NG-Test CARBA 5 is an in vitro rapid and visual multiplex immunochromatographic assay for the qualitative detection and differentiation of five common carbapenemases (KPC, OXA-48-like, VIM, IMP and NDM) from carbapenem non-susceptible pure bacterial colonies when grown on the following media:

- 5% sheep blood agar or MacConkey agar (16-24 hours) for testing Enterobacteriaceae and Pseudomonas aeruginosa
- HardyCHROM™ CRE agar (18-24 hours) for testing E. coli and KES (Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae complex and Serratia marcescens).

The NG-Test CARBA 5 is intended as an aid for infection control in the detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa in healthcare settings. NG-Test CARBA 5 is not intended to guide or monitor treatment for carbapenem non-susceptible bacterial infections. A positive or negative NG-Test CARBA 5 test result does not rule out the presence of other mechanisms of antibiotic resistance. NG-Test CARBA 5 should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.

**Summary and Principles**

β-Lactams are first-line antibiotics for the treatment of infections caused by Enterobacteriaceae. Nevertheless, since the beginning of their massive use in the 1940s, their efficacy has been challenged by the production of enzymes which inactivate them: the β-lactamases. Among them are carbapenemases which hydrolyze carbapenem antibiotics. Before the 1940s, most of the resistance to antibiotics was associated with the production of Extended-Spectrum β-Lactamases (ESBLs) belonging to classes A, B, C, and D of Ambler’s classification. Since then, studies have shown an increase in the production of carbapenemases among the Enterobacteriaceae family. Among those carbapenemases, the β-lactamase KPC (Class A) has spread worldwide in the 2000s. In addition, the metallo-β-lactams (MBL) of IMP type, VIM and NDM (Class B) as well as OXA-48 (Class D) have also expanded. These carbapenemases are mainly detected in hospital settings and are responsible for most of the nosocomial infections, raising major global health problems since their presence can be difficult to detect.

NG-Test CARBA 5 is an in vitro rapid and visual multiplex immunochromatographic assay that detects one or more of the five common types of carbapenemase enzymes (KPC, OXA-48-like (O), IMP (I), VIM (V), NDM (N)) in bacterial colonies. Liquid extraction buffer is used as a cell lysing solution when mixed with colonies. Monoclonal antibodies that individually recognize each of the five carbapenemases are immobilized on a nitrocellulose membrane. Free monoclonal antibodies are present in the sample pad and labelled with colloidal gold. Upon addition of colonies mixed with extraction buffer to the sample pad, the capillary action of the nitrocellulose draws the sample through the mobile antibodies and immobile antibodies on the test strip. The immobilized control antibodies capture any mobile antibodies that run through the sample pad and nitrocellulose without binding to other test lines. A positive result occurs when a red line appears on the control region (C) and one or more lines appear in the test regions (K, O, V, I, or N) and indicates that the sample contains one or more carbapenemases. A negative result occurs when only the control line is observed and indicates that the sample does not contain any of the 5 carbapenemases. If the control line does not appear, the test result is invalid.

**Reagents and materials supplied**

Each kit contains:
- 20 Test cassettes in aluminum pouches with desiccant
- 20 Eppendorf tubes
- 20 Disposable pipettes of 100 μL
- 1 Extraction buffer solution in a plastic bottle (4.5 mL)
- 1 Instructions for Use

**Materials required but not supplied**

- Timer
- Single use gloves
- Loop
- Vortex

**Precautions**

- In vitro diagnostic test. For professional use only.
- All the operations must be carried out according to good laboratory practices.
- The devices must remain in the sealed pouches until they are used.
- Handle the samples as if they were potentially infectious.
- After use, discard the device in an infectious waste container.
- Do not re-use the device.

**Storage and stability**

Store the devices in their sealed pouches between 4 and 30°C. Do not freeze.

**Culture and sampling**

The samples to be tested shall be obtained and handled according to standardised microbiology procedures.

**Operating procedure**

1. Wear protective gloves and standard personal protective equipment.
2. Bring the kit components to room temperature for at least 10 minutes.

**Preparing the sample**

1. Dispense 5 drops (150 μL) of extraction buffer in one of the microtubes provided into the kit.
2. From the agar culture, touch 3 colonies with a loop, and then suspend it in the microtube containing 150 μL of extraction buffer.
3. Close the microtube.
4. Vortex to homogenise the mixture before use.

**Carrying out the test**

1. Open the pouch, and take out the device. Once opened, use the test immediately.
2. Using the provided pipette, add 100 μL of the prepared mixture (sample must reach the black line indicated on the pipette) to accurately aspirate 100 μL in the sample well labelled “S”.

**Result interpretation**

1. **Negative result**
   - If only one red line appears in the control region (C): the sample does not contain any carbapenemase or contains carbapenemase(s) at a non-detectable level and must be interpreted as a negative result.

2. **VIM Positive**
   - If one red line appears in the control region (C) and one or several lines appear in the test regions K, O, V, I, or N: the sample contains one or several carbapenemases and must be interpreted as a positive result.

3. **Invalid result**
   - If the control line (C) does not appear, the test result is invalid. Insufficient sample volume or incorrect sample processing are the two most likely reasons for control line failure. Deterioration of the test kit may have occurred. Repeat the procedure using a new test. If the problem persists, do not use the kit and contact your distributor.

*Do not interpret the test results after 15 minutes.*
NG-Test CARBA 5

**Quality control**
An internal quality control is included in the test. When the control line develops, it confirms the sample volume was sufficient and the procedure was correct.

The following organisms are routinely used for testing at Hardy Diagnostics:

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae ATCC® BAA-1705*</td>
<td>Positive KPC Line</td>
</tr>
<tr>
<td>Klebsiella pneumoniae NCTC 13442</td>
<td>Positive OXA-48-like Line</td>
</tr>
<tr>
<td>Klebsiella pneumoniae NCTC 13439</td>
<td>Positive VIM Line</td>
</tr>
<tr>
<td>Escherichia coli NCTC 13476</td>
<td>Positive IMP Line</td>
</tr>
<tr>
<td>Klebsiella pneumoniae ATCC® BAA-2146</td>
<td>No Positive Test Lines</td>
</tr>
<tr>
<td>Klebsiella pneumoniae ATCC® BAA-1706</td>
<td>No Positive Test Lines</td>
</tr>
</tbody>
</table>

*According to CLSI document M100, Klebsiella pneumoniae ATCC® BAA-1705 may undergo a spontaneous loss of the plasmid encoding the carbapenemase, leading to false-negative QC results. To avoid false-negative QC results, K. pneumoniae ATCC® BAA-1705 as well as other carbapenemase-producing organisms, should be maintained on a carbapenem-containing medium or with a selective antimicrobial disk on non-selective agar prior to testing QC.

**Performances and characteristics**

**Clinical Evaluation**
Performance of NG-Test CARBA 5 was evaluated at three geographically diverse hospitals with prospectively-collected and stock bacterial isolates. The identification of carbapenemase production on NG-Test CARBA 5 was compared to another FDA-cleared device, Xpert Carba-R by Cepheid (PCR for KPC, OXA-48 or 181, IMP, VIM, NDM), modified carbapenem inactivation method (mCIM) and EDTA carbapenemase inactivation method (eCIM) as described by CLSI M100, S29, and antibiotic susceptibility testing results to ertapenem, imipenem, and meropenem. Identifiability and susceptibility of organisms were confirmed using FDA-cleared ID and AST systems. NG-Test CARBA 5 quality control was performed in parallel every day of testing.

A total of 310 organisms were tested against PCR (Xpert Carba-R, Cepheid) and phenotypic tests (mCIM, eCIM, and disk diffusion). One organism did not meet enrollment criteria because it was a species of Pseudomonas (Cornell 50) other than *P. aeruginosa* and was therefore excluded from the analysis. Of the remaining 309 organisms tested, a total of 240 Enterobacteriaceae (which provided 244 results since four isolates co-produced two carbapenemases) and 69 *P. aeruginosa* isolates were tested on NG-Test CARBA 5 with concordant results obtained by phenotypic testing paired with Xpert Carba-R results.

Performance was equivalent between blood and MacConkey agar. Table 1 indicates the PPA and NPA for each individual target separated out by organism group. The overall PPA for Enterobacteriaceae was 100.0% (97.6% - 100.0%) and the overall NPA was 95.5% (88.9% - 98.2%) (Table 2). The overall PPA for *P. aeruginosa* was 100.0% (77.2% - 100.0%) and the overall NPA was 94.6% (85.4% - 98.2%) (Table 3). *P. aeruginosa* with NDM (n=2) were evaluated analytically in the bench testing.

**Table 1. Performance of NG-Test CARBA 5 vs. the comparator method for all sites combined**

<table>
<thead>
<tr>
<th>Plate</th>
<th>Organism Group</th>
<th>Total Targets</th>
<th>Target Organism Group</th>
<th>Total Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae (ENT)</td>
<td>244</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No True Positive results for OXA-48-like and NDM for the *P. aeruginosa* organism group in multicentric clinical testing.

Lower bounds are below 90% due to the low prevalence of the OXA, IMP, and VIM carbapenemases. The claim of NG-Test CARBA 5 detection of OXA, IMP, and VIM carbapenemases is supported by analytical reactivity data.

For Enterobacteriaceae, three isolates were false positive for IMP on NG-Test CARBA 5 (positive IMP on NG-Test CARBA 5, positive mCIM, and negative Xpert Carba-R result). One isolate was a false positive for NDM on NG-Test CARBA 5 (positive NDM on NG-Test CARBA 5, positive mCIM, negative Xpert Carba-R result). For *P. aeruginosa*, three isolates were false positive for IMP on NG-Test CARBA 5 (positive IMP on NG-Test CARBA 5, positive mCIM, and negative Xpert Carba-R result).

All three isolates were confirmed to have the IMP-8 gene, making these true positives for IMP after discrepant analysis. IMP-8 is predicted to be detected by Xpert Carba-R based on in silico analysis but has not been demonstrated analytically.) One isolate was confirmed to have an NDM-1 gene making this isolate true positive for NDM after discrepant analysis. (NDM-1 is predicted to be detected by Xpert Carba-R based on in silico analysis and has been tested analytically.) After discrepant analysis, the Enterobacteriaceae overall PPA increased to 100.0% (97.6% - 100.0%) and the overall NPA increased to 100.0% (95.6% - 100.0%).

All three isolates were confirmed to have an IMP gene (IMP-7, IMP-15, and IMP-19) making these true positives for IMP after discrepant analysis. (IMP-7 is a known limitation of Xpert Carba-R. IMP-19 is predicted to be detected by Xpert Carba-R based on in silico analysis but has not been tested analytically. The ability of Xpert Carba-R to detect IMP-15 is unknown.) After discrepant analysis, the *P. aeruginosa* overall PPA increased to 100% (80.6% - 100%) and the overall NPA increased to 100% (93.2% - 100%).

Ertapenem disks were routinely used to maintain selective pressure for isolated colonies of retrospective Enterobacteriaceae isolates. No selective pressure was used for isolated colonies of retrospective *P. aeruginosa* isolates.

**Table 2. Agreement of NG-Test CARBA 5 with the composite reference method when testing Enterobacteriaceae**

<table>
<thead>
<tr>
<th>Enterobacteriaceae</th>
<th>Composite Reference Method</th>
<th>Total Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>156</td>
<td>4</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>244</td>
</tr>
</tbody>
</table>

**Table 3. Agreement of NG-Test CARBA 5 with the composite reference method when testing *P. aeruginosa***

<table>
<thead>
<tr>
<th><em>P. aeruginosa</em></th>
<th>Composite Reference Method</th>
<th>Total Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>53</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>62</td>
</tr>
</tbody>
</table>

Positive Percent Agreement (PPA) = 95.5% (95% CI: 98.3 - 100.0%)

Negative Percent Agreement (NPA) = 94.6% (95% CI: 85.4% - 98.2%)

Quality control results of the assay were positive by mCIM.

An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored blaIMP variant - 7, (ii) are predicted by in silico analysis but not analytically demonstrated to be detected by the assay (blaIMP - 15), or (iii) the reactivity of the assay is unknown (blaIMP variant - 15). Isolates were positive by mCIM.

**Table 4. Performance of NG-Test CARBA 5 vs. the comparator method for all sites combined**

<table>
<thead>
<tr>
<th>NG-Test CARBA 5</th>
<th>Composite Reference Method</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>156</td>
<td>4</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>244</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive Percent Agreement (PPA) = 95.5% (95% CI: 98.3 - 100.0%)

Negative Percent Agreement (NPA) = 94.6% (95% CI: 85.4% - 98.2%)

An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored blaIMP variant - 7, (ii) are predicted by in silico analysis but not analytically demonstrated to be detected by the assay (blaIMP - 15), or (iii) the reactivity of the assay is unknown (blaIMP variant - 15). Isolates were positive by mCIM.
The bacterial isolates used to evaluate NG-Test CARBA 5 from blood and MacConkey agar were also used internally to evaluate the performance of NG-Test CARBA 5 from HardyCHROM™ CRE agar. These results were compared to Xpert Carba-R, mCIM, and eCIM as described by CLSI M100, S29, and antibiotic susceptibility testing results to erapenem, imipenem, and meropenem. Identity and susceptibility of organisms were confirmed using FDA-cleared ID and AST systems. NG-Test CARBA 5 quality control was performed in parallel every day of testing.

Of the 186 organisms enrolled, one organism was not available for testing and was excluded from the analysis. Of the 185 organisms that fell under HardyCHROM™ CRE claims, 180/185 (97.3%) organisms (184 target results) were recovered from Raw stool, and 178/185 (96.2%) organisms (182 target results) were recovered from C&S Cary Blair stool onto HardyCHROM™ CRE. Table 4 indicates the PPA and NPA for each individual target separated out by organism group. The overall PPA from raw stool specimen inoculated to HardyCHROM™ CRE was 100.0% (97.4% - 100.0%) and the overall NPA was 90.2% (77.5% - 96.1%) (Table 5). The overall PPA from C&S Cary Blair stool specimen inoculated to HardyCHROM™ CRE was 100.0% (97.3% - 100.0%) (Table 6) and the overall NPA was the same as the raw stool specimen.

### Table 4. Performance of NG-Test CARBA 5 vs. the comparator method - Analysis by Target

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>Specimen Type</th>
<th>Organism Group</th>
<th>Target</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>PPA</th>
<th>low 95% 1</th>
<th>high 95% 2</th>
<th>NPA</th>
<th>low 95% 1</th>
<th>high 95% 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Stool</td>
<td>E. coli, KES</td>
<td>KPC</td>
<td>76</td>
<td>0</td>
<td>0</td>
<td>108</td>
<td>100</td>
<td>93.2</td>
<td>100</td>
<td>100</td>
<td>96.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXA-48-like</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>166</td>
<td>100</td>
<td>82.4</td>
<td>100</td>
<td>100</td>
<td>97.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>175</td>
<td>100</td>
<td>70.1</td>
<td>100</td>
<td>100</td>
<td>97.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMP</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>177</td>
<td>100</td>
<td>51.0</td>
<td>100</td>
<td>99.3</td>
<td>95.2</td>
<td>99.4</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDM</td>
<td>36</td>
<td>1</td>
<td>0</td>
<td>147</td>
<td>100</td>
<td>90.4</td>
<td>100</td>
<td>99.3</td>
<td>96.3</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>C&amp;S Cary Blair Stool</td>
<td>E. coli, KES</td>
<td>KPC</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>107</td>
<td>100</td>
<td>95.1</td>
<td>100</td>
<td>96.5</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXA-48-like</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>164</td>
<td>100</td>
<td>82.4</td>
<td>100</td>
<td>97.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>174</td>
<td>100</td>
<td>67.6</td>
<td>100</td>
<td>97.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMP</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>175</td>
<td>100</td>
<td>51.0</td>
<td>100</td>
<td>99.3</td>
<td>95.2</td>
<td>99.4</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDM</td>
<td>36</td>
<td>1</td>
<td>0</td>
<td>145</td>
<td>100</td>
<td>90.4</td>
<td>100</td>
<td>99.3</td>
<td>96.2</td>
<td>99.9</td>
<td>99.9</td>
</tr>
</tbody>
</table>

1Lower bounds are below 90% due to the low prevalence of the OXA, IMP, and VIM carbapenemases. The claim of NG-Test CARBA 5 detection of OXA, IMP, and VIM carbapenemases is supported by analytical reactivity data.

### Table 5. Agreement of NG-Test CARBA 5 with the composite reference method when testing bacterial growth on HardyCHROM™ CRE agar after seeded in Raw Stool

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Stool</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG-Test CARBA 5</td>
<td>143</td>
<td>41/37</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>143/143</td>
<td>100%</td>
<td>(95% CI: 97.4-100%)</td>
</tr>
<tr>
<td></td>
<td>(37/41 = 90.2% (95% CI: 77.5-96.1%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Percent Agreement (PPA)</td>
<td>143/143 = 100% (95% CI: 97.4-100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1An alternative PCR assay showed that the NDM false positive isolate harbored a blaqIM-1 variant. Isolate was positive by mCIM.

2An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored blaqIM-8/47 variant that is predicted by in silico analysis but not analytically demonstrated to be detected by the assay. Isolates were positive by mCIM.
ANALYTICAL REACTIVITY

NG-Test CARBA 5 was evaluated with ninety-two strains characterized to have a target carbapenemase. Each organism was incubated aerobically for 16 hours on sheep’s blood agar and MacConkey agar at 35°C or 18 hours on HardyCHROM™ CRE agar at 35°C. Each test was performed in triplicate from each type of media. NG-Test CARBA 5 test result was read 15 minutes after inoculating the buffer mixed with bacteria into the sample port. The operator was blinded to the expected result while setting up and interpreting the test. All organisms that yielded a negative NG-Test CARBA 5 result were further analyzed by modified carbapenemase inactivation method (mCIM, CLSI M100, S29). After the mCIM analysis, the final sensitivity for all target organisms evaluated was 88/92 (95.7%) from blood agar and 90/92 (97.8%) from MacConkey agar. After the mCIM analysis, the final sensitivity for all target organisms evaluated was 41/41 (100%) from HardyCHROM™ CRE agar. Two IMP-producing P. aeruginosa isolates (IMP-14 and IMP-18) were negative on NG-Test CARBA 5 but positive by mCIM. On blood agar only, two Proteus mirabilis strains resulted in false negative results.

### Table 7. Analytical Reactivity Summary

<table>
<thead>
<tr>
<th>Organism Group</th>
<th>Number of strains tested on Blood/ MacConkey</th>
<th>Target</th>
<th>Number of targets tested on Blood/ MacConkey agar</th>
<th>Number of targets Tested HC CRE</th>
<th>Variants Tested</th>
<th>Variants Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>66</td>
<td>KPC</td>
<td>17</td>
<td>8</td>
<td>2, 3, 4, 6, 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXA-48-like</td>
<td>12</td>
<td>7</td>
<td>48, 181, 163, 232 (48 type)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM</td>
<td>11</td>
<td>9</td>
<td>1, 4, 5, 6, 23, 27, 31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMP</td>
<td>8</td>
<td>7</td>
<td>4, 8/47, 26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDM</td>
<td>15</td>
<td>11</td>
<td>1, 5, 6, 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>68</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>26</td>
<td>KPC</td>
<td>5</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXA-48-like</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM</td>
<td>13</td>
<td>2, 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMP</td>
<td>6</td>
<td>1, 7, 14, 18, 19, 26</td>
<td>14, 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDM</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1NDM-1 and IMP-26 not detected in P. mirabilis growth from blood agar, but yielded positive results from MacConkey agar.
2IMP-8 and IMP-47 were determined to be the same protein based on sequence analysis by the Beta-Lactamase Database (http://www.blbd.eu/BLDB.php?prot=B1IMP).
3Isolates have targeted carbapenemase resistance genes but were negative by mCIM making them true negatives for carbapenemase production. They were also negative by NG-Test CARBA 5.

### Table 8. Resistance Mechanisms Evaluated with NG-Test CARBA 5 for Specificity

<table>
<thead>
<tr>
<th>Organism Group</th>
<th>Resistant mechanisms evaluated</th>
<th>Blood &amp; MacConkey agar</th>
<th>HardyCHROM™ CRE agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>ACT-1, ACT-2, AmpC, CT-X-M [1, 3, 8, 9, 14, 15, 22, 24, 30, 40, 55, 74, 75, 79, 124], DHA-1, ESBL, IMI, OmpK35, OmpK37, OXA [1, 2, 30], SHV [11(2b), 12(2b), 18, 28, 31, 89(2b), 108(15), 145, 179(1), 180(1p), 182(1u), O85(2b), SME, SME-2, TEM [1, 2(2b), 11(2b), 63(2b), 93(2b), 210(1u), O85(2b), let(A), tet(B)]</td>
<td>ACT-1, AmpC, CT-X-M [1, 3, 8, 9, 14, 15, 22, 24, 30, 40, 55, 74, 75, 79, 124], DHA-1, IMI, MIR-8, OXA, SME, TEM-129(2b), let(A)</td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>aaAD6, aAD8, apH(3'-1)b, catB7, CES-1, CES-5(c), OXA [10, 50], PAO, PDC [1, 5, 19, 35], PER-1, strA, strB, suI, tet(c), VEB-1, inducible AmpC</td>
<td>Vuta</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td>Vuta</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### INCUBATION STUDY

In order to confirm that NG-Test CARBA 5 delivered consistent results over a range of incubation time, twenty-two strains were tested from blood and MacConkey agar every two hours from 16 to 24 hours of incubation. Fifteen of the twenty-two organisms were also tested from HardyCHROM™ CRE every two hours from 16 to 24 hours. All organisms tested produced the expected result on NG-Test CARBA 5 at every time point tested. NG-Test CARBA 5 test result was read 15 minutes after inoculating the buffer mixed with bacteria into the sample port. The operator was blinded to the expected result while setting up and interpreting the test.

### REFRIGERATION STORAGE STUDY

In order to determine if agar media that has been stored in the refrigerator can be used with NG-Test CARBA 5, twelve strains were cultured and evaluated over time from refrigerated storage. Blood and MacConkey agar plates were inoculated directly with organisms (colonies) for the fresh culture, streaking for isolation. HardyCHROM™ CRE was inoculated with organisms at 3x10⁵ CFU/mL in raw stool and stool in C&S Cary Blair Transport Media, streaking for isolation with a 1µL loop. Each strain was incubated aerobically on sheep’s blood agar and MacConkey agar at 35°C and tested were performed after 16 to 24 hours of incubation (Day 0). Ten of the twelve organisms were also tested from HardyCHROM™ CRE after 18 to 24 hours of incubation. All organisms tested produced the expected result on NG-Test CARBA 5 for each day of refrigeration for up to 3 days. The operator was blinded to the expected result while setting up and interpreting the test.

### REPRODUCIBILITY

Prior to initiating the clinical study, a panel of 20 blinded isolates provided by Hardy Diagnostics was tested at three distinct study sites on five work days to demonstrate reproducibility and to document proficiency in the performance of the test. Agreement of ≥95% with known test results was required before proceeding with the study. The testing was done with at least one operator and two readers, blinded to each other’s results, per site. All target carbapenemase positive isolates tested (100%) were detected by NG-Test CARBA 5 on all days of the reproducibility study.
**Variants Detected by NG-Test CARBA 5**

Table 9 below shows which variants were evaluated from bacterial isolates in analytical and clinical testing. Variants that have been reported to be detected in publications are also listed. This list may not be exhaustive of all enzyme variants that may be detected by NG-Test CARBA 5.

**Table 9. Summary of Variants Detected by NG-Test CARBA 5**

<table>
<thead>
<tr>
<th>Enzyme Type</th>
<th>Assayed Variants</th>
<th>Variants Detected in US clinical trial (Table 1)</th>
<th>Variants Detected in publications</th>
<th>Variants Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPC</td>
<td>2, 3, 4, 6, 12</td>
<td>2, 3, 4, 12</td>
<td>2, 3, 4, 9, 23</td>
<td></td>
</tr>
<tr>
<td>VIM</td>
<td>1, 4, 5, 6, 23, 27, 31</td>
<td>1, 4, 5, 6, 23, 27, 31</td>
<td>1, 2, 4, 5, 19, 26, 27, 31, 39, 46, 51, 52, 54, 56, 58, 59</td>
<td></td>
</tr>
<tr>
<td>IMP</td>
<td>4, 8, 26</td>
<td>1, 4, 8</td>
<td>1, 4, 6, 7, 8, 10, 11, 22, 29</td>
<td>14</td>
</tr>
<tr>
<td>NDM</td>
<td>1, 5, 6, 7</td>
<td>1, 5, 6, 7</td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 9</td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPC</td>
<td>2, 5</td>
<td>Variants unknown</td>
<td>Variants unknown</td>
<td></td>
</tr>
<tr>
<td>OXA-48-like</td>
<td>2, 11</td>
<td>181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIM</td>
<td>2, 14, 19, 26</td>
<td>2</td>
<td>4, 7, 8</td>
<td>13, 14</td>
</tr>
<tr>
<td>NDM</td>
<td>1</td>
<td>Variants unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Publications and Analytical Testing*
Bibliography


Symbols

This test was developed in collaboration with the CEA*. The French Alternative Energies and Atomic Energy Commission is a key player in research, development and innovation.

ATCC is a registered trademark of the American Type Culture Collection.