ROSCO CE

Insert for Kit 98024

Neo-Rapid CARB Kit

(New Improved Version of 98021)

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LANGUAGE: English

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for detection of resistance mechanisms.

MANUFACTURE: ROSCO, 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 and Acinetobacter spp.

INTENDED USERS: Only to be used by professionals 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 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 color 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 (3 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 products.
The latest edition of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk
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:
E-mail: info@rosco.dk
Phone: +45 43 99 33 77

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 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 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: Triton X-100 10% Sol. TRIS-HCl Lysis 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: 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 Biomerieux'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 that the mixture of saline and lysis buffer and certainly much better that 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.
Use either Protocol 1 or Protocol 2.

Protocol 1.
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 ml of Triton X-100 10% sol. in 10 ml water and add 10 µl of this solution to the bacterial suspension.

Protocol 2.
Add one 10 µl loop of bacteria to a mixture of 150 µl of 0.9% NaCl sol + 50 µl TRIS-HCl lysis buffer (B-PER II). No pH regulation is necessary.
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 **or** 150 µl NaCl 0,9% sol +50 µl TRIS-HCl lysis buffer.
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.
Fernandez et al (19) took 8 drops (¨200 ul)of the positive blood culture bottles and inoculated 2 MH plates and incubated at 37 degrees in a CO2 **atmosphere for 4 hours.**The Carba NP test was performed on all Enterobacteriaceae,using as much inoculum as possible from the 2 MH agar plates.This rapid procedure could be used with the kit 98024.

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.Positivity within 15 min.,indicates high expression levels of carbapenemase gene.
In a few cases, an orange yellowish color or light yellow is obtained after incubation. This is a positive result too, if the negative control remains red.
Positives are those tests displaying any color change (to yellowish) compared to the Negative control in the incubation period (max 1 hour).
If the Negative Control CARB shows a light yellow color, 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.
Huber et al (17) found a sensitivity of 98% and specificity 95-100% with the Neo-Rapid CARB kit (98024) in Australia.
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 more than 2 years.
Paulussen(18) in a comparative study of the Neo-Rapid CARB kit against Rapidec CARBA NP and others,found a sensitivity of 98 % and a specificity of 100 %,while the Rapidec showed a specificity of 84.2 %.

Please notice: Suspect OXA-48 production, when the isolate is temocillin high level resistant (Temocillin 30 µg 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)	*Klebsiella pneumoniae* BAA 1705	*E. coli* ATCC 25922

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