Insert for Total ESBL + AmpC Confirm kit (98019)

REVISION:	DBV0037A
DATE OF ISSUE:	01-04-2013
LANGUAGE:	English

Total ESBL + AmpC Confirm kit
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 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: Only to be used by professionals and people trained to work with microbes and disc diffusion testing.

PRINCIPLE OF THE TEST:

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 each laboratory working with ROSCO's *Diagnostic products.*
Last edition of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk. Here more detailed information can also be found in ROSCO's User's Guide for DIATAB in English.
Instructions for Use and User's Guide can be obtained free of charge from your 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:

6 cartridges of tablets, formulated for maximum stability, each containing 50 tablets:
Cefotaxime + Cloxacillin, coded CTXCX
Cefotaxime + Clavulanate, coded CTX+C
Ceftazidime + Cloxacillin, coded CAZCX
Ceftazidime + Clavulanate, coded CAZ+C
Cefotaxime + Cloxacillin + Clavulanate, coded CTXCC
Ceftazidime + Cloxacillin + Clavulanate, coded CAZCC

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. Once a cartridge has been opened and in particular when placed in a dispenser, it should be kept at room temperature for up to 2 months. If necessary, when in use for a longer period than 2 months, the cartridges can be stored at 2-8°C. 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.

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 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.
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 of the different tablets

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 ia 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

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
ESBL	Cefotaxime + Cloxacillin Ceftazidime + Cloxacillin	>= 5 mm and/or	- >= 5 mm
AmpC	Cefotaxime + Clavulanate Ceftazidime + Clavulanate	>= 5 mm and/or	- >= 5 mm-
ESBL + AmpC	Cefotaxime + Cloxacillin Cefotaxime + Clav Ceftazidime + Cloxacillin Ceftazidime + Clav	>= 5 mm **and** >= 5 mm	 >= 5 mm **and** >= 5 mm

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 Diagnostica 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 CAZCC
ESBL incl. PER, VEB, GES CTXM, SHV etc.	Cefotaxime + Cloxacillin Ceftazidime + Cloxacillin	>= 5 mm and/or	>= 5 mm

REFERENCES: www.rosco.dk