Rapid detection of carbapenemase-producing *Enterobacteriaceae* strains

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1. **INTENDED USE**

The decreased susceptibility to carbapenems can be due either to the presence of specific enzymes (carbapenemases) in the bacteria or either to the modification in the membrane avoiding the penetration of the molecule inside the microorganism. β CARBA is a qualitative colorimetric test used for detecting strains with the decreased susceptibility to carbapenems due to the production of carbapenemases. The test may be performed directly with *Enterobacteriaceae* isolated colonies. β CARBA test is not intended to replace conventional antimicrobial susceptibility testing methods.

2. **SUMMARY AND EXPLANATION OF THE TEST**

Carbapenems are last-resort antibiotics, and the current dissemination of carbapenemases-producing *Enterobacteriaceae* (CPE) is extremely worrisome. All *Enterobacteriaceae* species may acquire genes coding for carbapenemases and CPE are frequently resistant to many other antibiotics leaving few treatment options. Carbapenemases are grouped into three classes according to their amino acid identity (Ambler classification):  
- Class A carbapenemases: mostly KPC-type;  
- Class B carbapenemases also called metallo-β-lactamases (MBL): mainly IMP, VIM and NDM types;  
- Class D carbapenemases: OXA-48 and its variants.

The β CARBA test allows rapid CPE detection, information of utmost importance for the determination of appropriate therapeutic schemes and the implementation of infection control measures. Interpretation of the test results should be made taking into consideration the patient's clinical context.

3. **PRINCIPLE OF THE PROCEDURE**

The principle of the β CARBA test is based on the change of color of a chromogenic substrate* in presence of CPE. The test is performed in a micro-tube from freshly isolated *Enterobacteriaceae* colonies. The interpretation must be performed within the following 30 minutes.

* Thanks to the contribution of Dr. Hideaki Hanaki of Kitasato University.

4. **REAGENTS**

4.1 *Description*

One kit including the R1, R2, R3 reagents and 25 micro-tubes

<table>
<thead>
<tr>
<th>Identification</th>
<th>Description</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Extraction solution (colorless)</td>
<td>One vial ready-to-use, 1mL</td>
</tr>
<tr>
<td></td>
<td>Preservative: 0.1% ProClin™ 300</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Lyophilised yellow chromogen mix</td>
<td>One vial, to be reconstituted with R3</td>
</tr>
<tr>
<td>R3</td>
<td>Suspension solution</td>
<td>One vial ready-to-use, 1.1mL</td>
</tr>
<tr>
<td></td>
<td>Preservative: 0.1% ProClin™ 300</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Storage and handling conditions
This kit should be stored at + 2-8 °C.
The reagents may be used up to the expiry date mentioned on the package.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Conservation (after first opening)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>3 months at + 2-8 °C</td>
</tr>
<tr>
<td>R2 (reconstituted solution)</td>
<td>3 months at + 2-8 °C</td>
</tr>
</tbody>
</table>

Ensure that the caps of the two vials are firmly tightened to avoid contamination or drying of the reagents. Store the vials in upright position.

5. WARNINGS AND PRECAUTIONS
For in vitro diagnosis use only
For healthcare professional use only

Health and safety instructions:
• This test kit should be handled by qualified personnel only, trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
• Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Hazardous laboratory, chemical or biological waste must be handled and discarded in accordance with all local, regional and national regulations.
• For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

Precautions related to the procedure
Do not use kits showing any sign of deterioration.

5.1. Preparation
• Ensure that the use of β CARBA test follows the procedure written into the package insert. Modification of the routine use can give incorrect results.
• Do not mix or associate reagents from different batches in the same run.
• Before use, homogenize by a brief vortexing each reagent R1 and R3.
• Ensure that the lyophilized reagent is in the bottom of the R2 vial prior reconstitution with R3 solution.
• Reconstitute lyophilized R2 with 1.1 mL of R3 solution (the rubber stopper must be discarded).
• Do not use reconstituted R2 if the color turns to red.
• Do not freeze the R1, R2 (reconstituted and not) and R3 reagents.
• Do not prepare in advance the R1 and R2 mixture.
• Do not use reagents after expiry date.
• Do not use Enterobacteriaceae colonies isolated from medium that has been incubated for more than 24 hours.
• Use only well-individualized colonies.

5.2. Handling
• Perform the test at room temperature (between 18-30°C).
• Shake the R1 and R2 vials before use.
• After use, store the vials R1 and R2 at +2-8°C in their box.
• Respect the amount of inoculum with a full 1µl loop.
• The interpretation of the color change must be performed within 30 minutes of incubation.

6. SPECIMENS
The test is performed with well-individualized Enterobacteriaceae colonies.
The colonies can be collected from the following media after 18-24 hours of incubation: Columbia Agar + 5% sheep blood, UriSelect™ 4, Drigalski, Trypto-Casein-Soy Agar. For use of other culture media, please contact Bio-Rad.
7. PROCEDURE

7.1. Materials required

7.1.1. Materials provided
• Micro-tubes
• Reagents R1 and R2 (R2 previously reconstituted with R3)

7.1.2. Materials required but not provided
• 1 µl loops
• Timer
• Incubator

7.2. Assay procedure
• Label the micro-tube.
• Add 40 µL of reagent R1 and 40 µL of reagent R2 into the micro-tube.
• Select *Enterobacteriaceae* freshly and well-individualized colonies and pick up with one 1 µl loop in order to fill it completely. The loop must be full. From a chromogenic medium, select colonies with the same homogenous color and morphology. Discharge completely the loop in the micro-tube.
• Homogenize the mix.
• Observe the color of the mix at T0.
• Incubate the micro-tube at 37°C (acceptable range: 33-39°C) for **30 minutes** and read the result.
• Note any change in color and interpret the result (negative/positive). The final interpretation of the color must be made within 30 minutes of incubation; however, a positive result can be observed in fewer than 30 minutes.

7.3. Quality control
• Negative control, e.g. *Escherichia coli* ATCC® 35218 (TEM-1 type penicillinase-producing strain).
• Positive control, e.g. *Klebsiella pneumoniae* NCTC 13438. This strain contains carbapenemase KPC-3 that confers resistance to carbapenems. In the routine testing, a well-characterized carbapenemase-producing clinical strain can be used.

7.4. Interpretation of results

<table>
<thead>
<tr>
<th>Color change to clear orange or red or purple</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No color change to clear orange or red or purple</td>
<td>Negative</td>
<td>Absence of carbapenemase production</td>
</tr>
</tbody>
</table>

8. PERFORMANCES CHARACTERISTICS

8.1. Precision Study
Within a period of 3 weeks, 4 strains were tested in triplicate 10 times by 2 different operators. Three different batches of β CARBA were tested for repeatability and intermediate precision. All tests showed expected results.

8.2. Performances
Two European studies were performed using collection strains well characterized with genotypic method. 298 *Enterobacteriaceae* strains were tested with β CARBA test. Among them, 91 were non carbapenemase-producing strains (expected negative results), 207 were carbapenemase-producing strains (expected positive results). The carbapenemase types were as follows: KPC (53), OXA-48-like (57), NDM (36), VIM (34), IMP (15), IMI (5), GES-5 (4) and *Klebsiella pneumoniae* isolates coproducing KPC & MBL carbapenemases (3).

β CARBA test was performed after culture of strains on Drigalski, Columbia Agar + 5% sheep blood or UriSelect™ 4 media for 18-24 hours.
8.2.1. **Sensitivity**

<table>
<thead>
<tr>
<th>Carbapenemase-producing isolates</th>
<th>Positive at 30 min</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>OXA-48-like</td>
<td>50</td>
<td>57</td>
</tr>
<tr>
<td>NDM</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>VIM</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>IMP</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>IMI</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>GES-5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>KPC &amp; MBL</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

- Sensitivity is equal to 89.4% (185/207) with 95% confidence interval [84.0; 94.7] % for carbapenemase-producing Enterobacteriaceae strains.
- A positive result before 30 min (reading at 15 minutes) was observed for 100%, 98.6% and 77.6% of true positive strains respectively for Columbia Agar + 5% sheep blood, UriSelect™ 4 and Drigalski media.

8.2.2. **Specificity**

<table>
<thead>
<tr>
<th>Non carbapenemase-producing isolates</th>
<th>Negative at 30 min</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89</td>
<td>91</td>
</tr>
</tbody>
</table>

- Specificity is equal to 97.8% (89/91) with 95% confidence interval [94.0; 100] % for non carbapenemase-producing strains.
- The two false positive strains were AmpC with decreased susceptibility to carbapenems.

9. **TEST LIMITATIONS**

- Using Enterobacteriaceae colonies isolated from Mac Conkey agar, CLED, BCP or EMB agar is not recommended.
- The test cannot be performed from colonies that color the mix at T0 in clear orange, red or purple .
- Some IMI- and GES-type carbapenemase-producing strains may not be detected.

10. **BIBLIOGRAPHY REFERENCES.**

2 - CLSI® M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Current Revision.