

# ROSCO DIAGNOSTICA

Rapid CARB Screen Kit 98021

REVISION:

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LANGUAGE:

English

## FOR IN VITRO DIAGNOSTIC USE ONLY

- PRODUCT GROUP:** Kits for detection of resistance mechanisms.
- MANUFACTURER:** ROSCO Diagnostica A/S, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark.
- INTENDED USE:** Tablets are used for *in vitro* screening of carbapenemase producing bacteria. The method is valid for Enterobacteriaceae and Pseudomonas aeruginosa.
- INTENDED USERS:** Only to be used by professionals and people trained to work with microbes and disc diffusion testing.
- PRINCIPLE OF THE TEST:** Potential carbapenemase producing bacteria are currently screened by the means of susceptibility testing of carbapenems (Imipenem, Meropenem and Ertapenem). Reduced inhibition zones around these carbapenems are used to indicate carbapenemase production. A rapid method is based on the identification of the hydrolysis of the beta-lactam ring of a carbapenem in the presence of an indicator. Utilizing this principle ROSCO Diagnostica has developed a new Diatab; Imipenem + Indicator (CARB). The test is performed quickly and the reading of the results is ready within 30 minutes to one hour, from the time the reaction is started. Thus, applying this kit, in the routine screening of carbapenemases, saves time and effort in the laboratory. The idea is to help the laboratory to perform their own carbapenemase screening.

## DETAILED

### INSTRUCTIONS:

ROSCO's detailed Instruction for Use of DIATABS should be available in each laboratory working with ROSCO's *Diagnostic products*. The latest edition of Instruction for Use can be seen in and/or printed out from ROSCO's website [www.rosco.dk](http://www.rosco.dk). Here more detailed information can also be found in ROSCO's User's Guide for Detection of resistance mechanisms 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](mailto:info@rosco.dk) or  
Fax: +45 43 52 73 74

### CONTENT AND FORMULATION:

Two vials with 6 mm tablets; Imipenem + Indicator (CARB), formulated for maximum stability, each containing 25 tablets equivalent to a total of 50 tests:  
One vial with 6 mm. tablets: CARB Negative Control Diatabs, 50 tablets.

### STORAGE/HANDLING:

Store at 2-8° C. Before use allow the vials to acclimatize for 30-60 minutes, in order to avoid condensation forming on the tablets. The expiry date on the vials only applies to vials with lids, stored at the correct temperature. Keep the vials well protected from light and avoid high humidity.

### 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.

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## MATERIALS REQUIRED BUT NOT PROVIDED:

Buffer B-PER II, Bacterial Protein Extraction Reagent.  
Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.

## PROCEDURE:

Use always fresh isolates. Otherwise, inoculate/incubate the isolate 2 times before testing.

Colonies should be taken from the following media: Mueller-Hinton Agar, Blood agar or TSA agar.

Do not use colonies from selective agars (Drigalski, Mc Conkey)

Add several loops of the strain to be tested (recovered from antibiogram) to 100 µl Tris-HCL 20 mmol/l lysis buffer (B-PER II; Bacterial Protein Extraction Reagent, Thermo Scientific). Standard Bug Buster reagent Tris buffered pH 7.5 (NOVAGEN) can also be used. Make a suspension of at least McFarland Standard 4.

Vortex the suspension for one minute and maintain at room temperature for 30 min.

Add 50 µl of the bacteria suspension in a tube to 100 µl saline solution and add 1 Imipenem + Indicator (CARB) and close the tube. Vortex for 1–2 seconds to disintegrate the tablet.

Incubate the test tube at 35-37 °C for 30 min, 1 hour and 2 hours, respectively.

The same process is repeated using the CARB Negative Control Diatab.

### **Blood cultures:**

Take 5 ml. of blood culture (positive for gram – negative bacilli) and centrifuge for 3 min. at 1500 g. Discard the pellet (red blood cells). The supernatant is recovered and centrifuged for 15 min. at 4000 g in order to pellet the bacteria.

Suspend the bacteria pellet in 150 µl TRIS – HCl lysis buffer and follow the procedure indicated.

### **Urine samples:**

Take 10 ml urine (positive for gram – negative bacilli) and centrifuge. Suspend the bacteria pellet in 150 µl TRIS – HCl lysis buffer and follow the procedure indicated.

## INTERPRETATION OF RESULTS:

A change of color from red to yellow indicates a positive reaction, indicating that the test strain possesses a carbapenemase.

If the reaction is positive after 30 minutes or 1 hour, it is not necessary to incubate further. The tube must be incubated for no more than 2 hours, because positive reactions may fade out.

In a few cases an orange colour or light yellow is obtained after incubation. Repeat the test using a higher inoculum.

If the Negative Control CARB shows a light yellow colour, report the result as uninterpretable, no matter the result of Imipenem+Indicator CARB.

Please notice: Some Enterobacteriaceae producing OXA-48 ( or similar showing MICs for imipenem  $\leq$  0.5 ug/ml may show a negative result with the Rapid CARB Screen kit. Suspect OXA-48 production when the isolate is high level resistant to temocillin (Temocillin 30 ug Neo-Sensitabs, zone < 12 mm).

### QUALITY CONTROL:

DIATABS	Positive	Negative
Imipenem + Indicator (CARB)	<i>Klebsiella pneumoniae</i> BAA 1705	<i>E. coli</i> ATCC 25922

## REFERENCES:

Nordmann P et al. Rapid detection of carbapenemase producing Enterobacteriaceae. Emerg. Infect. Dis. **18**, 1503-1507, 2012.

Nordmann P et al. Rapid detection of carbapenemase production in Pseudomonas. Presentation CI-078, ICAAC, 2012.

Tijet N et al : " Evaluation of the Carba NP Test for rapid detection of carbapenemase-producing Enterobacteriaceae and Ps aeruginosa." Antimicrob. Agents Chemother **57**, 4578-4580, 2013