

HCAI / AMR National Team

Technical Document

Environmental Testing for Carbapenemase Producing Enterobacterales (CPE)

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Introduction

Enterobacterales can persist in suitable environmental niches for extended periods. Environmental contamination has emerged as potential reservoir of CPE that may contribute to colonisation or infection of patients. In some cases Infection Prevention and Control (IPC) teams may consider it appropriate to test clinical environments for CPE. Sensitivity of detection of CPE from the environment is dependent on the sampling method and the analytical method. This document describes an approach to testing for environmental contamination with CPE that has been applied in one hospital with a significant yield of positive results. The approach is outlined here for consideration for use by other IPC teams and laboratories. The method has not been comprehensively compared with other approaches some of which may be equally of more effective.

Scope

This document is intended for use by IPC teams and laboratory practitioners.

Approach to Testing

Aseptic technique is essential for the following procedure.

Swab method for environmental sampling¹

Recommended: Flocked nylon swabs or macrofoam swabs for maximum recovery of organisms².

- 1. Perform alcohol rub hand hygiene, put on gloves.
- 2. Remove a swab from the sterile wrapping and moisten the tip by immersing it in a tube containing dilution liquid or neutralizing fluid.
- 3. Press the tip of the swab against the wall of the tube to remove excess liquid.
- 4. Place the sterile template on the surface to be investigated, place the tip of the swab on the surface inside the template and streak the area within, whilst rotating the swab between the thumb and forefinger in two directions at right angles to each other.
- 5. Put the swab into a tube containing 10 ml of dilution liquid (buffered saline/nutrient broth/ buffered peptone saline/ Brain Heart Infusion Broth) and aseptically break or cut off the stick.
- 6. After sample collection remove gloves and perform hand hygiene. Label the sample and submit for laboratory processing (skip to step 7 "Laboratory Processing")

Sponge method for environmental sampling¹

- 1. Perform alcohol rub hand hygiene, put on gloves.
- 2. Open the plastic bag or container containing the sponge.
- 3. Remove the sponge aseptically from its container with a sterile forceps or a sterile gloved hand. Moisten the cloth or sponge with sufficient quantity of diluent (without excess).

Note: Commercially available sponges are pre moistened with neutralizing buffer.

- 4. Sample the chosen surface in two perpendicular directions, changing the face of the cloth or sponge. Return the sponge to the sterile container and close.
- 5. After sample collection remove gloves and perform hand hygiene. Label the sample and submit for laboratory processing.

Laboratory Processing

- Add 100mls of diluents (buffered saline/nutrient broth/buffered peptone saline) to the transport container containing the sponge. Replace the lid and mix thoroughly by shaking/vortex for 30 seconds.
- 7. Incubate the container at 37°C in aerobic conditions for 24 hours.
- 8. Re-suspend broth (Vortex in safety cabinet) and inoculate the Chromogenic CPE agar with 10µls of broth (avoiding aerosols), streak out for discreet colonies.

Recommended: Use a selective Chromogenic agar that is optimised for types of CPE that are common in Ireland including OXA-48.

- 9. Incubate the plate at 37°C in aerobic conditions for 24 hours.
- 10. Read plate for suspect colonies and identify all isolates e.g. MALDI.
- 11. Test isolates of Enterobacterales for CPE enzymes/CPE encoding genes by molecular analysis/immuno-chromatography assay.
- 12. Send confirmed or suspect CPE to the National CPE Reference Laboratory Service.

Note: Bacteria of orders other than Enterobacterales may be recovered including *Acintetobacter spp.* and *Pseudomonas spp.* The relevance of these non- Enterobacterales will depend on the infection control issue under investigation but is not addressed further in this note on methods.

Appendix 1: Flow Chart of Environmental Testing for Carbapenemase Producing Enterobacterales

Swab/sponge areas for environmental testing

Incubate broth

37°C for 24

hours and subculture 10µl
onto
Chromagenic
CPE agar,
incubate 37°C
for 24 hour.

Identify all suspect colonies and perform rapid analysis (molecular/immuno-chromatography assays)

References:

- 1. BS ISO 18593:2004 Microbiology of food and sanimal feeding stuffs- Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
- 2. S. Galvin, A. Dolan, O. Cahill, S. Daniels, H. Humphreys. Microbial monitoring of the hospital environment: why and how? *Journal of Hospital Infection* 2012; **82**: 143-151.
- 3. Acknowledgement: This document is based on work performed by B. Hananhoe, Surveillance Scientist, Galway University Hospital.