AmpC).</p> |

Frøding et al (2) using the EUCAST disc diffusion test with Neo-Sensitabs (ROSCO ESBL detection kits) evaluated the accuracy of reading EUCAST disc diffusion after 6 hours' incubation. They concluded that inhibition zone reading at 6 hours, is an accurate method for detecting ESBLs in Enterobacteriaceae.

QUALITY CONTROL:

Although ROSCO Diagnostica A/S produces, by far, the most stable diffusion discs (tablets) it is necessary to perform regular quality control. This should be done with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination tablets against the negative control (i.e. E. coli ATCC 25922), should be within 3 mm.

As positive Q. C. stains the following may be used:
Enterobacter cloacae NCTC 13406, AmpC positive
Klebs. pneumoniae ATCC 700603, ESBL positive
Enterobacter cloacae ATCC BAA-1143 AmpC positive

Table 1: Enterobacteriaceae

| | | Cefotaxime + Cloxacillin + Clavulanate CTXCC | Ceftazidime + Cloxacillin + Clavulanate CAZCC | AMC PTZ |
|-------------------------------|---------------------------|---|--|---------------|
| ESBL | Cefotaxime + Cloxacillin | >= 5 mm and/or | - | |
| | Ceftazidime + Cloxacillin | | >= 5 mm | |
| AmpC | Cefotaxime + Clavulanate | >= 5 mm and/or | - | |
| | Ceftazidime + Clavulanate | | >= 5 mm- | |
| ESBL + AmpC | Cefotaxime + Cloxacillin | >= 5 mm and | | |
| | Cefotaxime + Clav | >= 5 mm | | |
| | Ceftazidime + Cloxacillin | | >= 5 mm and | |
| | Ceftazidime + Clav | | >= 5 mm | |
| ESBL Complex mutant phenotype | Cefotaxime + Cloxacillin | >= 5 mm and / or | | AMC resistant |
| | Ceftazidime + Cloxacillin | | >= 5 mm | PTZ resistant |

AMC = Amoxicillin + Clavulanate

PTZ = Piperacillin + Tazobactam

P. aeruginosa and Acinetobacter

For P. aeruginosa and Acinetobacter spp. only ESBL detection is relevant, because they intrinsically produce AmpC or cephalosporinases.

The detection of ESBLs in P. aeruginosa and Acinetobacter may be difficult because they produce cephalosporinases, that mask the effect of ESBLs. The use of cloxacillin containing media, that inhibits cephalosporinase activity, allow the detection of synergy between 3rd generation cephalosporins and clavulanate. ROSCO has developed cloxacillin containing tablets that permit their use in current MH agar plates. (See table 2)

Table 2: P.aeruginosa and Acinetobacter

| | | Cefotaxime + Cloxacillin + Clavulanate CTXCC | Ceftazidime + Cloxacillin + Clavulanate CAZC |
|--|---|--|---|
| ESBL incl. PER, VEB, GES CTXM, SHV etc. | Cefotaxime + Cloxacillin Ceftazidime + Cloxacillin | >= 5 mm and/or | >= 5 mm |

REFERENCES:

1. Ulstad CR: Carriage of ESBL/AmpC-producing or ciprofloxacin non-susceptible E. coli and Klebsiella spp in healthy people in Norway. Antimicrobial Resistance and Infection Control 5, 57, 2016.
2. Frøding I et al: Rapid EUCAST disc diffusion testing of MDR E. coli and K. pneumoniae: inhibition zones for ESBL can be readily read after 6 hours' incubation. J Antimicrob Chemother. Dec 2016, ahead of print.