

# NG-Test CARBA-5 for the detection of carbapenemase enzymes expressed by CPOs

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## INTRODUCTION

Carbapenems (members of the  $\beta$ -lactam family of antibiotics) are effective against both Gram positive and negative bacteria and are often used as a last resort against multi-drug-resistant infections.  $\beta$ -lactam antibiotics are challenged by the emergence of  $\beta$ -lactamases; enzymes with the ability to hydrolyse the  $\beta$ -lactam ring and deactivate the antibiotic.  $\beta$ -lactamases with the ability to hydrolyse carbapenem antibiotics are carbapenemases, with the 5 most prevalent being the families KPC, NDM, IMP, VIM, OXA-48.

The emergence of carbapenemase producing organisms (CPOs), is an increasing concern in healthcare. The American Centre for Disease Control and Prevention (CDC) in 2019 classed CPOs as an urgent threat to public health<sup>1</sup> and the UKHSA has launched a national framework, setting out a range of measures to implement and minimise the impact of CPE in terms of cost, healthcare operation, and public health.

At the University Hospital of Wales (UHW) Cardiff, once a CPO is suspected from conventional culture and sensitivity testing, the isolate is sent for molecular confirmatory testing using the Cepheid GeneXpert CARBA-R test and referred to the Specialist Antimicrobial Chemotherapy Unit (SACU).

The turnaround time for the Cepheid is ~50 minutes, costs £18.85 per test, with molecular controls costing £264.25 per run (to be run for acceptance testing of kits, post-maintenance and intervention, and at a minimum once a month), and the assay possesses limitations for some IMP variant detection.

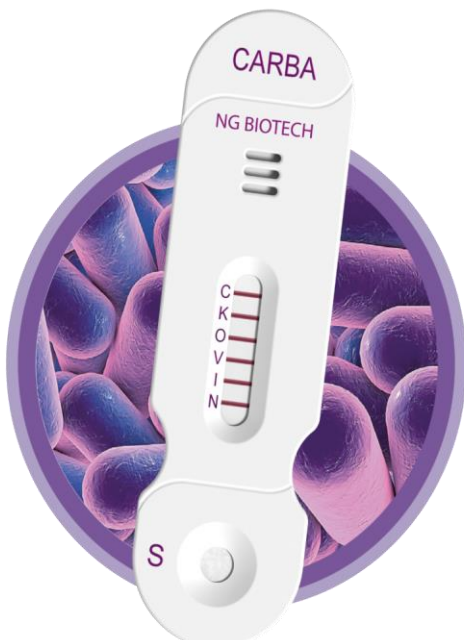
The NG-Test CARBA-5, is a lateral flow assay for the detection of carbapenemase enzymes expressed by CPOs. This assay takes 15 minutes, and costs £16.15 per test, with no additional cost for controls, as cultured NCTC organisms are used.

During this project, CARBA-5 was verified as a test alongside the procedures already in place for identification of CPOs, including culture, molecular testing using the Cepheid GeneXpert CARBA-R assay, and referral to SACU. The aim was to implement this test in the Bacteriology laboratory at UHW in place of the Cepheid CARBA-R assay, as a quicker and more cost-effective method.

## METHODS

**Phase one** - testing 5 NCTC strains each possessing one of the 5 carbapenemase enzymes:

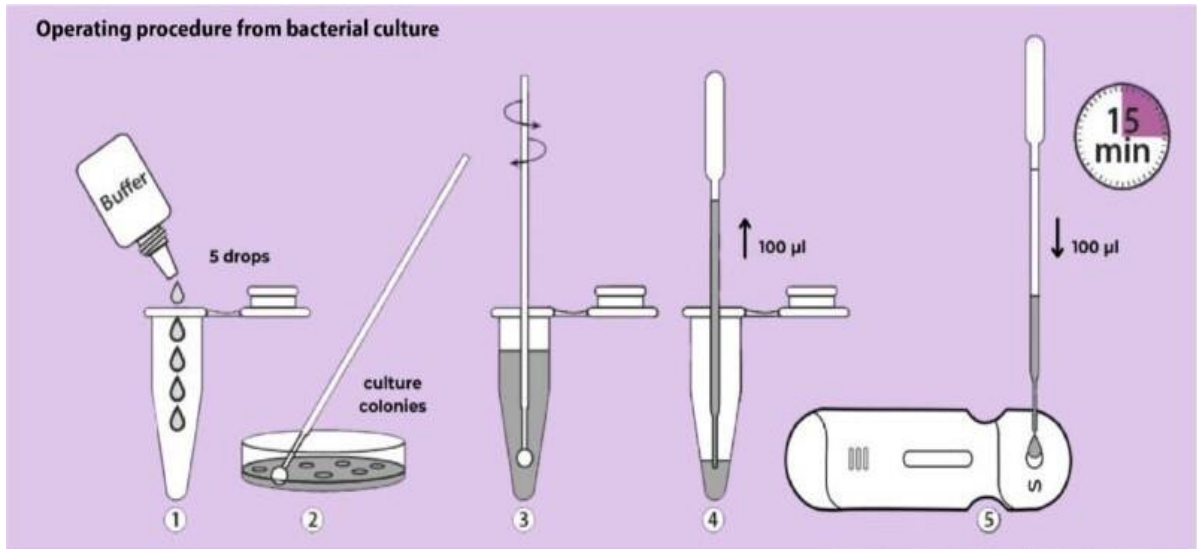
- *Klebsiella pneumoniae* NCTC 13440 (**VIM**-1)
- *Klebsiella pneumoniae* NCTC 13443 (**NDM**-1)
- *Klebsiella pneumoniae* NCTC 13438 (**KPC**-3)
- *Klebsiella pneumoniae* NCTC 13442 (**OXA**-48)
- *Escherichia coli* NCTC 13476 (**IMP**-1-like)



**Phase two** - 25 patient samples tested using the CARBA-5 and the CARBA-R. 10 of these were stored samples provided by SACU as a blind panel, the rest were a combination of stored isolates from 2023 of interest to IPC (5), and patient samples as they arrived in the lab (10). The result provided by SACU through their in-house PCR was taken as definitive, and their Whole Genome Sequencing (WGS) results used to provide context to the results analysis and discussion.

**From pure suspected CPO colonies, the CARBA-5 test is carried out as follows:**

1. Dispense 5 drops of extraction buffer into the kit-provided microtube.
2. Pick pure CPO colonies by tapping 3 colonies with a 1 $\mu$ l loop from the plate and emulsify in the buffer. Close the microtube and vortex to homogenise.
3. Open the lateral flow test and use immediately.
4. Using the disposable pipettes provided with the kit, fill the pipette to the line (100 $\mu$ l) with sample mixture from the microtube.
5. Dispense the entire volume into the sample well of the lateral flow labelled "S."
6. Read the results after 15 minutes and interpret.



## RESULTS



### Phase one

The CARBA-5 and CARBA-R assays were in 100% agreement with the expected results for the 5 control organisms. CARBA-5 was able to detect all 5 carbapenemases on one test, as well as individually and in combinations of 2 and 3 targets at once.

### Phase two

25 patient samples were tested simultaneously using the CARBA-5 lateral flow, and the Cepheid CARBA-R assay. 10 of these were a blind panel of stored patient samples provided by SACU, and 15 were patient samples. Once the 15 patient samples were tested, they were referred to SACU for confirmation.

## DISCUSSION

The CARBA-5 showed a sensitivity and specificity of 100%. The CARBA-R showed a sensitivity of 82% and a specificity of 100%. The CARBA-R generated 3 false negative results by failing to detect the carbapenemase IMP.

| NG-Test CARBA-5 |          |          |          |       |
|-----------------|----------|----------|----------|-------|
| Cepheid CARBA-R |          | Positive | Negative | Total |
|                 | Positive | 14       | 0        | 14    |
|                 | Negative | 3        | 8        | 11    |
|                 | Total    | 17       | 8        | 25    |

Results of note include:

- 2 *P.aeruginosa* samples containing IMP-14 and IMP-8 were detected by the CARBA-5 and SACU as IMP, but were not detected by CARBA-R. The CARBA-R package insert limitations state that the assay will generate a negative IMP result when testing samples containing IMP-7, IMP-13, or IMP-14 gene sequences. The insert sheet also states, "caution should be taken using the Xpert assay in areas with a high prevalence of CRE carrying blaIMP-8." Other studies have also shown that the GeneXpert CARBA-R test has been unable to identify the blaIMP-8 variant when tested<sup>2</sup>. These IMP variants are uncommon in Wales, with the most common variant being IMP-4 according to SACU WGS information. However, in an outbreak scenario, the ability to detect a wide range of variants is important. A negative result does not preclude the presence of other carbapenemases: CPOs not a part of the assay will not be detected and will affect the clinical sensitivity.
- An *E.coli* sample gave a negative result on both the CARBA-5 and the CARBA-R. The intended SACU result was positive for the carbapenemase GES, however this is not a target of either assay, so has been counted as a true negative result when determining the analytical sensitivity for both assays.
- The testing carried out on isolates of interest to IPC were 5 *K.pneumoniae* patient isolates that were investigated in 2023 as they were resistant to ertapenem (and some to imipenem and meropenem as well). Carbapenem resistance was found to be due to CTX-M type Extended Spectrum Beta-Lactamase (ESBL), efflux and porin loss by SACU, and confirmed as non-CPOs. All 5 isolates gave a negative result on the CARBA-5 and the CARBA-R as expected, in agreement with SACU.
- An *E.hormaechei* isolate was tested on CARBA-5 and CARBA-R, then referred to SACU. The CARBA-5 and the SACU report confirmed IMP. However, the CARBA-R, despite detecting IMP at a Ct of 39, sent out a "Not Detected" report. Cepheid was unable to provide information regarding cut-off points for Ct values or offer advice on results reporting in the future if similar cases arise with high Ct values.

## SUMMARY

To summarise, the CARBA-5 is quicker, more cost-effective, and more sensitive than the CARBA-R test when considering the Cepheid IMP limitations. The test is easy to perform and can be easily integrated into the laboratory workflow, and as of January 2025 has been implemented as part of Cardiff Bacteriology routine testing.

The CARBA-5 test allows for quick and accurate detection of CPO organisms, and these results highlight the importance of a rapid, sensitive and specific test. Rapid CPO detection allows IPC to make important decisions regarding patient isolation and treatment, and to prevent and control potential CPO outbreaks on hospital wards, ultimately improving patient outcome and public health.

|                  | NG-Test CARBA-5 | Cepheid CARBA-R |
|------------------|-----------------|-----------------|
| Sensitivity      | 100%            | 82%             |
| Specificity      | 100%            | 100%            |
| Turnaround Time  | 15 minutes      | 50 minutes      |
| Cost per test    | £16.15          | £18.85          |
| Additional costs | N/A             | £264.25*        |

\*Cost of running CARBA-R controls once including 5 cartridges

## REFERENCES

1. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
2. Huang,Y, *et al.* (2022). "Evaluating NG-Test CARBA 5 Multiplex Immunochromatographic and Cepheid Xpert CARBA-R Assays among Carbapenem-Resistant Enterobacteriales Isolates Associated with Bloodstream Infection". Microbiology Spectrum. 10(1).
3. Cepheid Xpert CARBA-R package insert
4. Mentasti.M *et al.* (2019). "Rapid detection of IMP, NDM, VIM, KPC and OXA-48-like carbapenemases from Enterobacteriales and Gram-negative non-fermenter bacteria by real-time PCR and melt-curve analysis." European Journal of Clinical Microbiology & Infectious Diseases. 32(9).