UK NEQAS: Development Of A Carbapenemase-Producing Organisms (CPO) EQA Scheme

Mohammed Islam, Shila Seaton, Anisa Hussain, Jennifer Henderson
UK NEQAS for Microbiology, National Infection Service, UK Health Security Agency, London

Schematic in the development of a new EQA scheme

Justify EQA is relevant to clinical needs → Background research including sending questionnaire to labs → Design specimens → Perform stability, homogeneity and viability studies → Carry out pilot studies with interested registered labs → Collate and analyse results for evidence to support the delivery of the EQA → Proceed to a live scheme

Justify EQA is relevant to clinical needs

External Quality Assurance (EQA) is a pivotal aspect of clinical patient care in ensuring that medical laboratories are able to deliver the best possible care to patients. The development of a new EQA scheme in identifying Carbapenemase Producing Organisms (CPO's) was expressed to be a clinical need in a survey where 327 out of 394 responders expressed an interest in a potential scheme for CPO detection.

Carbapenems are antimicrobials often used as a 'last resort' for treating life-threatening nosocomial infections. CPOs hydrolyse carbapenems via the production of the enzyme carbapenemase, causing drug treatment to become ineffective[1]. In 2017, the World Health Organization published their first ever list of 'priority pathogens' which considered carbapenem resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and*, Enterobacterales* priority 1 critical organisms for urgent need of new antimicrobial agents[2].

An increase in the use of carbapenems has led to the development of resistance through acquisition of carbapenemase enzymes. Therefore there is currently a need for an external quality assessment (EQA) scheme to assess the performance of laboratories providing a service in the detection of the 'big five' CPOs – VIM, NDM, KPC, OXA-48, and IMP.

Background Research

- In 2017, a questionnaire was sent to participants of the 'Antimicrobial Susceptibility' and ' Community Medicine' EQA schemes provided by UK NEQAS for Microbiology.
- Questions included:
  - Whether the lab screens for CPO.
  - What screening methodology and genotypic tests are used.
  - Whether the lab would be interested in participating in a pilot study.
- Results from the questionaire showed that chromogenic agar was more commonly used compared to traditional methods such as overnight TSB (Tryptic Soy Broth) incubation.
- Carbapenemase detection using the Cepheid-GeneXpert: Carba-R PCR was most commonly used.
- 327 labs expressed an interest in taking part in the pilot EQA distributions.

Design Specimens

A comparison study was performed by preparing simulated specimens in both lyophilised and swab format, each containing organisms possessing one of the five dominant carbapenemases (NDM, OXA-48, IMP, VIM, KPC). The vials and swabs were stored at two temperatures to examine the viability and stability of the target organisms over an eight week period.

Choice of sample medium

- To compare the viability, homogeneity and stability of lyophilised specimens (our gold standard) compared to swabs (liquid amies, dry swab inoculated with brain heart infusion broth, and fecal transwab).
- Swabs were chosen for further evaluation, as this is the most sensitive medium for CPO detection[3].

Stability testing

Swabs (liquid amies, dry swab and fecal transwab) were inoculated with a prepared bacterial suspension, containing the CPO.

- The simulated specimens were then stored at ambient temperature (21°C) and cold storage (4°C).
- Specimens were tested by inoculation onto both MacConkey and mSuperCARBA chromogenic agar, in duplicate.
- A lateral flow test was performed using a CARBA-5 assay to identify the carbapenemase present (Figure 2).
- This was carried out at 10 time points, which included 24 and 48 hours post preparation, followed by weekly for a total of 8 weeks.

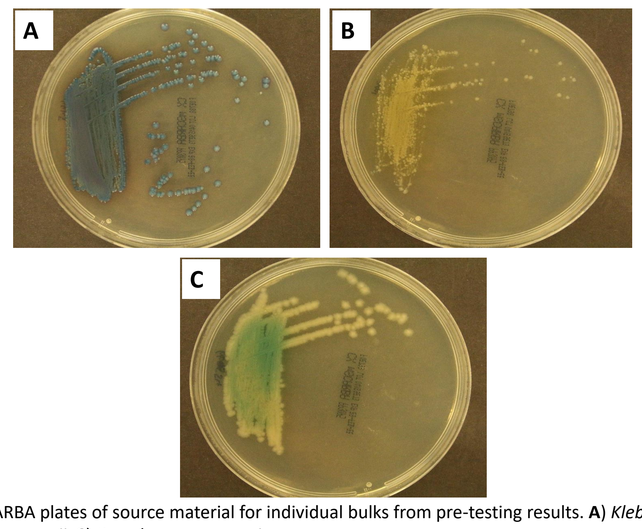

Figure 1: mSuperCARBA plates of source material for individual bulks from pre-testing results. **A**) *Klebsiella pneumoniae*. **B**) *Acinetobacter baumannii*. **C**) *Pseudomonas aeruginosa*

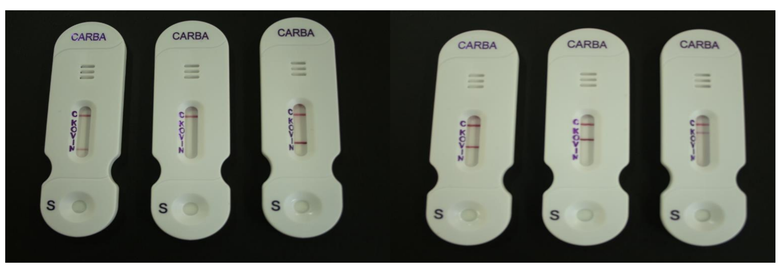

Figure 2: CARBA-5 genotypic pre-testing results of the organisms of stability testing material

Perform Stability, Homogeneity and Viability Studies

1st study

- 6 specimens sent in total, containing either positive or negative material.
- 1 specimen positive for each of the 'big five' CPOs and one negative bulk was included in the pilot scheme.
- 4 different sample mediums were tested (lyophilised samples, liquid amies swab, fecal transwab, dry swab in brain heart infusion broth).

2nd study

- 6 specimens were sent, with the addition of commensals.
- Focused on liquid amies swab only.

Table 1: Results of the 1st study from week 1 and week 8 comparing growth yield and genotypic results

Sample Type	Week 1			Week 8		
	Growth on MacConkey Agar	Growth on Chromogenic Agar	Carba-5 Result	Growth on MacConkey Agar	Growth on Chromogenic Agar	Carba-5 Result
Freeze dried specimen						
Ambient Temperature	+++	++++	KPC	++++	++++	KPC
Dry Swab						
Ambient Temperature	++++	++++	KPC	+++	+++	KPC (faint line)
4°C	++++	++++	KPC	No Growth	No Growth	N/A
Liquid Amies Transwab						
Ambient Temperature	+++	++++	KPC	++++	++++	KPC
4°C	++++	++++	KPC	++++	++++	KPC

- The freeze dried specimen and liquid amies transwab provided expected and consistent results throughout the 8 week period of stability testing. However the dry swab showed less favorable results with false negative results displayed at 4°C at week 8 (Table 1).
- The dry swab also displayed no growth of the test organism from week five in three other bulks.
- The CARBA-5 assay correctly identified the CPO up to 8 weeks post preparation, with the dry swab demonstrating the least favourable results (faint bands).
- Swabs stored at ambient temperature demonstrated greater organism stability after 8 weeks in the growth of the pathogen compared to swabs stored at 4°C.

Carry Out Pilot Studies

Table 2: Intended results for the pilot distributions 5150, 5157 and 5162.

Distribution no.	Specimen No.	Organism	Carbapenemase Detected
5150	**7064**	*Klebsiella pneumoniae*	KPC
	7065	*Enterobacter cloacae*	NDM
5157	**7092**	*Pseudomonas aeruginosa*	IMP
	7093	*Klebsiella pneumoniae*	OXA-48
5162	**7136**	*Acinetobacter baumannii* Commensals: *Enterococcus faecalis, Proteus irabilis*	Negative
	7137	*Klebsiella pneumoniae* Commensals: *Escherichia coli, Citrobacter freundii*	VIM

- The pilot distributions included CPO's that were selected for the stability testing study (Table 1).
- Commensals were added to distribution 5162, in order to better replicate patient samples received by testing laboratories.

Analyse Pilot Distribution Results

Table 3: Results from the pilot distributions for the lyophilized and liquid amies swab respectively.

Pilot Distribution	Specimen Number	Intended Result	Lyophilised format (%) correct	Rectal swab (%) correct
5150	**7064**	KPC	96	95
	7065	NDM	94	95
5157	**7092**	IMP	84	85
	7093	OXA-48	86	85
5162	**7136**	Negative	91	91
	7137	VIM	92	92

- Lyophilised samples are comparable to the simulated rectal swab type, as seen in the results from Table 3.
- The 5157 distribution has a low percentage for correct returned results. It was noticed that many labs returned results of 'no growth' and all these labs used the same agar plates from the same manufacturer.
- Specimen number 7136 was a negative sample, however 9% of participants returned false positive results. This could have an impact on patient results, demonstrating the importance of an EQA for CPO detection.
- The most commonly used test identified was the Xpert Carba R, with 36% of participants using this method.
- The NG-TEST CARBA 5 assay was used by 21% of participants. This kit was also used during the stability testing study.

Discussion

The most appropriate swab type was identified to be the liquid amies swab as it demonstrated the most consistent results after 8 weeks from the 1st and 2nd stability studies.

- The liquid amies swabs were able to produce similar results to those of the lyophilised vials, containing the same organism. However, there were a greater number of participants who did not report results for the liquid amies swab, as some participants were unsure on how to process the sample.
- Distribution 5157 had lower than expected correct intended results, however it was observed many laboratories used the Brilliance CRE agar to culture the sample, which produced false negative results (Table 3).
- The presence of false positive and false negative results throughout the stability and pilot studies could have an impact on patient results, so underlines the importance of an EQA for CPO detection.

Conclusion

- Analysis of participant results has demonstrated the swab format to an appropriate simulated specimen for distribution in this new EQA. This provides a more authentic sample type for clinical diagnostic laboratories.
- Participation in an EQA is a valuable tool in the quality assurance of CPO testing in the diagnostic laboratory and demonstrates the validity of comparing collated data between laboratories.
- EQA is also an important tool in providing evidence of competence.

Acknowledgments

Thank you to the team at UK NEQAS for Microbiology at UKHSA Colindale for their excellent work in the development of the EQA in the detection of CPO's in clinical specimens. Thank you also to the laboratories that participated in the pilot distributions.

References

1) Peaper, D., Kulkarni, M., Tichy, A., Jarvis, M., Murray, T. and Hodsdon, M., 2013. Rapid detection of carbapenemase activity through monitoring ertapenem hydrolysis in Enterobacteriaceae with LC–MS/MS. *Bioanalysis*, 5(2), pp.147-157
2) Olivia Lawe Davies, O. and Bennett, S., 2021. *WHO publishes list of bacteria for which new antibiotics are urgently needed*. [online] Who.int. Available at: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> [Accessed 15 September 2021]
3) GOV.UK. 2021. *of bacteria with carbapenem hydrolysing ß lactamases (carbapenemases)*. [online] Available at: <https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-wit*UK SMI B 60: detection* h-carbapenem-hydrolysing-lactamases-carbapenemases> [Accessed 20 September 2021]