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MAST® CARBA PAcE

PACE-ID

Intended Use
For the rapid detection of carbapenemase producing Enterobacterales, *Pseudomonas*, OXA 48 and 23-like enzyme production in *Acinetobacter.*

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents
- **Vial PEL.** Freeze dried pellet* - 4 vials containing inhibitors and lysis components, each designed for 12 tests.
- **Vial RB.** Reconstitution buffer* - 4 vials containing chromogenic indicator resuspension buffer, each sufficient for 12 tests.
- Plastic 0.5 mL tubes, sufficient for 48 tests.

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening. Once reconstituted, test solution stored at 2 to 8°C, must be used within 4 weeks.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard and aseptic techniques. To be used by only trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to product safety data sheets.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST Group Ltd. culture media, table top vortexes, pipettes, incinerators and incubators, etc.

Procedure
1. Reconstitute the pellet by tipping the entire contents of vial RB into vial PEL.
2. Allow the pellet to fully dissolve at room temperature for 1 minute and mix contents by gently vortexing for 10 seconds. Reconstituted solution should be yellow, if the solution is any other colour do not use.
3. Dispense 250 µL of reconstituted solution into the tubes provided. One tube per test.
4. Using a pure, fresh culture of the test organism, take an approximate 1 to 5 µL loopful of organism, and add to the tube containing test solution. Mix well by vortexing for 20 seconds.
   Note: to obtain distinct results, ensure that the bacterial resuspension is similar to the turbidity of a 3.0 to 3.5 McFarland standard; Approx. 10^9 CFU/mL.
5. Incubate at 35±1°C for 10 minutes.
6. Record the colour of the test solution immediately or up to 20 minutes after incubation.

Please refer to corresponding steps on the image page.

Interpretation of results
If a colour change is recorded; from yellow to orange/red, record the organism as demonstrating carbapenemase activity.
If no colour change is recorded; solution remains yellow, record the organism as negative for carbapenemase activity.

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and another to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organism	Result
Acinetobacter baumanii NCTC 13301	Orange/Red Carbapenemase positive
Pseudomonas aeruginosa NCTC 13437	Orange/Red Carbapenemase positive
Acinetobacter lwoffi ATCC® 15309	Remains Yellow Carbapenemase negative
Pseudomonas aeruginosa ATCC® 25668	Remains Yellow Carbapenemase negative
Klebsiella pneumoniae NCTC 13438	Orange/Red Carbapenemase positive

Limitations
1. Colonies isolated from indicator media are not recommended.
2. This product only detects the presence of a carbapenemase, differentiation can be carried out by using a suitable genotypic or phenotypic test (for example **MAST**DISCS® *Combi Carba Plus*; D73C).
3. Some GES-type carbapenemases might be difficult to detect.
4. To avoid potentially erroneous results, ensure that equipment used for testing is free of contamination.
5. Test results must be recorded within 20 minutes following the initial 10 minute incubation.
6. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

References
Bibliography available on request.

Acknowledgement
HMRZ compound used in this product was developed by Dr. Hideaki Hanaki of Kitasato, Institute, Japan.

IFU159 GB 08/20 V3

*Formulation may change to meet performance criteria

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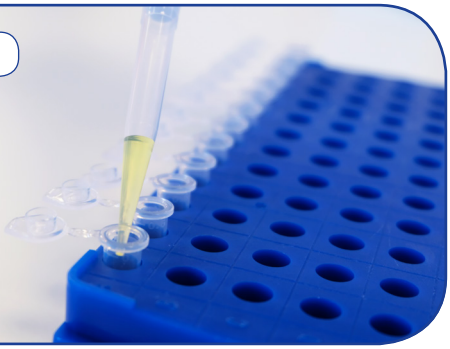
Mast Group

1

2

3



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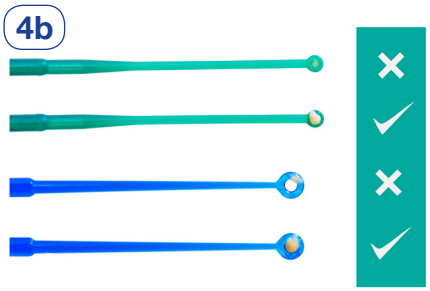

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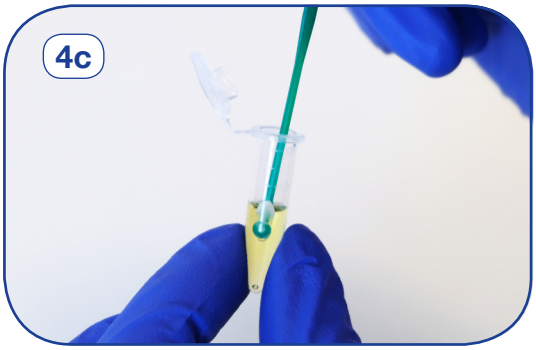

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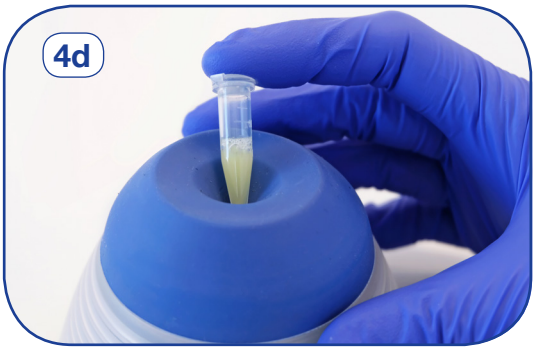

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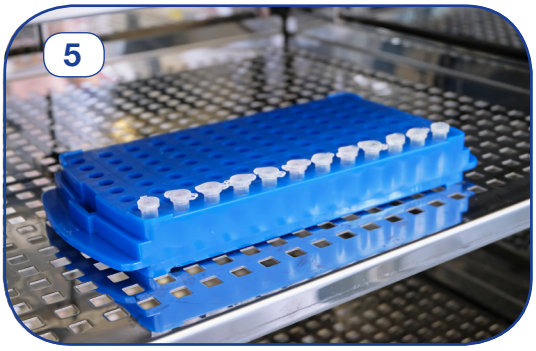

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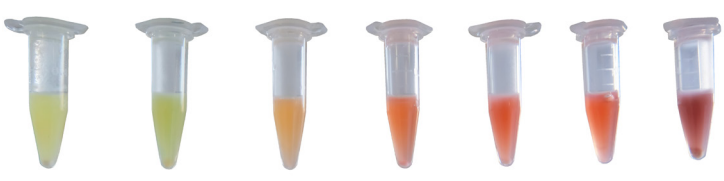

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MASTDISCS® *Combi*
Extended Spectrum Beta-Lactamase (ESβL) Detection Set (CPD10)

D67C

Intended use
For the detection of extended spectrum beta-lactamases (ESβLs) in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*
3 paired sets of cartridges per pack, each cartridge containing approximately 50 discs.

Set 1	CAZ30	Ceftazidime 30 μg discs
	CAZCV	Ceftazidime 30 μg +clavulanic acid 10 μg discs
Set 2	CTX30	Cefotaxime 30 μg discs
	CTXCV	Cefotaxime 30 μg + clavulanic acid 10 μg discs
Set 3	CPD10	Cefpodoxime 10 μg discs
	CPDCV	Cefpodoxime 10 μg +clavulanic acid 1 μg discs

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® culture media, Mueller-Hinton agar, swabs, forceps, callipers etc., as well as an incubator capable of maintaining 35 ± 2°C.

Procedure
1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard.
2. Using a sterile swab spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the Clinical and Laboratory Standards Institute (CLSI) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each type of disc onto the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 2°C for 17 ± 1 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre. Discs showing no zone of inhibition should be recorded as 6 mm.

Interpretation of results
Compare the zone of inhibition for each cephalosporin alone and when in combination with clavulanic acid. An increase in zone diameter of ≥5 mm in the presence of clavulanic acid for any or all of the sets indicates the presence of ESβL in the test organism.

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination disc with clavulanic acid and corresponding cephalosporin only disc against ESβL-negative control organism *E. coli* ATCC® 25922 should be equal or show no greater difference in diameter than ±2 mm. Any greater difference implies malfunction or deterioration. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain:

Test Organism	Result
Escherichia coli NCTC 13351	Positive
Escherichia coli NCTC 13352	Positive
Escherichia coli NCTC 13353	Positive
Klebsiella pneumoniae ATCC® 700603	Positive
Escherichia coli ATCC® 25922	Negative

Limitations
D67C is not suitable for testing *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results do not mix cartridges from different batches and ensure all discs in the set are tested on the same plate.

References
Bibliography available on request.

IFU185 GB 03/21 V6

*Formulation may change to meet performance criteria

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MASTDISCS® *Combi*
AmpC Detection Set

D69C

Intended use
For the detection of AmpC β-lactamase enzyme production in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*
3 cartridges per pack, each cartridge containing approximately 50 discs.

Cartridge A	Cefpodoxime 10 μg + AmpC inducer
Cartridge B	Cefpodoxime 10 μg + AmpC inducer + ESβL inhibitor
Cartridge C	Cefpodoxime 10 μg + AmpC inducer + ESβL inhibitor + AmpC inhibitors

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® culture media, Mueller-Hinton agar, swabs, forceps, callipers etc., as well as an incubator capable of maintaining 35 ± 1°C.

Procedure
1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard in physiological saline.
2. Using a sterile swab, spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each disc onto the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 1°C for 18 ± 2 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre. Discs showing no zone of inhibition should be recorded as 6 mm.

Interpretation of results
Interpret results by comparing inhibition zone diameters in the sequence described below:

Step 1 - Compare the zone of inhibition of the cefpodoxime plus AmpC inducer disc (**A**) to the zones of inhibition of each of the cefpodoxime plus inducer and inhibitor discs (**B, and C**).
If all zones are within 3 mm of each other, record the organism as negative for AmpC production.

Step 2 - Subtract **A** from **C**, **A** from **B** and **B** from **C.**

If **C** – **A** and **C** – **B** is ≥5 mm the organism is demonstrating AmpC activity. This should be considered as a positive result

If **C** – **A** and **B** – **A** is ≥5 mm and zones of Discs **B** and **C** have a maximum difference of 4 mm then the organism may be demonstrating another resistance mechanism.

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained against a negative control organism *E. coli* ATCC® 25922, should be equal or show no greater difference in diameter than ±3 mm. Any greater difference implies malfunction or deterioration. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain:

Test Organism	Result
Escherichia coli ATCC® 25922	Negative
Escherichia coli DSMZ 22316 (Plasmid AmpC)	Positive
Enterobacter cloacae NCTC 13406 (Derepressed AmpC)	Positive
Enterobacter cloacae NCTC 13405 (Inducible AmpC)	Positive

Limitations
D69C is not suitable for use with *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results do not mix cartridges from different batches of D69C and ensure all discs in the set are tested on the same plate.

The formulation is designed to detect all types of AmpC production. The ESβL inhibitor is present to prevent this enzyme affecting results when an isolate contains both AmpC and ESβL enzymes. Although ESβL inhibitor is contained in discs B and C, this product **cannot** be used for ESBL detection.

References
Bibliography is available on request.

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MASTDISCS® *Combi*
Carba plus

D73C

Intended use
For the detection of carbapenemase and OXA-48 enzyme production in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*
5 cartridges per pack, each cartridge containing approximately 50 discs:

Cartridge A	Penem
Cartridge B	Penem + MβL inhibitor
Cartridge C	Penem + KPC inhibitor
Cartridge D	Penem + AmpC inhibitor
Cartridge E	Temocillin + MβL inhibitor

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® culture media, Mueller-Hinton agar, swabs, forceps, callipers, etc., as well as an incubator capable of maintaining 35 ± 1°C.

Procedure
1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard in physiological saline.
2. Using a sterile swab, spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each disc on to the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 1 °C for 18 ± 2 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre, **ignoring any microcolonies in the zone**. Discs showing no zone of inhibition should be recorded as 6 mm.

Interpretation of results
To interpret results based on observed zones of inhibition, use the D73C calculator. The calculator is available for download and can be accessed via www.mast-group.com, in the registered members section. Alternatively, results can be interpreted manually by comparing inhibition zone diameters as described below:
Compare the zone of inhibition of the penem disc (A) to the zones of inhibition of each of the penem plus inhibitor discs (B, C and D).
If disc B **only** shows a zone difference ≥5 mm than disc A (C - A and D – A should be <5 mm), record the organism as demonstrating MβL activity.
If disc C **only** shows a zone difference ≥5mm than disc A (B – A and D – A should be <5 mm), record the organism as demonstrating KPC activity.
If discs C and D both show significant zone differences (≥5 mm) compared to disc A (B – A should be <4 mm), record the organism as demonstrating AmpC activity coupled with porin loss (impermeability). If no synergy is obtained between discs A, B, C and D and disc E shows a zone of inhibition of ≤10 mm, record the organism as demonstrating OXA-48 activity. **If an equivocal or negative result is generated but resistance to disc A is shown, the organism may still be expressing a carbapenemase enzyme. Molecular testing or MAST**DISCS® ***ID*** **D74 Indirect Carbapenemase Test (ICT) can be performed to verify this.**

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination discs with inhibitor and corresponding penem only disc against negative control organism *E. coli* ATCC® 25922 should be equal or show no greater difference in diameter than ±2 mm. The zone diameter for disc E should be >10 mm. Any deviation implies malfunction or deterioration. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain:

Test Organism	Result
Klebsiella pneumoniae NCTC 13440	MβL Positive
Klebsiella pneumoniae NCTC 13438	KPC Positive
Klebsiella pneumoniae NCTC13442	OXA-48 Positive
Escherichia coli ATCC® 25922	Negative

Limitations
D73C is not suitable for detection of carbapenemase production in *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results, do not mix cartridges from different batches of D73C and ensure all discs in the set are tested on the same plate. D73C may give equivocal results against clinical isolates that have acquired complex co-resident carbapenemase mediated resistance mechanisms. Users are obliged to always use the latest version of the D73C calculator.

References
Bibliography available on request.

IFU190 GB 03/21 V8

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MASTDISCS® *Combi*
ESβL Detection Set (EUCAST)

D76C

Intended use
For the detection of extended spectrum beta-lactamases (ESβLs) in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*
2 paired sets of cartridges per pack, each cartridge containing approximately 50 discs:

Set 1	CTX5	Cefotaxime 5 μg discs (x1)
	CTXCV	Cefotaxime 5 μg + clavulanic acid 10 μg discs (x1)
Set 2	CAZ10	Ceftazidime 10 μg discs (x1)
	CAZCV	Ceftazidime 10 μg + clavulanic acid 10μg discs (x1)

Storage and shelf life
Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

Precautions
For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

Materials required but not provided
Standard microbiological supplies and equipment such as loops, MAST® culture media, Mueller-Hinton agar, swabs, forceps, callipers etc., as well as an incubator capable of maintaining 35 ± 1°C.

Procedure
1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard in physiological saline.
2. Using a sterile swab, spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each disc onto the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 1 °C for 18 ± 2 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre. Discs showing no zone of inhibition should be recorded as 6 mm.

Interpretation of results
Compare the zone of inhibition for the cephalosporin disc to that of the corresponding cephalosporin plus clavulanic acid combination disc. An increase in zone diameter of ≥5mm in the presence of clavulanic acid for one or both of the sets indicates the presence of ESβL in the test organism.

Quality control
Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using combination disc plus clavulanic acid and corresponding cephalosporin only disc against ESβL-negative control organism *E. coli*. ATCC® 25922 should be equal or show no greater difference in diameter than ±2mm. Any greater difference implies malfunction or deterioration. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain:

Test Organism	Result
Escherichia coli NCTC 13351	Positive
Escherichia coli NCTC 13353	Positive
Escherichia coli ATCC® 25922	~~ESβL~~ Negative

Limitations
D76C is not suitable for testing *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results do not mix cartridges from different batches and ensure all discs in the set are tested on the same plate.

References
Bibliography available on request.

IFU194 GB 03/21 V3

*Formulation may change to meet performance criteria