Rapid detection of carbapenemase activity in *Enterobacteriaceae*

The Carba NP (Nordmann-Poirel) test using bacterial colonies

*To be used with enterobacteriaceae showing any slight decreased susceptibility to any carbapenem (imipenem, meropenem, ertapenem)*

"A similar protocol can be used for the detection of carbapenemase activity from *P.aeruginosa* colonies"

**Protocol**

1. Add 100 μl of 20 mM Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent, Thermo Scientific, Pierce) in each of two 1.5 ml eppendorf tubes

2. Resuspend a 1/4 to 1/3 calibrated oese (10 μl) of bacterial colonies in each of those 100 μl of 20 mM Tris-HCl lysis buffer. (bacterial colonies may be recovered directly from the antibiogram around disk of carbapenem performed according to the disk diffusion techniques).

NB : Carba NP test was validated with bacterial colonies grown on Mueller-Hinton agar plates (Becton Dickinson, Le Pont de Chaix, France), blood agar plates, trypticase soy agar plates, and most of selective media used for carbapenemase producers screening. Carba NP test **CANNOT** be performed with bacterial colonies grown on Drigalski or McConkey plates.

Recommended medium : trypticase soy agar supplemented with ZnSO₄ at 70 μg/ml

3. Check that bacterial colonies have been correctly resuspended. If necessary mix up and down with a pipette

4. Add (i) 100 μl of Solution A in the first eppendorf tube and (ii) 100 μl Solution A + imipenem 6 mg/ml in the second 1.5 ml eppendorf tube.

5. Incubate at 37°C for a maximum of 2 hours

6. Optical reading of the color of each tube

**Interpretation :**

<table>
<thead>
<tr>
<th></th>
<th>No antibiotic</th>
<th>Imipenem</th>
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<tbody>
<tr>
<td>No carbapenemase</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Carbapenemase producer</td>
<td>Red</td>
<td>Orange/Yellow</td>
</tr>
<tr>
<td>Not interpretable</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
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</table>
Usually, the time required for obtaining positive results is as follows:
- KPC producers: 2 to 30 min
- OXA-48 like producers: 20 min to 1h
- Metallo-β-lactamases (NDM, VIM, IMP): 15 min to 1h

**Material**

- 1.5 ml Eppendorf tubes
- Imipenem sodium salt (Sigma-Aldrich) or Imipenem + cilastatin (drug used for patient treatment).
- B-PERII, Bacterial Protein Extraction Reagent (Thermo Scientific, Pierce), Cat: 78260.
- ZnSO₄, 7H₂O (Sigma-Aldrich, Cat: 221376)
- Negative (wild-type *E. coli*) and positive (*K. pneumoniae* OXA-48 or *K. pneumoniae* KPC-2) controls.

**Preparation and storage of Solution A**

1. Prepare a concentrated solution of red phenol 0.5% w/v
2. Mix 2 ml of the concentrated red phenol solution (strongly vortex before pipetting to resuspend the solution) in 16.6 ml of distilled water
3. Adjust the pH at 7.8 by adding drops of a NaOH solution (1 N)
4. Add 180 µl of ZnSO₄ (Sigma-Aldrich, Cat: 221376) 10 mM to obtain a final concentration of 0.1 mM

Solution A is stable at room temperature for 1 week and may be kept at -20°C for several months.

**Solution A + imipenem (6 mg/ml) has to be prepared extemporaneously.**

**However, batches of imipenem powders can be weighted and prepared in advance and kept at 4°C for two weeks if solution A is not added.**

- 600 µl of solution A is needed for each test (solution A, solution A + imipenem, positive and negative controls).