ROSCO DIAGNOSTICA

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**Rapid CARB Blue Kit (98023)**

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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 has been developed for Acinetobacter spp., Enterobacteriaceae and Pseudomonas spp. Oxacillinases from Acinetobacter can be detected by this kit, while they are not detected in kits using lysis buffer.

**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 1 new Diatabs; Imipenem (x2)+ Brthymol Blue The test is performed quickly and the reading of the results is ready within 15 minutes to one hour, from the time the reaction is started. No lysis buffer needed. 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. 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 or
Fax: +45 43 52 73 74

**CONTENT AND FORMULATION:**

One vial with: Imipenem (x2)+Brthymol Blue, formulated for maximum stability, containing 50 tablets equivalent to a total of 50 tests:
One vial with: CARB Negative Control Blue 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. Vials may be opened and closed several times without affecting the potency or shelf-life of the tablets. Keep the vials well protected from light and avoid high humidity. The long shelf-life is due to the use of crystalline imipenem powder.

**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:**

0.9% NaCl solution adjusted at pH 8.5 (using 0.01 N NaOH solution).
**Please notice**: The pH value of this solution may fall during storage. Therefore, test always the pH value before use and adjust to pH 8.5, using 0.01 N NaOH.

**No lysis buffer needed.**
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, Columbia blood agar, TSA agar or Mueller-Hinton from BD. Other MH brands must be supplemented with ZnSO4 to a final concentration of 70 mg/liter.
Do not use agars containing glucose, maltose, sucrose because these sugars may be fermented by the strains to be tested resulting in an acidic suspension of bacteria that may affect the results of the Rapid CARB Blue test (false positives).
Zinc ions in MH agar are absolutely necessary for detection of VIM and NDM metalo-beta-lactamases. Some MH agars, such as Biomerieux's do not contains enough zinc ions and give false negative results.
Do not use colonies from selective agars (Drigalski, Mc Conkey).
Add one 10 ul loop of the strain to be tested (recovered from antibiogram) to 200 µl 0.9% NaCl solution at pH 8.5.
In case of Acinetobacter use 2x 10 ul loops of the strain.
Vortex the suspension for one minute and maintain at room temperature for 30 min.
Add 1 Imipenem(x2) +Brthymol Blue tablet and close the tube. Vortex for 1–2 seconds to disintegrate the tablet.
Incubate the test tube at 35-37 °C for 15 min, 30 min or 1 hour, respectively.
The same process is repeated using CARB Negative Control Blue Diatab.

**Blood cultures:**

**Protocol 1.**
Make a subculture: Take 2 ml nutrient broth containing 0.12 ug/ml imipenem ** and add 5 drops (75 ul) of the positive blood culture. Incubate for 2 hours at 37 degrees. Centrifugate at 10.000 g for 5 minutes. Wash the pellet in sterile water.
Suspend the bacteria pellet in 200 ul 0.9% NaCl pH 8.5 and follow the procedure indicated.
** Imipenem to make a 0.12 ug/ml sol. can be provided as an Imipenem 300 ug Diatabs

a) 1 Imipenem 300 ug Diatabs is added to 5 ml 0.9 % NaCl sol, Vortex for 1 minute and maintain at room temperature for 30 min (60 ug IMP/ml).
b) Dilute 1 ml supernatant into 4 ml 0.9 % NaCl sol (12 ug IMP/ml).
**c)** Add 20 ul of Sol b (12 ug IMP/ml) to 2 ml Nutrient broth (0.12 ug/ml).

**Protocol 2**
Transfer 0.5 ml positive blood culture to 2 tubes and add 50 ul Triton 10 % solution to each tube, Vortex and incubate 5 min at room temperature.
Centrifuge at 13.000 x g for 2 min and discard supernatant.
Resuspend the bacterial pellet in 500 ul distilled water (bacterial colonies must be

properly resuspended).
Centrife at 13.000 x g for 2 min and discard supernatant.
Resuspend the bacterial pellet in 200 ul 0.9 % NaCl sol at pH 8.5.To one of the tubes add the Imipenem(2)+bromthymol Blue Diatab and add the Negative Control Diatab to the other tube.Vortex 1 – 2 seconds to disintegrate the tablet and incubate for 15 min,30 min or 1 hour at 37 degrees Celsius.

**Urine samples:**
Take 10 ml urine (positive for gram – negative bacilli) and centrifuge. Suspend the bacteria pellet in 200 µl 0.9% NaCl pH 8.5 and follow the procedure indicated.

**INTERPRETATION OF RESULTS:**

A change of color from blue to yellow indicates a positive reaction, indicating that the test strain possesses a carbapenemase.
If the reaction is positive after 15 minutes or 30 min, the test is finished (it is not necessary to incubate further). The tube must be incubated for no more than 1 hour, because positive reactions may fade out.
If the test suspension is green yellow and the negative Control is blue, indicates a positive reaction. It is seen with oxacillinases from Acinetobacter.
If the test suspension is yellow and the Negative Control is green, indicates a positive result.
If the Negative Control Blue CARB shows a light yellow colour, report the result as uninterpretable, no matter the result of Imipenem + Brthymol Blue.

Please notice: Some Enterobacteriaceae producing OXA-48 (or similar) showing MICs for imipenem < 0.5 ug/ml may show a negative result with the Rapid CARB Blue kit. Suspect OXA-48 production when the isolate is high level resistant to temocillin (Temocillin 30 ug Neo-Sensitabs, zone < 12 mm). Some OXA-48-like are not true carbapenemases (OXA-163,OXA-405) and will produce a negative test result. They can be differentiated from true carbapenemases using the Temocillin Neo-Sensitabs. True carbapenemases show resistance to Temocillin Neo-sensitabs, while OXA-163, OXA-405) are susceptible to Temocillin (zone > 12 mm).

| | **Imipenem(2) + Brthymol Blue**<br>**All species** | | **Negative Control Blue**<br>**All species** |
|---|---|---|---|
| Carbapenemase<br>POSITIVE | Yellow<br>Yellow<br>Green yellow | and | Green / Blue<br>Green / Blue<br>Blue |
| Carbapenemase<br>NEGATIVE | Blue<br>Green | and | Blue<br>Green |

**QUALITY CONTROL:**

| **DIATABS** | **Positive** | **Negative** |
|---|---|---|
| Imipenem(2) + Brthymol Blue (CARB) | *Klebsiella pneumoniae* BAA 1705 | *E. coli* ATCC 25922 |
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**REFERENCES:**

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3) Pasteran F et al: Comparison of the in-house Blue Carba Test (BCT) with the Rapid CARB Blue Kit for detection of carbapenemase-producing Gram-negative bacilli.Presentation at ECCMID 2015 (Copenhagen).

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