

Insert for Kit 98023**Rapid CARB Blue Kit****REVISION:** DBV0043G**DATE OF ISSUE:** 06.05.2024**LANGUAGE:** English

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for detection of resistance mechanisms.**MANUFACTURER:** Rosco Diagnostica ApS, Stensmosevej 24A, DK-2620 Albertslund, 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:** To be used only by professionals, qualified laboratory personnel and people trained to work with microbes and disc diffusion testing.**TEST PRINCIPLE:** 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. The Imipenem stability in the Rosco Diatabs (3 years) compares with the instability of Imipenem solution (2-4 days) from competitor products.**DETAILED INSTRUCTIONS:** Rosco Diagnostica's detailed *Instruction for Use for Detection of resistance mechanisms* should be available in laboratories working with Rosco Diagnostica's Diagnostic products.
Latest version of Instruction for Use can be found at Rosco Diagnostica's website www.rosco-diagnostica.com
User's Guide can be obtained free of charge from your local distributor on request, or from Rosco Diagnostica:
E-mail: info@rosco-diagnostica.com
Phone: +45 93 40 65 65**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 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:

0.9% NaCl solution adjusted at pH 8.5 (8.3-8.7) (using 0.01 N NaOH solution).

Please notice: The pH value of this solution may fall during storage. Therefore, always test 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 metallo-beta-lactamases. Some MH agars, such as Biomerieux's do not contain enough zinc ions and give false negative results.

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

Add one 10 µl loop of the strain to be tested (recovered from antibiogram) to 200 µl 0.9% NaCl solution adjusted to pH 8.5 (8.3 - 8.7) using 0.01 N NaOH.

In case of *Acinetobacter* use 2x 10 µl 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**

Transfer 0.5 ml positive blood culture to 2 tubes and add 50 µl 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 µl distilled water (bacterial colonies must be properly resuspended).

Centrifuge at 13.000 x g for 2 min and discard supernatant.

Resuspend the bacterial pellet in 200 µl 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.

Nastro et al (9) describes a variation of the method, which allows the detection of carbapenemases (*Enterobacteriaceae*, *P. aeruginosa*, *Acinetobacter*) **after 4 hours incubation**, from a haze of bacterial growth obtained from a positive blood culture, with a sensitivity of 98.1 % and specificity of 100 %.

Paulussen (10) in a comparative study of Rapid CARB Blue against Rapidec Carba NP found a sensitivity of 97 % and a specificity of 100 % with the Rapid CARB Blue, while the Rapidec showed a specificity of 84.2 %.

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 color, report the result as uninterpretable, no matter the result of Imipenem + Brthymol Blue.

If the results are difficult to interpret use the following modifications:

- 1) Holding the tube in vertical orientation above eyelevel and inspecting the bottom of the tablet for yellow color (positive) and
- 2) the comparisons of test and negative control tubes by viewing them side by side, tilted gently to horizontal and examined in bright light above a white background.

Please notice: A few Enterobacteriaceae producing OXA-48 (or similar) showing MICs for imipenem < 0.25 µg/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 µg 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 All species		Negative Control Blue All species
Carbapenemase POSITIVE	Yellow Yellow Green yellow	and	Green / Blue Green / Blue Blue
Carbapenemase NEGATIVE	Blue Green	and	Blue Green

QUALITY CONTROL:

DIATABS	Positive	Negative
Imipenem(2) + Brthymol Blue (CARB)	<i>Klebsiella pneumoniae</i> BAA 1705	<i>E. coli</i> ATCC 25922

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