Insert for Kit 98008

ESBL + AmpC Screen ID Kit

REVISION: DBV0032J

DATE OF ISSUE: 09.02.2017

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 *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: This combination screen test is designed for the routine screening of ESBL and/or AmpC producers. ESBL + AmpC Screen Kit consists of four cartridges of disc diffusion tablets: one cartridge of tablets with Cefotaxime alone and three cartridges of tablets with Cefotaxime combined with either Clavulanate (ESβL inhibitor) or Cloxacillin (AmpC inhibitor) and Cefotaxime combined with both Clavulanate and Cloxacillin. If an organism is suspected of ESβL and/or AmpC activity it can be shown by a difference in the inhibition zone of the cephalosporin alone and in combination with the inhibitors. The choice of cephalosporin is made to have the highest sensitivity and specificity with a single agent.
For the highest sensitivity and specificity, the laboratory should include results for all third generation cephalosporins. All isolates with reduced susceptibility to these, or Aztreonam should be suspected of being ESBL producers.
For confirmatory AmpC and ESBL tests ROSCO recommends the use of our kits intended for these purposes; AmpC Confirm Kit and Total ESBL Confirm Kit.

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: 4 cartridges, each containing approximately 50 tablets, formulated for maximum stability:

- Cefotaxime 30 µg, coded CTX30
- Cefotaxime 30 µg + Clavulanate, coded CTX+C
- Cefotaxime 30 µg + Cloxacillin, coded CTXCX
- Cefotaxime 30 µg + Clavulanate + Cloxacillin, coded CTXCC

STORAGE/HANDLING: 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.

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.

PRECAUTIONS: For *in vitro* 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.

REQUIRED BUT NOT PROVIDED MATERIALS: 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 Screen 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 zone. No zone around a tablet corresponds to a 9 mm inhibition zone.

Frøding et al (4) using the EUCAST disk diffusion test with Neo-Sensitabs (Rosco ESBL Detection kits) evaluated the accuracy of reading EUCAST disc diffusion after 6 hours' incubation (compared to normal 18 hours). They concluded that **inhibition zone reading at 6 hours** is an accurate method for detecting ESBLs in Enterobacteriaceae.

INTERPRETATION OF RESULTS: The results are interpreted by comparing the inhibition zones of the different tablets.

1. Compare the zone of inhibition of the Cefotaxime 30 µg tablet to the zones of inhibition of each of the Cefotaxime 30 µg + inhibitor(s) combination tablets. If all zones are within 3mm of each other, record the organism as neither expressing ESβL nor AmpC activity.
   Frøding et al (4) using the EUCAST disk diffusion test with Neo-Sensitabs (Rosco ESBL Detection kits) evaluated the accuracy of reading EUCAST disk diffusion after 6 hours incubation (compared to normal 18 hours). They concluded that inhibition zone reading at 6 hours, is an accurate method for detecting ESBLs in Enterobacteriaceae.

2. Measure the inhibition zones around Cefotaxime 30 µg (CTX30) and Cefotaxime 30 µg + Cloxacillin(CTXCX) and compare with Cefotaxime 30 µg + Clavulanate(CTX+C) and Cefotaxime 30 µg + Clavulanate + Cloxacillin (CTXCC), respectively. If both CTX+C – CTX30 and CTXCC – CTXCX is ≥ 5mm and both the values in step 3) are <5 mm, the organism is demonstrating ESβL activity alone.

3. Measure the inhibition zones around Cefotaxime 30 µg + Clavulanate (CTX+C) and Cefotaxime 30 µg (CTX30) and compare with Cefotaxime 30 µg + Clavulanate + Cloxacillin (CTXCC) and Cefotaxime 30 µg + Cloxacillin(CTXCX), respectively. If both

CTXCC – CTX+C and CTXCX – CTX30 ≥ 5mm and the values in step 2) is < 5mm, the organism is demonstrating AmpC activity alone.

4. Measure the inhibition zones around Cefotaxime 30 µg (CTX30) and Cefotaxime 30 µg + Cloxacillin (CTXCX) and compare with Ceftaxime 30 µg + Clavulanate(CTX+C) and Cefotaxime 30 µg + Clavulanate + Cloxacillin(CTXCC), respectively. If CTXCC – CTXCX ≥ 5mm (ESBL) and CTX+C – CTX30 < 5mm (AmpC), the organism is demonstrating both ESβL and AmpC activity.

Please notice that Clavulanate has two different functions:

1) It inhibits ESBL and
2) It induces AmpC. This explains why CTX+C-CTX30 < 5mm, if the isolate possesses an AmpC

5. Use table 1 to assist in the interpretation

Procedure for reading the results.

Step 1: If B-A and/or D-C ≥ 5 mm: ESBL positive.
Otherwise ESBL is negative.

Step 2A: Isolate found ESBL positive:
If D-B ≥ 5 mm and/or B-A < 5 mm: AmpC positive.
Consequently, isolate ESBL + AmpC positive

Step 2B: Isolate found ESBL negative:
If C-A and/or D-B >= 5 mm: AmpC positive.
Consequently, isolate ESBL negative and AmpC positive
Otherwise negative for both ESBL and AmpC

A calculator program is available.

QUALITY CONTROL: Although ROSCO produces 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 tablet 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:
Enterobacter cloacae NCTC 13406, Amp C positive
Enterobacter cloacae ATCC BAA – 1143, Amp C positive
Klebs. pneumoniae ATCC 700603 ESBL positive

Table 1.

		Cefotaxime CTX30 A	**Cefotaxime+Clav. CTX+C B**	**Cefotaxime+Cloxa. CTXCX C**
ESβL	CTX+C or B CTXCC D	≥ 5 mm -	- <5 mm	- ≥ 5 mm
AmpC	CTXCX or C CTXCC D	≥ 5 mm -	- ≥ 5 mm	- <5 mm
ESβL + AmpC	CTX+C and B CTXCC D	<5 mm (AmpC) -	- ≥ 5 mm (AmpC)	- ≥ 5 mm (ESBL)

Neither ESβL nor AmpC: All zones within 3mm of each other. CTXCC = Cefotaxime + Clavulanate + Cloxacillin.

Note: "-" means that the difference is irrelevant for the mechanism (i.e. the difference between CTXCC compared to CTX30 is irrelevant for the detection of ESBL).

REFERENCES:

1. Harila I: Evaluation of 5 different methods for detection of ESBL-producing Enterobacteriaceae(Swedish) Umeå University 2015.
2. Nayar R et al: Antibiotic impregnated tablets for screening ESBL and AmpC beta-lactamases.IOSR Journal of Pharmacy 2, 207-209, 2012.
3. Scapaticci M et al: Epidemiology of ESBL, AmpC and Class A carbapenemases at San Camillo Hospital of Treviso (Italy). Between april 2012 and march 2014. Microbiologia Medica 31, 4622, 2016.
4. Frøding I et al: Rapid EUCAST disc diffusion testing of MDR E. coli and K. pneumoniae: inhibition zones for ESBL can be reliably read after 6 h. incubation. J Antimicr Chemother Dec 20 2016, ahead of print.