

**An optimised work-flow to reduce time-to-detection of carbapenemase-producing Enterobacteriaceae (CPE) using direct testing from rectal swabs.**

C. O'Connor<sup>1,2</sup>, M.G. Kiernan<sup>2</sup>, C. Finnegan<sup>1</sup>, M. O'Hara<sup>1</sup>, L. Power<sup>1</sup>, N.H. O'Connell<sup>1,2</sup>, C.P. Dunne<sup>2</sup>

<sup>1</sup> Department of Clinical Microbiology, University Hospital Limerick, Limerick, Ireland.

<sup>2</sup> Centre for Interventions in Infection, Inflammation & Immunity (4i) and Graduate-Entry Medical School, University of Limerick, Limerick, Ireland.

**Corresponding author:**

Prof Colum Dunne

Graduate Entry Medical School

University of Limerick

Limerick

Ireland

Email: [colum.dunne@ul.ie](mailto:colum.dunne@ul.ie)

Tel: +353 61 234703

**Keywords:** Carbapenem-producing Enterobacteriaceae, polymerase chain reaction, eSwab™, Check-Direct CPE®

**Abbreviations:**

Carbapenem-producing Enterobacteriaceae	CPE
Deoxyribonucleic acid	DNA
Imipenem-hydrolyzing beta-lactamase	IMP
<i>Klebsiella pneumoniae</i> carbapenemase	KPC
Mass spectrometry	MS
Matrix-assisted laser desorption/ionization time-of-flight	MALDT-TOF
New Delhi metallo-beta-lactamase-1	NDM
Oxacillin-hydrolysing	OXA-48
Polymerase chain reaction	PCR
Standard operating procedure	SOP
University Hospital Limerick	UHL
Verona integrin-encoded metallo beta-lactamases	VIM

## Abstract

Rapid detection of patients with carbapenem-producing Enterobacteriaceae (CPE) is essential for the prevention of nosocomial cross-transmission, allocation of isolation facilities and to protect patient safety. Here, we aimed to design a new laboratory work-flow, utilising existing laboratory resources, in order to reduce time-to-diagnosis of CPE. A review of the current CPE testing processes and of the literature was performed to identify a real-time commercial polymerase chain reaction (PCR) assay that could facilitate batch testing of CPE clinical specimens, with adequate CPE gene coverage. Stool specimens (210) were collected; CPE-positive inpatients (n=10) and anonymised community stool specimens (n=200). Rectal swabs (eSwab™) were inoculated from collected stool specimens and a manual DNA extraction method (QIAamp® DNA Stool Mini Kit) was employed. Extracted DNA was then processed on the Check-Direct CPE® assay. The three step process of making the eSwab™, extracting DNA manually and running the Check-Direct CPE® assay, took <5 minutes, 1 hour 30 minutes and 1 hour 50 minutes, respectively. It was time efficient with a result available in under 4 hours, comparing favourably with the existing method of CPE screening; average time-to-diagnosis of 48/72 hours. Utilising this CPE work-flow would allow a 'same-day' result. Antimicrobial susceptibility testing results, as is current practice, would remain a 'next-day' result. In conclusion, the Check-Direct CPE® assay was easily integrated into a local laboratory work-flow and could facilitate a large volume of CPE screening specimens in a single batch, making it cost-effective and convenient for daily CPE testing.

## Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) are prevalent worldwide in all areas of healthcare, with isolates and outbreaks reported in acute care facilities<sup>1-4</sup> including neonatology and paediatrics,<sup>5, 6</sup> respite/convalescence facilities<sup>7</sup> and long-term care facilities.<sup>8</sup> Early detection and infection control strategies are key factors for successfully restricting onward transmission of CPE.<sup>9</sup> The Mid-West of Ireland has the highest rates of CPE in Ireland with 140 first isolates detected between 2009 and 2015, predominantly *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae (n=123).<sup>10</sup> For clinicians and antimicrobial pharmacists, CPE pose a major challenge at the bedside as they significantly limit antimicrobial prescribing.<sup>11</sup> Morbidity and mortality are both increased in CPE infections, particularly bloodstream and respiratory infections,<sup>12</sup> with mortality rates ranging from 38-57%,<sup>9</sup> and poor outcomes in survivors,<sup>13-15</sup> emphasising the need for commencement of timely targeted antimicrobial therapy to increase patient survival.

Culture-based techniques using selective agars for the identification of CPE detect carbapenem-resistant organisms with minimal laboratory 'hands on' bench time, but they cannot differentiate either the carbapenemase type or the Enterobacteriaceae species present with additional phenotypic and/or molecular testing required.<sup>16, 17</sup> Within our laboratory, our current CPE screening process, utilising selective chromogenic agar (CHROMagar™ KPC, Paris, France) and GeneXpert® Carba-R for in-house molecular confirmation of CPE positive specimens (Cepheid, Buckinghamshire, United Kingdom), had an average turnaround time of 48/72 hours from specimen arrival in the laboratory to final CPE result for the clinician. The 48/72 hour delay in diagnosis was in keeping with other centres worldwide using selective cultures,<sup>18-20</sup> but was resulting in use of empiric broad-spectrum antimicrobials and an inefficient use of limited isolation facilities.

PCR-based techniques, facilitating direct testing from rectal swabs, have demonstrated efficiencies in the identification of CPE-positive isolates and a welcome reduction in laboratory turnaround time.<sup>21</sup> Our aim was to establish, under usual laboratory working conditions and current available resources, how quickly a CPE diagnosis could be reached in-house from the time of receipt of the clinical specimen in the laboratory to a CPE result. The workflow was designed to utilise direct screening from rectal swabs (eSwab™ 480CE, Copan, Italy), a manual DNA extraction method (QIAamp® DNA Stool Mini Kit, Qiagen, United Kingdom) and the installation of the Check-Direct CPE® assay onto an existing LightCycler® 480 Instrument (Roche Diagnostics Limited, United Kingdom). The Check-Direct CPE® assay (Check-Points, Wageningen, The Netherlands) enables the simultaneous detection of four of the most frequently detected carbapenemase enzymes (*bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub>) using direct testing from rectal swabs, and has been shown to reduce time-to-diagnosis with good sensitivity and specificity.<sup>22</sup>

Previous publications using the Check-Direct CPE® assay have utilised an automated DNA extraction methodology; NucliSENS® easyMAG® extraction kit, bioMérieux, France.<sup>23-25</sup> To the best of our knowledge, this is the first report of the use of a manual DNA extraction method with the Check-Direct CPE® assay.

## **Results**

### *Epidemiological*

From 195 anonymised community samples that underwent further processing, 58% (n=112) were from females. The median age of positive patients was 62.4 years (range 20-94 years). 186 samples (95%) were from general practitioner clinics from patients living in community

dwellings. Nine stool samples were received from long-term care facilities (LTCF) of which six were public institutions.

### *Technical*

A significant improvement in time-to-diagnosis versus the existing culture-based processes employed in the laboratory was demonstrated using our newly designed CPE laboratory workflow. The three step process of making the eSwab™, extracting DNA manually and running the Check-Direct CPE® assay, taking <5 minutes, 1 hour 30 minutes, 1 hour 50 minutes respectively, was time efficient with a result available in under 4 hours. A key factor in the ability to provide a result in under 4 hours was no delay in waiting to use the Lightcycler® instrument. While our process of making the eSwab™ was used as a surrogate for rectal swabs taken from patients, the time taken to perform a rectal swab on a patient is 5-7 minutes following our local standard operating procedure (SOP). When the time taken to transport the rectal swabs from clinical areas to the laboratory is accounted for, usually 10-20 minutes depending on time of day, a CPE results was still possible in under 4 hours. Antimicrobial susceptibility testing and an Enterobacteriaceae species identification using MALDI-TOF MS is still necessary on any positive isolate identified on the *Check-Direct* CPE® assay.

The Check-Direct CPE® assay was able to process larger batches of clinical specimens in comparison to the GeneXpert® Carba-R, making it suitable for use as part of a CPE screening programme. The assay was user-friendly and the interface on the Lightcycler® was easy to navigate. For clinicians and scientists with no previous or limited PCR experience, the pre-PCR sample preparatory steps required minimal hands-on time, with all steps occurring in an intuitive fashion.

A challenge encountered with our new workflow was that DNA could not be extracted from a number of samples (n=45, 26%) when assessed on the SPECTROstar Nano LVis Plate. This was attributed to a combination of overly diluted samples and other inhibitory factors within the stool specimens such as trace elements of blood in the patient's stool specimen or undigested food particles. Of the 45 stool specimens, 27% (n=12) were recorded as being a liquid consistency on collection, even before dilution. A review of the results from the other 128 community specimens from which DNA was positively extracted displayed a mean DNA concentration of 38.6 ng/µl. Interestingly, 8% (n=10) of these specimens were also classified as having a liquid consistency on collection yet DNA extraction was possible. This was an indication of involvement of other inhibitory factors.

### *Microbiological*

While the aim of this study was not primarily to identify new positive CPE cases, it is worth noting that no positive CPE isolates were detected from culture (n=390 plates; n=195 COLOREX mSuperCARBA™, n=195 chromID® CARBA SMART) following 24 hours incubation. One positive isolate (a KPC) was found using the Check-Direct CPE® assay as shown in Figure 1. This specimen had a Ct value of 32.54, which fell within the positive specimen value range.

### **Discussion**

Our aim in performing this study was to design a new laboratory-work flow, utilising existing laboratory physical and human resources, with the aim of expediting the diagnosis of CPE. The new laboratory work-flow we had designed achieved this objective. A major contributing factor to this improvement in time-to-detection was the use of testing directly from rectal

swabs, with the removal of traditional culturing processes, and the ability to perform screening using PCR in larger batches than previously using the GeneXpert<sup>®</sup> Carba-R. From a clinical perspective, the old CPE workflow in use within the laboratory was suboptimal as the protracted time to diagnosis was leading to prolonged empiric broad-spectrum antimicrobial use pending CPE screening results, poor patient flow through the hospital, challenges in the allocation of scarce isolation facilities and unnecessary anxiety for patients in awaiting CPE screening results for a minimum of two days.

By removing the use of selective chromogenic screening agar, which are historically labour intensive, with variable sensitivity,<sup>26</sup> this reduced physical work within the laboratory associated with culturing, moving agar plates from incubators to benches for further phenotypic and molecular testing. The availability of physical bench space for other laboratory routine work is also achieved as is the redeployment of scientists from culturing to other tasks.

Rectal swabs are currently considered to be the most sensitive approach to detect colonisation with multi-drug resistant enteric Gram-negatives<sup>8, 27</sup> but are wholly dependent on healthcare workers who are performing rectal swabbing at ward level ensuring the presence of visible faecal staining on the swab, as a surrogate markers for quality/amount of faecal material present, and in turn the likelihood of a good DNA yield on extraction. In this regard, any permanent move to direct testing from rectal swabs would require education to be provided to clinical ward staff. There are of course clinical scenarios where, despite the best efforts of those performing rectal swabs, rectal swabbing can be very challenging, for example, in patients with a large body mass index and those who are highly dependent with minimal independent mobility, it is often a perineum swab that is received. The minimal storage



requirements of the eSwab™ makes them a more attractive sampling method also as they are slightly smaller in size than the rectal swabs currently used for screening.

A consideration in selecting a commercial assay for this study was the range of CPE enzyme types detectable and an acceptance that no assay on the market at present has the ability to detect novel carbapenemases or to provide full coverage for all enzyme variants, in particular the OXA-48-like variants. Additionally, no assay as yet can provide an identification to Enterobacteriaceae species level. The most prevalent carbapenemases worldwide are IMP, VIM, OXA-48, NDM and KPC, with the latter three being most commonly identified in Ireland.<sup>28</sup> The Check-Direct CPE® assay had the ability to detect and differentiate between *bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub>, the same range as with the current GeneXpert® Carba-R, but the larger volumes of sample that could be processed in each batch was an attractive feature of the Check-Direct CPE® assay. In 2015, 9493 CPE screens were processed at UHL (average 792 per month), accounting for 20% of the laboratory workload, demonstrating our need for a cost-effective screening solution. A potential future problem that may emerge if the epidemiology of CPE in Ireland changes is that if more IMP isolates begin to appear within the region neither, the Check-Direct CPE® assay will not have capability to detect these isolates.

From a practical perspective, consideration for the use of an automated DNA extraction method is perhaps warranted given the challenges encountered with the manual DNA extraction. Any extraction method employed must be able to successfully extract DNA from all consistencies of stool specimens for CPE testing.

## **Conclusions**

The accurate and timely detection of CPE is crucial for appropriate patient management and for the rapid implementation of infection prevention and control measures. The integration of a molecular commercial assay for batch CPE screening significantly reduces time-to-detection. Changing an existing CPE work-flow must address the potential costs involved, annual numbers of CPE screens processed by the laboratory, CPE gene coverage and whether an automated DNA extraction system can be utilised to ensure optimal results.

## **Patients and Methods**

### *Setting*

The Department of Clinical Microbiology at the University Hospital Limerick (UHL) provides a centralised microbiology service for six acute hospital sites and for general practitioners working within the area. Two full-time consultant microbiologists and one clinical microbiology specialist registrar are employed. The UHL laboratory team is comprised of 35 regular laboratory scientists in addition to two surveillance scientists. The hospital group serves a population of 400,000 people.

### *Ethical approval*

Ethical approval to complete this study was granted by the HSE Mid-Western Regional Hospital Research Committee in December 2015.

### *Existing microbiological and molecular methods for the detection of CPE*

CPE surveillance is currently performed on stool samples or rectal swabs. KPC selective chromogenic agar is used (CHROMagar™ KPC; Fannin, Catalog No. W11498) with an overnight incubation. MALDI-TOF MS (Bruker Diagnostics) identification is performed on all colonies, as previously described.<sup>29</sup> Antimicrobial susceptibility testing is performed using

broth microdilution (ARIS Sensititre<sup>®</sup> system (Thermo Fisher Scientific Inc, Massachusetts, USA). Isolates with elevated carbapenem minimum inhibitory concentration (MIC), as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, are further evaluated using the modified hodge test (MHT). Commercially available diagnostic kits consisting of meropenem discs supplemented with  $\beta$ -lactamase inhibitors meropenem + dipicolinic acid, meropenem + boronic acid and meropenem + cloxacillin (Rosco Diagnostica A/S; Catalog no. 98006) are used to phenotypically distinguish CPE isolates. In-house molecular confirmation is performed using GeneXpert<sup>®</sup> Carba-R.

#### *A new CPE detection laboratory work-flow*

The Check-Direct CPE<sup>®</sup> assay (Serosep, Catalog no. 18-0080) is a multiplex real-time PCR based on the TaqMan fast advanced molecular beacon technique with fluorescent DNA probes labelled with four different fluorophores (FAM, VIC, Texas Red or Cy5) for the detection of *bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub> and an internal extraction/inhibition control. This assay was installed onto an existing LightCycler<sup>®</sup> 480 PCR machine already in use within the laboratory for Entericbio realtime test panels (Serosep) for the detection of gastrointestinal pathogens. The Check-Direct CPE<sup>®</sup> assay was validated prior to the commencement of this study using positive CPE clinical isolates obtained from a reference laboratory.

The use of eSwabs<sup>™</sup>, a flocked nylon swab applicator (eSwab<sup>™</sup> Copan; Medical Supply Company, Catalog no. 480CE), is recommended for performing direct testing from rectal/perianal swabs in the Check-Direct CPE<sup>®</sup> user manual (Version 2.3, Issued 01-09-2004). At the time of this study, eSwabs<sup>™</sup> were only approved for use within the hospital for environmental sampling. As a consequence, convenience duplicate rectal swabbing from

patients could not be arranged to provide a sample for testing for the study. In order to obtain faecal material from which to extract DNA, a variety of stool specimens were collected, stool specimens from known CPE-positive inpatients (n=10) and anonymised community stool specimens (n=200). There were 195 community stool specimens were suitable for further analysis (Figure 1). Stools were classified on collection as either formed or of a liquid consistency. From these stool specimens, eSwabs™ were inoculated as per manufacturer's instructions. Stoma bag samples were also accepted. Stools were collected over a four-week period. Each eSwab™ was inoculated from a fresh stool sample and frozen at -20 °C, pending further processing.

The Check-Direct CPE® user manual (Version 2.3, Issued 01-09-2004) specified that the Check-Direct CPE® assay was validated for use using the NucliSENS® easyMAG® Extraction kit (bioMérieux). This automated DNA extraction system was not available within our laboratory. As an alternative, a manual DNA extraction method (QIAamp® DNA Stool Mini Kit; Qiagen, Catalog no. 51504) was employed for DNA extraction following manufacturer's guidelines (QIAamp® DNA Stool Handbook 06/2012) with some modifications. Briefly, stool lysis buffer (Buffer ASL) was added to approximately 200 mg of frozen rectal swab diluted in liquid amies buffer (eSwab™) and mixed until homogenous. The sample was incubated at 70 °C for 5 min followed by centrifugation as per the protocol. An InhibitEX tablet was added to the supernatant and vortexed until completely dissolved and mixed followed by centrifugation as per the manufacturer's protocol. The supernatant was added to proteinase K; additional lysis buffer (Buffer AL) and 96% ethanol were subsequently added before incubation at 70 °C. DNA was then isolated following a series of centrifugations with QIAamp spin columns and buffer solutions. DNA was eluted in 100 µL elution buffer (Buffer

AE) and quantity and quality was assessed using a Spectrostar Nano plate reader LVis plate function (BMG Labtech). Extracted DNA was stored at -20 °C for subsequent PCR.

With regard to pre-PCR sample preparation, 15 µl of the Check-*Direct* CPE<sup>®</sup> assay PCR (qPCR) mix was added to each PCR well in-use. In addition, 10 µl of positive and 10 µl negative controls supplied with the kit, which already contained the internal control (a control DNA molecule provided as part of the kit), were used. Extracted DNA (known CPE positive or negative – 10 µL) was added to each well plus 5 µl of the internal control. DNA extracted from inpatient stool samples already confirmed as CPE positive were also used to confirm if our DNA extraction process could be used to extract CPE positive DNA. Amplification curves were analysed using the ‘High Confidence’ option as recommended by the manufacturer examining for typical sigmoidal amplification seen with positive test results and cycle threshold (C<sub>t</sub>) values were read.

Opportunistically as large volumes of stool samples were being collected, it was decided to trial two new agar plates for the laboratory at this time also; COLOREX mSuperCARBA<sup>™</sup> (E&O Laboratories Ltd; Syntec, Catalog no. PP3095) and chromID<sup>®</sup> CARBA SMART (bioMérieux, Catalog no. 414685). Culturing on these new agar plates also afforded a means of comparing the performance of the Check-*Direct* CPE<sup>®</sup> assay.

## **Funding**

This study was funded by a research grant awarded by the Irish Society of Clinical Microbiologists, which is supported by Pfizer Ireland.

## **Acknowledgements**

The authors thank the staff of the National Carbapenemase Producing Enterobacteriaceae Reference Laboratory Service (CPEMLS) at University Hospital Galway, Ireland, for providing CPE positive isolates for validation of the assay and the staff of the Departments of Clinical Microbiology and Infection Prevention and Control, UHL, for their support of this study.

DRAFT

## References

1. Lemmenmeier E, Kohler P, Bruderer T, Goldenberger D, Kleger GR, Schlegel M. First documented outbreak of KPC-2-producing *Klebsiella pneumoniae* in Switzerland: infection control measures and clinical management. *Infection* 2014; 42:529-34;469 <http://dx.doi.org/10.1007/s15010-013-0578-9>
2. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. *Archives of Internal Medicine* 2005; 165:1430-5;470 <http://dx.doi.org/10.1001/archinte.165.12.1430>
3. Schwaber MJ, Lev B, Israeli A, Solter E, Smollan G, Rubinovitch B, Shalit I, Carmeli Y. Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America* 2011; 52:848-55;471 <http://dx.doi.org/10.1093/cid/cir025>
4. Lomaestro BM, Tobin EH, Shang W, Gootz T. The spread of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New York. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America* 2006; 43:e26-8;472 <http://dx.doi.org/10.1086/505598>
5. Drew RJ, Turton JF, Hill RL, Livermore DM, Woodford N, Paulus S, Cunliffe NA. Emergence of carbapenem-resistant Enterobacteriaceae in a UK paediatric hospital. *The Journal of Hospital Infection* 2013; 84:300-4;473 <http://dx.doi.org/10.1016/j.jhin.2013.05.003>
6. Giuffre M, Bonura C, Geraci DM, Saporito L, Catalano R, Di Noto S, Nociforo F, Corsello G, Mammina C. Successful control of an outbreak of colonization by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* sequence type 258 in a neonatal intensive care unit, Italy. *The Journal of Hospital Infection* 2013; 85:233-6;474 <http://dx.doi.org/10.1016/j.jhin.2013.08.004>
7. Ben-David D, Masarwa S, Navon-Venezia S, Mishali H, Fridental I, Rubinovitch B, Smollan G, Carmeli Y, Schwaber MJ. Carbapenem-resistant *Klebsiella pneumoniae* in post-acute-care facilities in Israel. *Infection Control and Hospital Epidemiology* 2011; 32:845-53;475 <http://dx.doi.org/10.1086/661279>
8. Lin MY, Lyles-Banks RD, Lolans K, Hines DW, Spear JB, Petrak R, Trick WE, Weinstein RA, Hayden MK. The importance of long-term acute care hospitals in the regional epidemiology of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America* 2013; 57:1246-52;497 <http://dx.doi.org/10.1093/cid/cit500>
9. Carmeli Y, Akova M, Cornaglia G, Daikos GL, Garau J, Harbarth S, Rossolini GM, Souli M, Giamarellou H. Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control. *Clinical Microbiology and Infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2010; 16:102-11;477 <http://dx.doi.org/10.1111/j.1469-0691.2009.03115.x>
10. O'Connor C, O'Connell NH, Commane M, O'Donovan E, Power L, Dunne CP. Limerick: forever associated with five lines of rhyme or infamous for irrepressible carbapenemase-producing Enterobacteriaceae for all time? *The Journal of Hospital Infection* 2016; 93:155-6;478 <http://dx.doi.org/10.1016/j.jhin.2016.03.008>

11. Akova M, Daikos GL, Tzouvelekis L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clinical Microbiology and Infection* : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2012; 18:439-48;479 <http://dx.doi.org/10.1111/j.1469-0691.2012.03823.x>
12. Lee GC, Burgess DS. Treatment of *Klebsiella pneumoniae* carbapenemase (KPC) infections: a review of published case series and case reports. *Annals of Clinical Microbiology and Antimicrobials* 2012; 11:32;480 <http://dx.doi.org/10.1186/1476-0711-11-32>
13. Borer A, Saidel-Odes L, Riesenberk K, Eskira S, Peled N, Nativ R, Schlaeffer F, Sherf M. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia. *Infection Control and Hospital Epidemiology* 2009; 30:972-6;482 <http://dx.doi.org/10.1086/605922>
14. Daikos GL, Petrikkos P, Psychogiou M, Kosmidis C, Vryonis E, Skoutelis A, Georgousi K, Tzouvelekis LS, Tassios PT, Bamia C, et al. Prospective observational study of the impact of VIM-1 metallo-beta-lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. *Antimicrobial Agents and Chemotherapy* 2009; 53:1868-73;483 <http://dx.doi.org/10.1128/aac.00782-08>
15. Tzouvelekis LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clinical Microbiology Reviews* 2012; 25:682-707;484 <http://dx.doi.org/10.1128/cmr.05035-11>
16. Gazin M, Paasch F, Goossens H, Malhotra-Kumar S. Current trends in culture-based and molecular detection of extended-spectrum-beta-lactamase-harboring and carbapenem-resistant Enterobacteriaceae. *Journal of Clinical Microbiology* 2012; 50:1140-6;485 <http://dx.doi.org/10.1128/jcm.06852-11>
17. Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. *Journal of Clinical Microbiology* 2008; 46:3110-1;486 <http://dx.doi.org/10.1128/jcm.00249-08>
18. Savard P, Carroll KC, Wilson LE, Perl TM. The challenges of carbapenemase-producing Enterobacteriaceae and infection prevention: protecting patients in the chaos. *Infection Control and Hospital Epidemiology* 2013; 34:730-9;487 <http://dx.doi.org/10.1086/671003>
19. Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant Enterobacteriaceae from surveillance rectal swabs. *Journal of Clinical Microbiology* 2011; 49:2239-42;488 <http://dx.doi.org/10.1128/jcm.02566-10>
20. Vrioni G, Daniil I, Voulgari E, Ranellou K, Koumaki V, Ghirardi S, Kimouli M, Zambardi G, Tsakris A. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. *Journal of Clinical Microbiology* 2012; 50:1841-6;489 <http://dx.doi.org/10.1128/jcm.06848-11>
21. Hindiyyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, Vax M, Ben David D, Tal I, Rahav G, et al. Rapid detection of blaKPC carbapenemase genes by real-time PCR. *Journal of Clinical Microbiology* 2008; 46:2879-83;490 <http://dx.doi.org/10.1128/jcm.00661-08>
22. Nijhuis R, Samuelsen O, Savelkoul P, van Zwet A. Evaluation of a new real-time PCR assay (Check-Direct CPE) for rapid detection of KPC, OXA-48, VIM, and NDM



- carbapenemases using spiked rectal swabs. *Diagnostic Microbiology and Infectious Disease* 2013; 77:316-20;491 <http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.007>
23. Huang TD, Bogaerts P, Ghilani E, Heinrichs A, Gavage P, Roisin S, Willems E, Verbruggen AM, Francart H, Denis O, et al. Multicentre evaluation of the Check-Direct CPE(R) assay for direct screening of carbapenemase-producing Enterobacteriaceae from rectal swabs. *The Journal of Antimicrobial Chemotherapy* 2015; 70:1669-73;499 <http://dx.doi.org/10.1093/jac/dkv009>
  24. Saegeman V, Van den Eynde J, Niclaes L, De Ridder D, Schuermans A, Glupczynski Y. Performance of different culture methods and of a commercial molecular assay for the detection of carbapenemase-producing Enterobacteriaceae in nursing homes and rehabilitation centers. *European Journal of Clinical Microbiology & Infectious Diseases* : official publication of the European Society of Clinical Microbiology 2015; 34:991-7;492 <http://dx.doi.org/10.1007/s10096-015-2318-9>
  25. Lau AF, Fahle GA, Kemp MA, Jassem AN, Dekker JP, Frank KM. Clinical Performance of Check-Direct CPE, a Multiplex PCR for Direct Detection of bla(KPC), bla(NDM) and/or bla(VIM), and bla(OXA)-48 from Perirectal Swabs. *Journal of Clinical Microbiology* 2015; 53:3729-37;493 <http://dx.doi.org/10.1128/jcm.01921-15>
  26. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V. Identification and screening of carbapenemase-producing Enterobacteriaceae. *Clinical Microbiology and Infection* 2012; 18:432-8;500 <http://dx.doi.org/10.1111/j.1469-0691.2012.03815.x>
  27. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerging Infectious Diseases* 2011; 17:1791-8;496 <http://dx.doi.org/10.3201/eid1710.110655>
  28. E M, M C. Carbapenemase Producing Enterobacteriaceae Ireland Annual Report 2014. In: Saolta, ed. Ireland: Saolta, 2015.
  29. O'Connor C, Fitzgibbon M, Powell J, Barron D, O'Mahony J, Power L, O'Connell NH, Dunne C. A commentary on the role of molecular technology and automation in clinical diagnostics. *Bioengineered* 2014; 5:155-60;494 <http://dx.doi.org/10.4161/bioe.28599>

## Figure Legends

**Figure 1.** Work flow for detection of carbapenemase-producing Enterobacteriaceae incorporation of two methods.

DRAFT

**Table 1.** Performance of two commercial CPE detection kits compared.

	GeneXpert® Carba-R	Check-Direct CPE® assay on a LightCycler® 480 Instrument
Cost of one cartridge/kit (excluding VAT)	€44.00 (ex-VAT)	€950.50 (ex-VAT)
Additional costs/kit needed	None	QIAamp DNA Stool Mini kit for 50 extractions €220.00 (ex-VAT)
Molecular method	Real-time PCR	Real-time PCR
Range of specimens that can be tested	Bacterial culture, rectal swabs/stool specimens	Bacterial culture or rectal swabs/stool specimens
Carbapenemase enzymes covered within the assay	KPC, OXA-48, VIM, NDM, IMP	KPC, OXA-48, VIM, NDM
Carbapenemase enzymes not covered within the assay		IMP
Pre-PCR hands-on preparation time	<5 minutes	<5 minutes
Number of CPE clinical samples that can be performed simultaneously in one run	4 (with current local machine set-up)	96-well PCR plates used, therefore potentially 96 specimens in one run
Total assay run time	53 minutes	1 hour 50 minutes
Time until result available	48/72 h	< 4 h
Number of samples that can be performed from one kit purchased as per manufacturer	10	48

VAT = value added tax

PCR = polymerase chain reaction

IMP = imipenem-hydrolyzing beta-lactamase

KPC = *Klebsiella pneumoniae* carbapenemase

OXA-48 = Oxacillin-hydrolysing

VIM = Verona integrin-encoded metallo beta-lactamases

NDM = New Delhi metallo-beta-lactamase-1

DRAFT