ROSCO DIAGNOSTICA

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Insert for ESBL Confirm kits 98011 – 98012 – 98014

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ESBL Confirm kits

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for Beta-lactamase identification

MANUFACTURER: ROSCO Diagnostica A/S, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark.

INTENDED USE: Tablets are used for *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: Only to be used by professionals and people trained to work with microbes and disc diffusion testing.

PRINCIPLE OF THE TESTS:

ROSCO has developed a series of confirmatory (and screening) kits for the routine detection of ESBL producing isolates among the species Enterobacteriaceae. The choice of test kit depends on the isolate to be tested, and whether or not it produces chromosomal AmpC.
Therefore, the isolate should be identified beforehand, and assigned in to one of the two following groups:

Group 1:
E. coli, Klebsiella spp., P. mirabilis, Salmonella spp. and Shigella spp.

- Recommendation CLSI/EUCAST: Use of Cefotaxime and Ceftazidime and their combination with Clavulanate.

- If the isolate is, Cefoxitin resistant (zone < 19mm) also test for Cefepime and Cefepime+ Clavulanate as well.

Group 2:
Enterobacter spp., Serratia spp., Providencia spp., C. freundii, Morg. morganii and Hafnia alvei (all producing chromosomal AmpC)

- Recommendation EUCAST: Use Cefepime and Cefepime + Clavulanate

Use of the Total ESBL Confirm Kit includes detection of all Enterobacteriaceae, including chromosomal AmpC producers.
Use of the ESBL Confirm (acc. to CLSI/EUCAST) Kit includes detection of isolates in group 1 and use of the ESBL Confirm (Chrom. AmpC) Kit includes detection of isolates belonging to group 2. Furthermore, the ESBL Screen Kit allows for the routine screening of ESBL producing bacteria in all Enterobacteriaceae.

Generally, isolates showing the following zones of inhibition should be suspected of producing ESBL's:
<= 21 mm with Cefotaxime 5 ug and/or ≤ 22 mm with Ceftazidime 10 ug.
<= 27 mm with Cefotaxime 30 ug and/or<= 22 mm with Ceftazidime 30 ug.
and/or <= 17 mm with Cefpodoxime 10 ug.

DETAILED INSTRUCTIONS:

ROSCO's detailed Instruction for Use for "Detection of resistance mechanisms" should be available in each laboratory working with ROSCO's Identification discs
Latest edition of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk.
Instructions for Use and User's Guide can be obtained free of charge from the local distributor on request, or from ROSCO Diagnostica A/S:
E-mail: info@rosco.dk or
Fax: +45 43 52 73 74

CONTENT AND FORMULATION:

1. **Total ESBL Confirm Kit (98014)**
   Comprises one cartridge (50 tests) each of the following:
   Cefotaxime 30 µg
   Cefotaxime 30 µg + Clavulanate
   Ceftazidime 30 µg
   Ceftazidime 30 µg + Clavulanate
   Cefepime 30 µg
   Cefepime 30 µg + Clavulanate
   Testing of any kind of Enterobacteriaceae, including chromosomal AmpC producers.

2. **ESBL Confirm (acc. to CLSI/EUCAST) Kit (98011)**
   Comprises one cartridge (50 tests) each of the following
   Cefotaxime 30 µg
   Cefotaxime 30 µg + Clavulanate
   Ceftazidime 30 µg
   Ceftazidime 30 µg + Clavulanate
   Testing of Enterobacteriaceae Group 1 (without chromosomal AmpC) according to CLSI/EUCAST.

3. **ESBL Confirm (Chrom. AmpC) Kit (98012)**
   It comprises two cartridges (100 tests) each of the following:
   Cefepime 30 µg
   Cefepime 30 µg + Clavulanate
   Testing of Enterobacteriaceae Group 2 (possessing chromosomal AmpC) according to EUCAST.

4. **ESBL Screen Kit (98013)**
   It comprises two cartridges (100 tests) of the following:
   Cefpodoxime 10 µg
   Cefpodoxime 10 µg + Clavulanate.

STORAGE/HANDLING:

Store at 2-8°C in the box provided or unopened cartridges until the expiry date shown on the product label. Allow the cartridges to acclimatize to room temperature for 30-60 minutes before the lid is removed from the cartridge. Cartridges may be opened and closed several times during use, without affecting the shelf-life of the tablets. Always seal the cartridges with the original green lid, and never place a dispenser in the refrigerator. When stored at 2-8°C the cartridges should be allowed to acclimatize, as described above, before use. The long shelf-life is due to the use of crystalline substances.

PRECAUTIONS:

For *in vitro* diagnostic use only. Safety precautions should be taken and aseptic techniques 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.

MATERIALS REQUIRED BUT NOT PROVIDED:

Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.

PROCEDURE:

1. Using a fresh, pure culture prepare a suspension of the organism to be tested equivalent to McFarland 0.5
2. Using a sterile swap or Drigalski spatula spread the suspension uniformly over the entire area of a Mueller Hinton susceptibility agar plate.
3. Using a single tablet 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 Kit can be tested on the same plate.
4. Incubate at 35±1°C for 18±2 hours (overnight).
5. Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone.

INTERPRETATION OF RESULTS: The results are interpreted by comparing the inhibition zones around the different diffusion discs/tablets. Compare the zone of inhibition around the Cephalosporin + Clavulanate combination with the zone around the Cephalosporin alone. If the inhibition zone diameter around the combination disc is ≥ 5 mm larger than the one around the single cephalosporin disc/tablet the isolate is showing ESBL production.

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 plus the cephalosporin alone disc against the negative control (i.e. *E. coli* ATCC 25922), should be within 3 mm. Any greater difference indicates that the product has lost activity and should not be used.

As positive Q.C. strains the following may be used:
Klebsiella pneumoniae ATCC 700603,ESBL positive,(except cefepime)
Enterobacter cloacae NCTC 13464, ESBL positive (use Cefepime and FEP+C)

REFERENCES:

www.rosco.dk