

Carbapenemase Activity Test (CAT) Discs - D71C

Introduction

D71C is a carbapenemase activity test disc comprising of discs impregnated with a penem antibiotic. D71C can detect the presence of carbapenemase activity and can also indicate the presence of OXA-48 enzymes.

Method

Surface inoculate a susceptibility test agar plate with a suspension of the test organism. Place a Mastdiscs™ ID CAT disc on to the inoculated medium. Incubate the plate at 35°C to 37°C for 16 to 20 hours. Note any zones of inhibition that are observed.

Interpretation

| Resistance mechanism | Zone interpretation |
|-----------------------------|---|
| Carbapenemase production | No zone of inhibition |
| No carbapenemase production | Clear zone of inhibition |
| OXA-48 production | “Double zone” of inhibition i.e. zone of inhibition with colonies growing within the zone |

Quality Control

Uniformity

Discs randomly chosen from the batch are placed on an assay medium plate which has been surface inoculated with an organism sensitive to the antibiotic. Plates are incubated at 35°C to 37°C for 16 to 20 hours. All zones produced by the discs should be within 3mm of each other.

Performance

Discs are placed on the surface of assay medium plates which have been surface inoculated with recommended QC strains dependent on the method being followed. Plates are incubated at 35-37°C for 16-20 hours. Zones of inhibition for each disc should be as specified in the interpretation criteria specific for the resistance mechanism present.

Bioburden

A sample of discs are aseptically added to the surface of nutrient agar and incubated at 35°C to 37°C for 40 hours. There should be no signs of contamination after 40 hours incubation.

Stability

Long term stability tests are undertaken to ensure that the performance of the product remains within specified tolerances, under recommended storage conditions over the period between manufacture and stated expiry date. Studies are based on “Real Time” testing of retained product samples of the same material formulation, container and closure system as utilized in products supplied to the customer.

Performance Evaluation Data

Day *et al.* (2013) showed that faropenem 10ug discs have a sensitivity of 99% and specificity of 94% for predicting carbapenemase activity i.e. no zone of inhibition.

Internal testing on a small number of isolates showed that that discs were 100% sensitive for predicting carbapenemase activity i.e. no zone of inhibition.

| Resistance | Number of isolates | | | |
|------------|--------------------|---------|-------------|--------------------------|
| | Total | No zone | Double zone | Clear zone of inhibition |
| OXA | 11 | 1 | 10 | 0 |
| KPC | 9 | 9 | 0 | 0 |
| MBL | 11 | 11 | 0 | 0 |
| ESBL | 8 | 0 | 0 | 8 |
| AMPC | 7 | 0 | 0 | 7 |
| None | 1 | 0 | 0 | 1 |
| Total | 47 | 21 | 10 | 16 |

Reference

Day KM, Pike R, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Woodford N, Perry JD. Use of faropenem as an Indicator of Carbapenemase Activity in the Enterobacteriaceae. *JCM* 2013; **51**(6), 1881-1886

Repeatability

Eighteen discs of each disc type from a number of batches of D71C were tested against a freshly cultured strain of the negative control *Escherichia coli* ATCC® 25922.

| Product Code | Lot number | Zone diameter variation of 18 discs (mm) | |
|--------------|------------|--|---------|
| | | Minimum | Maximum |
| D71C | 14012 | 26 | 28 |
| D71C | 14014 | 26 | 27 |
| D71C | 14015 | 27 | 28 |
| D71C | 14016 | 26 | 27 |

Reproducibility

A batch of D71C (14012) was tested at weekly intervals against freshly cultured isolates of carbapenemase producers, non-carbapenemase producers and OXA-48 producers. The following results were obtained:

| Organism | Strain information | Zone of inhibition | | | |
|------------------------------|--|--|--|--|--|
| | | Week 1 | Week 2 | Week 3 | Week 4 |
| <i>Escherichia coli</i> | ATCC®25922 – negative for carbapenemase production | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition |
| <i>Escherichia coli</i> | NCTC 13352 – ESBL producer | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition |
| <i>Escherichia coli</i> | Wild type - KPC producer | No zone of inhibition | No zone of inhibition | No zone of inhibition | No zone of inhibition |
| <i>Escherichia coli</i> | Wild type - OXA-48 producer | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) |
| <i>Enterobacter cloacae</i> | NCTC 13406 – AmpC producer | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition | Clear zone of inhibition |
| <i>Klebsiella pneumoniae</i> | NCTC 13440 – MBL producer | No zone of inhibition | No zone of inhibition | No zone of inhibition | No zone of inhibition |
| <i>Klebsiella pneumoniae</i> | NCTC 13442 – OXA-48 producer | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) | Double zone of inhibition (colonies growing within zone) |

External Evaluations

Results from external evaluations have not yet been received.