

Neo-Rapid CARB Kit 98024

(New Improved version of 98021)

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English

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for detection of resistance mechanisms.**MANUFACTURER:** ROSCO Diagnostica ApS, Stensmosevej 24, DK-2620 Albertslund, Denmark.**INTENDED USE:** Tablets are used for *in vitro* screening of carbapenemase producing bacteria. The method is valid for Enterobacteriaceae and Pseudomonas aeruginosa and Acinetobacter spp.**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) +Indicator(CARB) . The test is performed quickly and the reading of the result is ready within 15 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. The higher content of imipenem in the Neo-Rapid CARB (98024) results in a stronger colour development and easier differentiation between positives and negatives and also results in a higher sensitivity of the method. The imipenem stability in the Rosco Diatabs (2 years), should be compared with the instability of imipenem solution (2 – 4 days) in the CARBA NP test.

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-diagnostica.com . 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 Aps:

E-mail: : info@rosco-diagnostica.com or

Phone: +45 93 40 65 65

CONTENT AND FORMULATION:Two vials with 6 mm tablets; Imipenem (x2) + 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. 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:

Triton X-100.

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: Columbia blood agar or TSA agar or MH agar from BD. Other MH agar brands must be supplemented with ZnSO₄ to a final concentration of 70 mg/liter.

Zinc ions in MH agar are absolutely necessary for detection of VIM and NDM metallo-beta-lactamases. Some MH agars, such as Bioré's do not contain enough zinc ions and give false negative results.

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

Willey et al (9) found that using 200 µl of 0.9 % NaCl alone (without lysis buffer) at pH 8.5 adjusted with 0.01 N NaOH gave better results than the mixture of saline and lysis buffer and certainly much better than the newly introduced Rapidec Carba NP.

Pasteran et al (16) found that the use of Triton X-100 at 0.1 % instead of lysis buffer, gave an enhanced detection of carbapenemase producers directly from bacterial cultures. This procedure will also be effective in detecting oxacillinases in *Acinetobacter*. In the case of *Acinetobacter* use 2 x 10 µl loop of bacteria.

Add one 10 µl loop of the strain to be tested (from antibiogram) to 200 µl of 0.9% NaCl adjusted with 0.01 NaOH to pH 8.5. Dilute 2.14 gram Triton X-100 in 100 ml water and add 10 µl of this solution to the bacterial suspension.

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

Add 1 Imipenem (x2) + Indicator (CARB) 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 and 1 hour, respectively.

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

Blood cultures:**Protocol 1.**

Transfer 0.5 ml positive blood culture to 2 tubes and add 50 µl of Triton X-100 10 % solution to each tube, Vortex and incubate 5 min at room temperature.

Centrifuge at 13.000xg for 2 min and discard supernatant.

Re-suspend the bacterial pellet in 500 µl distilled water (bacterial colonies must be properly re-suspended).

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

Re-suspend the bacterial pellet in 200 µl NaCl 0.9 sol **at pH 8.5**

To one of the tubes add the Imipenem(x2)+indicator (CARB) tablet and to the other

tube add the CARB Negative Control Diatabs. Vortex 1-2 seconds to disintegrate the tablet and incubate for 15min, 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 a mixture of 200 µl 0.9 % NaCl sol **at pH 8.5** 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 15 minutes or 30 min., the test is finished (it is not necessary to incubate further), because positive reactions may fade out.

In a few cases, an orange yellowish colour or light yellow is obtained after incubation. This is a positive result too, if the negative control remains red.

Generally, positives are those tests displaying any color change from that of the Negative control in the incubation period (max 1 hour).

If the Negative Control CARB shows a light yellow colour, report the result as uninterpretable, no matter the result of Imipenem(x2)+Indicator(CARB).

If the results are difficult to interpret (13) use the following modifications: 1) holding the tube in vertical orientation above eye level and inspecting the bottom of the tablet for yellow color (positive) and 2) the comparison 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. If the result remains unclear, the test is repeated with a higher inoculum.

AbdelGhani et al (13) in a comparative study with Carba NP, found that the Neo-Rapid CARB kit (98024) exhibited 100 % specificity and 99 % sensitivity.

Bou Casals J (15) criticizes the comparative study of Dortet et al on rapid colorimetric tests. Dortet et al have a patent for NP Carba transferred to Bio-Merieux. Dortet et al have used an obsolete kit (98021) in their study, while kit 98024: Neo-Rapid CARB kit have substituted kit 98021 more than 6 months ago. Bou Casals reports that kit 98024 contain twice as much imipenem as 98021 and uses much less lysis buffer than its forerunner and has a shelf-life of 2 years.

Please notice: Suspect OXA-48 production, when the isolate is temocillin high level resistant (Temocillin 30 ug Neo-Sensitabs, zone < 12 mm).

Some OXA-48 like beta-lactamases are not true carbapenemases (OXA-136,OXA-405) and they will show a negative result with the test. They can be differentiated from true carbapenemases, because they show Temocillin susceptibility (zone > 12 mm), while the OXA-48-like carbapenemases are Temocillin resistant.

QUALITY CONTROL:

DIATABS	Positive	Negative
Imipenem(x2)+Indicator(CARB)	<i>Klebsiella pneumoniae</i> BAA 1705	<i>E. coli</i> ATCC 25922

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