Carbapenemase-producing organisms (CPOs) are multidrug resistant and have been shown to be associated with increased morbidity and mortality. Rapid detection can guide clinical management and allow implementation of appropriate infection control procedures.

The Royal Free London NHS Foundation Trust is a tertiary referral centre with a diverse, international patient population. In April 2013, the Royal Free implemented a CPO screening programme of selected universal screening in some areas (intensive care, private, renal and liver units), together with risk factor-based screening in others (haematology, oncology, stroke and infectious disease units). In addition, any patients with identified risk factors for CPO and contacts of positive cases are screened.

The Royal Free London NHS Foundation Trust has a low prevalence of CPO (less than 1% of all patients tested in 2015), and the most commonly detected genotype is OXA-48, followed by NDM, VIM, KPC and OXA-23. In addition, occasionally more unusual types are isolated, including IMP, IMI and GES-5.

Carbapenemase-producing organisms are difficult to detect in diagnostic laboratories because of the many different genotypes that can be associated with a wide range of phenotypes. Standardised methodology has not been established, and current approaches adopted include:

- Culture-based methods are often cost-effective and easy to implement, but can lack specificity (particularly for OXA-48 detection) and are often slow and laborious.
- Molecular-based detection methods are rapid, but most assays available to diagnostic laboratories are not able to detect the large number of genotypes present worldwide; therefore, only those deemed to be the most relevant are detected.
- Methods based on the hydrolysis of a carbapenem and the subsequent detection of hydrolysis products are rapid, cost-effective and, in theory, have the ability to detect positive isolates regardless of the genotype present. However, there is debate about the most suitable carbapenem for use as the substrate, and assays have been linked with poor sensitivity for some carbapenemase types (particularly OXA-48).

The RESIST-3 O.K.N. is a lateral-flow immunochromatography assay (Coris BioConcept) that detects OXA-48-, KPC- and NDM-type carbapenemases from isolates within 20 minutes (Fig 1). Therefore, this study aims to evaluate the RESIST-3 O.K.N. assay for use in the routine diagnostic laboratory for the detection of CPOs.

**Methodology**

A total of 100 multidrug-resistant isolates were tested by the RESIST-3 O.K.N. assay. Samples included 95 clinical isolates (22 NDM [10 Escherichia coli, seven Klebsiella spp., two Acinetobacter baumannii, one Pseudomonas aeruginosa, one Citrobacter freundii and one Enterobacter spp.], 24 OXA-48 [11 Escherichia coli, nine Klebsiella spp., two Enterobacter spp.,
and two Serratia spp.), 19 VIM [16 P. aeruginosa, two Providencia spp., and one Escherichia coli], four OXA-23 [all Acinetobacter spp.], three KPC [all Klebsiella spp.], four NDM + OXA-48 [three Klebsiella spp. and one E. coli], one OXA23 + NDM [A. baumannii], one IMI [Enterobacter asburiae], one IMP [Pseudomonas aeruginosa], nine extended-spectrum β-lactamase [ESBL]-producing organisms [seven Escherichia coli, two K. pneumoniae], three derepressed AmpC [all Enterobacter spp.], four inducible AmpC [one K. oxytoaca, one E. cloacae, one C. freundii and one Serratia marcescens], and five control organisms.

All clinical isolates were sent to the reference laboratory (AMRHAI, Public Health England [PHE]) for confirmation of CPO status.

Isolates were tested by the RESIST-3 O.K.N. following the manufacturer’s instructions. In brief, bacterial colonies from an overnight subculture on cystine lactose electrolyte-deficient (CLED) agar were suspended in 10 drops of LY-A buffer, which was mixed to homogenise the solution. Three drops of the bacterial suspension were added to the sample well of the cassette, and the results were read after 15 minutes.

Results
The RESIST-3 O.K.N. assay correctly identified 28/28 OXA-48 (100% sensitivity, 87.7–100 CI), 26/27 NDM (96.3% sensitivity, 81.0–99.9 CI; 100% specificity, 94.7–100.0 CI), and all three KPC (100% sensitivity, 29.2–100 CI; 100% specificity, 96.0–100.0 CI) -positive isolates (Table 1).

The results were available within 20 minutes, with less than two minutes’ hands-on time. The strips were easy to read, although NDM-positive results were often weaker than OXA-48 and KPC results.

The assay was able to detect isolates carrying multiple carbapenemase types; the four isolates positive for OXA-48 and NDM included in this study were all correctly identified as OXA-48- and NDM-positive.

Overview of RESIST-3 O.K.N.
Method
The test is performed from a fresh culture in a Petri dish or in a blood culture bottle. Following a standard immunochromatography protocol, the test requires three minutes’ hands-on time and 15 minutes running time. No equipment is needed.

Validation
Compared to molecular methods and in various evaluations, the first two RESIST kits (OXA-48 and KPC) consistently gave 100% sensitivity and specificity. Now combining these two products and adding NDM in a single test, sensitivity and specificity remain at 100%

Presentation
Each RESIST kit contains 20 tests. The cassettes are packaged individually and dilution buffer and tubes are included. The product is stored at room temperature and has a shelf life of 12 months from the date of manufacture.

Table 1. OXA-48, KPC and NDM results obtained with the RESIST-3 O.K.N.

<table>
<thead>
<tr>
<th>Reference laboratory result</th>
<th>RESIST-3 O.K.N.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OXA-48</td>
<td>KPC</td>
<td>NDM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>Total</td>
<td>Pos</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
<td>0</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>67</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>67</td>
<td>95</td>
<td>3</td>
</tr>
</tbody>
</table>

The false-negative result obtained was from an NDM-positive K. pneumoniae isolate that was highly mucoid (Fig 2).

The correct result was obtained for the five control isolates included in the study.

Discussion and conclusions
The RESIST-3 O.K.N. performed well for OXA-48, KPC and NDM detection, with high sensitivity and specificity for each target. The assay was simple to perform, and results were easy to interpret and available within 20 minutes.

The missed NDM was from a highly mucoid K. pneumoniae isolate. Highly mucoid strains have been associated with false-negative results from other CPO assays that include a lysis step. However, adaptations to the methodology of some tests, in order to improve detection from mucoid isolates, have been described.

As not all carbapenemase types are detected by this assay (e. VIM, IMP, GES), users should consider the local prevalence of different genotypes, and their patient population, to decide on the suitability of this assay in their setting.

The Royal Free London NHS Foundation Trust serves a diverse patient population, and currently has a low prevalence of CPO. The RESIST-3 O.K.N. has now been implemented into the routine diagnostic laboratory as part of an algorithm aimed at improving detection of all carbapenemase genotypes, while optimising laboratory workflow.

Further reading

*Health Services Laboratories and †Royal Free London NHS Foundation Trust.

Further information is available from:
BioConnections
Unit 10 Brindley Court, Victoria Business Park Knypersley, Stoke-on-Trent ST8 7PP Tel: +44 (0)1782 516010 Fax: +44 (0)1782 510733 Email: welcome@biocconnections.co.uk Web: www.biocconnections.co.uk

Fig 2. Mucoid NDM-positive K. pneumoniae isolate that was negative by RESIST-3 O.K.N.