Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

MAST ISOPLEX® *CRE-ART*

FOR RESEARCH AND IN VITRO DIAGNOSTIC USE

DNA/LYO5 10 tests.

Intended Use

MAST ISOPLEX® *CRE-ART* kit is an *in vitro* diagnostic kit for professional use. It is based on loop-mediated isothermal amplification (LAMP) technology for the qualitative detection and differentiation of Carbapenem-resistant Enterobacterales (CRE) and other Carbapenem resistant bacteria isolated via overnight culture from a clinical specimen.

Principles of the examination method

The MAST ISOPLEX® *CRE-ART* is for the qualitative detection and differentiation of OXA-48, OXA-23, OXA-24/OXA-40, KPC, VIM, NDM and IMP DNA. The assay comprises of an 8-tube strip where each individual tube is targeted to one of the specific DNA targets (seven of the eight tubes). An inhibition control DNA is provided in the kit and is to be added prior to commencement of the assay. The inhibition control assay is conducted in tube 8 of the 8-tube strip to identify possible inhibition of DNA amplification in the sample and to confirm integrity of the assay reagents.
The LAMP assay involves use of specific primers to 8 distinct regions of the target DNA, a DNA polymerase with strand displacement activity, substrates and an intercalating fluorochrome dye at a constant temperature (63°C for MAST ISOPLEX® *CRE-ART).* Due to the high specificity of LAMP the presence of amplified product can indicate the presence of the target DNA in 30 minutes.

Components

Table 1: Kit components

Kit code	Content	Number	Volume	Lid colour	Additional packaging information
IC DNA	Inhibition control DNA	1 tube	Dried clear pellet	Green	Contained in a single re-sealable pouch with 2 desiccant bags
RB3	Reconstitution buffer	1 tube	1.5 mL	Orange	Tube is held in a tight fit bespoke insert
WTR	Molecular Grade Water	1 tube	1.5 mL	Black	Tube is held in a tight fit bespoke insert
CRE STRIP	Specific target LAMP assay reagent pellet	10 x 8-tube strip. Each tube individually numbered (1 to 8)	White pellet	Clear	CRE STRIP are individually packaged in a resalable pouch containing 2 desiccant bags

Each CRE STRIP tube number indicates the specific target being detected as described in Table 2.

Table 2: CRE STRIP tube definition:

Tube number	Target detected
1	OXA-48
2	OXA-23
3	OXA-24/OXA-40
4	KPC
5	VIM
6	NDM
7	IMP
8	Inhibition control

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Additional required equipment

1. Appropriate real-time PCR instrument or equipment capable of isothermal DNA amplification and detection of amplified product using fluorescence (see Table 3 for instruments suitable for use with MAST ISOPLEX® *CRE-ART.*
2. Microbiological culture media for isolation of single colony growth from a patient specimen (see table 3 for growth media used for colony isolation from a specimen during development of MAST ISOPLEX® *CRE-ART*.
3. Nuclease free 1.5 mL assay reaction tubes for use in resuspension of the isolated colony (see Examination procedure)
4. Desktop centrifuge with a rotor to hold 1.5 mL assay reaction tubes.
5. Plate centrifuge or microfuge/centrifuge with an insert for 8-tube strip (4Ti tube strip).
6. Standard DNase free supplies such as assay reaction tubes, pipettes and pipette tips.
7. Disposable powder-free gloves.
8. Adjustable calibrated pipettes capable of dispensing volumes from 10 µL to 200 µL.
9. Nuclease free toothpick or pipette tip.
10. Ice bucket with ice or cold block.
11. Heater unit with heating block to hold 1.5 mL assay reaction tubes and can maintain temperature at 95°C for five minutes.
12. Personal protective equipment (laboratory coat, gloves and eye protection (for specimen preparation).
13. Dependent upon the amplification instrument used, a holder for 8-tube strips (4Ti tube strip is used in CRE STRIP) may be required (see the relevant manufacturer instructions for use).

Table 3: Real-time PCR equipment and microbiological media plates

Real-time PCR Manufacturer	**Model**
MAST - Qiagen	TS 2.4
Applied Biosystems	ABI 7500 FAST, ABI 7500
Microbiological media supplier	**Media**
E&O Laboratories Ltd	Columbia Agar, Mueller Hinton
Mast Diagnostica GmbH	CHROMagar™ KPC/CHROMagar™ ESBL, CHROMagar mSuperCARBA

Reagent Preparation

Prepare Inhibition Control DNA (ICDNA) as follows:

1. Spin tube briefly in a microcentrifuge to ensure lyophilized DNA is at the bottom of the tube.
2. Add 200 µL of molecular grade water (WTR) and leave to dissolve for 5 minutes.
3. Mix gently by pipetting up and down several times.
4. Place on ice or cold block until used or aliquot into smaller volumes (10 µL required per sample being tested) for longer term storage.

Bacterial colony isolation from a clinical specimen shall be conducted following safe laboratory guidelines and standard microbiological practice.

Storage and Shelf life after first opening

1. MAST ISOPLEX® *CRE-ART* kits are shipped at ambient temperature.
2. Store the unopened kit at 2°C. to 30°C.
3. Protect reagents from direct sunlight.
4. Reagents can be used until the date of expiration. Following expiry product quality is not guaranteed.
5. Following resuspension of ICDNA the solution can be stored at 2°C to 8°C if to be used on the same day. For longer term storage after reconstitution, store in small aliquots (10 µL volume required per test sample) at minus 20°C to prevent multiple freeze-thaw cycles.
6. RB3 and WTR are kept at 2°C to 30°C after opening until the expiry date.
7. On day of conducting tests re-constituted CRE-STRIP should be held at 2°C to 8°C during assay set-up.

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Warnings and Precautions

1. Tests shall be conducted by professionals trained in the operation of the required equipment and molecular diagnostic assays.
2. The user must ensure integrity of packaging, labelling and component content before first use (any issues shall be reported to the manufacturer).
3. Do not use MAST ISOPLEX® *CRE-ART* kit after date of expiration.
4. Specimens from which colony isolation will be derived shall be treated as infectious and/or biohazardous in accordance with local/national guidelines.
5. Appropriate personal protective equipment shall be worn
6. Precautions should be taken to prevent microbial and nuclease contamination of MAST ISOPLEX® *CRE-ART* reagents and specimens to be tested.
7. Use nuclease free assay reaction tubes, pipette tips and toothpick(s)
8. Reaction tubes shall be kept closed at all times following addition of reagents and discarded without opening following use, according to local health and safety guidelines.
9. To avoid any contamination with the amplified product, never open CRE STRIP tube(s) after amplification.
10. Do not vortex reaction tubes as this can lead to shear stress causing damage to the enzyme and bubble formation that can interfere with fluorescence detection.
11. Ensure all assay reaction tubes are free from scratch(es) or cracks prior to use.
12. Dedicated work areas to segregate a) colony isolation/specimen handling; b) examination procedure and c) real-time amplification and detection shall be available with the necessary equipment required allocated to each area.
13. Ensure the assay reaction tube are placed on ice (or ice block) following incubation at 95°C for 5 minutes to prevent inactivation of the CRE STRIP components upon addition of the assay reaction tube content.
14. Ensure assay reaction tubes containing the specimen, RB3 and ICDNA are tightly closed prior to incubation at 95°C
15. Care should be taken when operating heating equipment, follow local and/or safety national guidelines.
16. Ensure all equipment is fully installed, calibrated and maintained according to manufacturer guidelines.

Primary sample collection, handling and storage

The primary sample to be used with MAST ISOPLEX® *CRE-ART* is an isolated bacterial colony cultured from a clinical specimen. Tests should be conducted following overnight culture of the clinical specimen to obtain an isolated colony.

Examination procedure

1. Pipette 90 µL of RB3 buffer in a 1.5 mL assay reaction tube.
2. Using a toothpick or pipette tip, touch the surface of an isolated colony (~0.2 McFarland or 10^8cfu/mL) from an overnight culture plate and immerse into 1.5 mL assay reaction tube (step 1) and mix gently.
3. Add 10 µL of reconstituted ICDNA to the mixture in the assay reaction tube. Mix by pipetting gently up and down at least five times.
4. Ensuring the assay reaction tube is closed, place the assay reaction tube at 95°C for 5 min.
5. After heating, immediately remove the assay reaction tube and place on ice or in a cold block for 5 minutes
6. After cooling, spin the reaction mix briefly in a centrifuge for ~ 5 seconds at ~6000rpm
7. Add 10 µL of the reaction mix to each tube of the CRE STRIP.
8. Spin the CRE STRIP in a plate centrifuge for 5 seconds at ~2500rpm
9. Place the CRE STRIP in the chosen instrument for isothermal amplification (if necessary ensure the appropriate 8 tube strip holder is used).
10. Set the instrument to run the LAMP assay:
    Refer to the relevant equipment user manual for guidance on setup and programming of the instrument
    a. Set assay parameters, these will differ dependent upon instrument used

Table 4: Assay parameters

Instrument	**Parameters**			
ABI 7500 FAST ABI 7500	Temperature: 63°C	Assay Time: 30minutes	Cycle time: 1 minute	Background reference: None
TS2.4	Select protocol CreArt_Eng_3			

Note for instruments not listed user should follow the temperature and assay time for ABI7500/FAST

b. Select detection FAM detection channel

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Control procedure

MAST ISOPLEX® *CRE-ART* kit (each lot) are tested against predefined specifications to ensure the consistency of product quality in accordance with Mast Group Limited EN ISO13485:2016 'Medical devices – Quality Management Systems – Requirements for regulatory purposes' and IVDD 98/79/EC 'In vitro diagnostic medical devices'.

CRE STRIP tube 8 acts as a quality control indicator for each CRE STRIP ran as this assay should always produce a positive result. This assay targets the ICDNA added to the test specimen prior to heat treatment. A positive result in tube 8 indicates heat treatment did not denature the DNA, the CRE STRIP components are performing as anticipated and ICDNA was added to the specimen prior to heat treatment. A negative result in CRE STRIP tube 8 indicates DNA degradation during heat treatment or ICDNA has not been added to the specimen or CRE STRIP reagents are not functioning appropriately. In the case of a negative result ensure the instrument set up and test procedure is followed correctly and that the reagents have not reached date of expiration prior to repeating the assay.

To ensure contamination is not introduced during the examination procedure, an assay can be conducted in the absence of ICDNA or specimen. Replace the volume of ICDNA or specimen with WTR provided in the MAST ISOPLEX® *CRE-ART* kit. All tubes should give a negative result. A positive result indicates the presence of non-specific DNA, ensure all equipment and working areas are contamination free before repeating the assay. If a similar result is seen contact Mast Group Limited Technical Support.

To ensure CRE STRIP tubes are performing appropriately positive control DNA specific to each of the tube targets can be used in place of test specimen. A positive result should be detected in each tube where positive control is added. If a negative result is seen this indicates inhibition of LAMP assay or failure to add the positive control DNA. Repeat the assay. Contact Mast Group Limited Technical Support for more information on positive control DNA.

Table 5: Interpretation of controls

	CRE STRIP (tube number)								Result interpretation
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Control specimen									
No template control (ICDNA only added)	-	-	-	-	-	-	-	+	Valid result
No template control (ICDNA only added)	+	+	+	+	+	+	+	+	Invalid result. Repeat test ensuring all equipment is DNA free
Positive control DNA +ICDNA	- In any tube (tubes 1 to 7)							+	Invalid result: Repeat test ensuring control DNA is added

+ denotes a positive amplification and Ct < 30; - denotes a negative amplification and no Ct value

Interpretation of results

The analysis of each test is conducted by the instrument software. In general, a positive result is indicated by a clear difference in intensity output at a given point in time when compared to a no template control. In order for a result to be valid a positive result shall be detected in Tube 8 (ICDNA). If CRE STRIP tube 8 gives a negative result the test should be repeated in the presence of ICDNA.

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

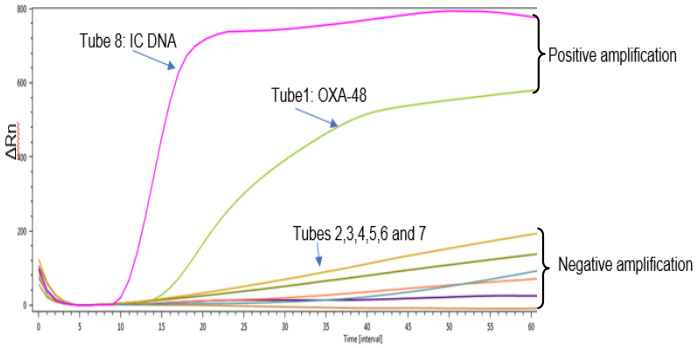
Table 6: MAST ISOPLEX® *CRE-ART* result interpretation

CRE STRIP (tube number)								Result interpretation
1	2	3	4	5	6	7	8	
+	-	-	-	-	-	-	+	Valid result: Positive for OXA-48
-	+	-	-	-	-	-	+	Valid result: Positive for OXA-23
-	-	+	-	-	-	-	+	Valid result: Positive for OXA-24/OXA40
-	-	-	+	-	-	-	+	Valid result: Positive for KPC
-	-	-	-	+	-	-	+	Valid result Positive for VIM
-	-	-	-	-	+	-	+	Valid result: Positive for NDM
-	-	-	-	-	-	+	+	Valid result: Positive for IMP
-	-	-	-	-	-	-	+	Valid result: Negative specimen (No target DNA in sample)
+/-	+/-	+/-	+/-	+/-	+/-	+/-	-	Invalid result. Repeat testing ensuring ICDNA is added to the test sample prior to sample heat treatment
+ in more than one tube (tubes 1 to 7)							+	Valid result: Positive for more than one target DNA

+ denotes a positive amplification and Ct < 30; - denotes a negative amplification and no Ct value

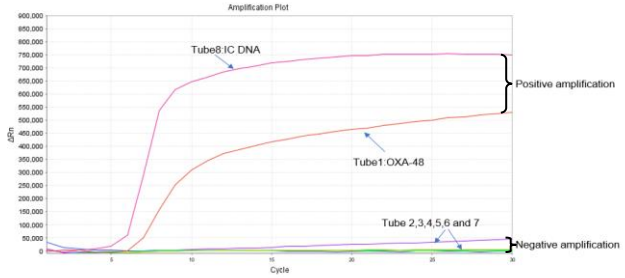
In the case of a query result it is recommended to re-test the sample (in duplicate to confirm the result) or the results should be confirmed using an alternative method.
A positive is indicated by a rapid increase in the fluorescent intensity in a relative short period of time and within the 30minutes assay time whereas a negative result shows no rapid increase in fluorescence intensity over the same period of time (refer to Figure 1 to 2).

Figure 1: Amplification plot of MAST ISOPLEX® *CRE-ART* kit using *Klebsiella pneumoniae* containing OXA48 gene tested on TS2.4



Note: 1time interval is equivalent to 0.5minutes thus amplification plot generated over 30minute assay time.

Figure 2: Amplification plot of MAST ISOPLEX® *CRE-ART* kit using *Klebsiella pneumoniae* containing OXA48 gene tested on ABI 7500 FAST real time cycler.



Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Performance characteristics

Traceability and values assigned to calibrators and trueness-control materials

Calibration, servicing and maintenance of equipment used in MAST ISOPLEX®*CRE-ART* is conducted in accordance with local and national guidelines. Quality control of all constituent components are tested and validated in accordance with local and national guidelines.

Analytical Sensitivity

The analytical sensitivity of MAST ISOPLEX®*CRE-ART* was determined using plasmid pEX-A218 with insert target gene DNA. Tests were conducted on 10-fold serial dilutions of plasmid DNA ranging from 10pg to 0.001fg. A total of 8 replicates were conducted for each plasmid at each concentration range and the data analysed using Probit analysis.

Table 7: Analytical sensitivity (based on plasmid containing specific target sequence)

Target gene	Probit value Femtograms/µL (95% confidence interval)	Probit value DNA copy number/µL (95% confidence interval)
OXA-48	0.13fg/µL (0.07 to 0.75)	44 copies/µL (22.5 to 244.2)
OXA-23	0.28 fg/µL (0.15 to 1.19)	92 copies/µL (49.7 to 386.7)
OXA-24/OXA-40	2.1 fg/µL (1.05 to 2.02)	683 copies/µL (344 to 6576)
KPC	0.1 fg/µL (0.05 to 0.58)	35 copies/µL (17.4 to 187.4)
VIM	0.1 fg/µL (0.05 to 0.62)	35 copies/µL (16.6 to 195.7)
NDM	0.2 fg/µL (0.08 to 1.36)	53 copies/µL (27.6 to 442.7)
IMP	2.7 fg/µL (1.12 to 27.8)	879 copies/µL (364.8 to 9035.3)

Table 8: Analytical sensitivity (based on Carbapenemase producing bacterial strains)

Strain	ACTC/ATCC identifier	Target gene family	Probit value cfu/µL (95% confidence interval)
Klebsiella pneumoniae	NCTC 13422	OXA-48	4 cfu/µL (2.7 to 10.9)
Acinetobacter baumanii	NCTC 13424	OXA-23	57 cfu/µL (16.71 to 925)
Acinetobacter baumanii	NCTC 13302	OXA-24/OXA-40	43 cfu/µL (18.4 to 1043.5)
Klebsiella pneumoniae	NCTC 13438	KPC	12 cfu/µL (7.0 to 53.2)
Klebsiella pneumoniae	NCTC 13439	VIM	12 cfu/µL (5.5 to 72.6)
Klebsiella pneumoniae	ATCC BAA-2472	NDM	15 cfu/µL (9.8 to 140.3)
Escherichia coli	NCTC 13476	IMP	5 cfu/µL (2.7 to 25.5)

Analytical Specificity

MAST ISOPLEX®*CRE-ART* provides 100% analytical specificity when tested using the target specific plasmid used in the analytical sensitivity testing and did-not cross react with any of the species (with known β-lactamase) listed (refer to Table 9).

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Table 9: Cross-reactivity testing

Species	Known B-lattamase	Species	Known B-lattamase
Klebsiella pneumoniae	DHA-1, SVH-5	*Klebsiella pneumoniae*	DHA-1, SHV
Klebsiella pneumoniae	DHA-1	*Klebsiella pneumoniae*	DHA-1, CTX-M
Klebsiella pneumoniae	CTX-M-15	*Klebsiella pneumoniae*	Fox-3
Klebsiella pneumoniae	SVH-5	*Klebsiella pneumoniae*	Fox
Klebsiella pneumoniae	CTX-M	*Escherichia coli*	Lat-3
Escherichia coli	CTX-M 1	*Escherichia coli*	TEM 2, CTX-M 9
Klebsiella pneumoniae	TEM-29, SHV 14	*Salmonella livingstone*	acc-1
Klebsiella pneumoniae	dha-1	*Escherichia coli*	lat-4
Enterobacter cloacae	CTX-M9	*Enterobacter cloacae*	SHV 12
Klebsiella pneumoniae	CTX-M9, SHV-36	Salmonella species	SHV 12
Escherichia coli	TEM 10	*Escherichia coli*	TEM 9
Escherichia coli	TEM 4	*Klebsiella pneumoniae*	SHV 2
Klebsiella pneumoniae	SHV 3	*Klebsiella pneumoniae*	SHV 2 SHV 5
Klebsiella oxytoca	K1	*Klebsiella pneumoniae*	SHV 18
Proteus mirabilis	CMY	*Proteus mirabilis*	ACC-1
Proteus mirabilis	CMY-3	*Pseudomonas aeruginosa*	Imi-R no carbapenemase
Pseudomonas aeruginosa NCTC 10662	No carbapenemase	*Acinetobacter baumannii*	No carbapenemase
Escherichia coli	CTX-M3	*Escherichia coli*	CTX-M 23
Klebsiella pneumoniae	SHV 12	*Escherichia coli*	TEM III
Escherichia coli	CTX-M 33	*Escherichia coli*	CTX-M 15
Proteus mirabilis	CTX-M1		

Precision

The precision of MAST ISOPLEX® *CRE-ART kit* was determined in terms of repeatability (intra batch variation using a single batch and six tests performed over a single day, refer to Table 10a and 10b) and reproducibility (inter batch variation – three batches tested, refer to Table 11a and 11b).

As LAMP is not a quantitative test the measure of precision is not an absolute measure of test performance in terms of time to positivity. The data generated shows for repeatability the standard deviation range is 0.35 to 1.94 for the various targets with % CV ranging from 2.29 to 12.31 and reproducibility the range for standard deviation is 0.21 to 3.02 and % CV from 2.1 to 17.96.
A %CV value <10 is considered very good, 10-20 is good, 20-30 is acceptable and >30 is considered not acceptable, therefore the values obtained are good.

Table 10a: Repeatability of MAST ISOPLEX® *CRE-ART* kit for specific targets

Target	Average Ct value	Standard deviation	Coefficient of variation (%CV)
OXA-48	15.13	1.12	7.67
OXA-23	15.18	1.87	12.31
OXA-24/OXA-40	19.21	1.94	10.07
KPC	15.34	0.35	2.29
VIM	12.10	1.08	8.88
NDM	10.66	0.47	4.43
IMP	14.23	1.27	8.91

Table 10b: Repeatability for Inhibition Control DNA (ICDNA)

Target	Average Ct value	Standard deviation	Coefficient of variation (%)
ICDNA	11.59	1.35	11.61

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Table 11a: Reproducibility of MAST ISOPLEX® *CRE-ART* kit

Target	Average Ct value	Standard deviation	Coefficient of variation (%CV)
OXA-48	13.2	1.17	8.86
OXA-23	12.99	1.05	8.03
OXA-24/OXA-40	17.98	3.02	17.96
KPC	14.52	0.57	3.92
VIM	10.34	1.44	13.93
NDM	9.99	0.21	2.1
IMP	13.03	0.58	4.43

Table 11b Reproducibility of MAST ISOPLEX® *CRE-ART* kit for Inhibition Control DNA (ICDNA)

Target	Average Ct value	Standard deviation	Coefficient of variation (%)
ICDNA	10.63	0.27	2.52

Diagnostic performance characteristics

MAST ISOPLEX® *CRE-ART* kit diagnostic performance characteristics (refer to Table 12a and 12b) were determined on 248 clinical isolates, comprising Enterobacterales (n=203), *Acinetobacter* species (n=37) and *Psuedomonas aeruginosa* (n=8). Isolates harbouring carbapenemases not targeted by the assay which were not detected are classified as true negatives. Work was conducted by the Microbiology department, Newcastle upon Tyne Hospitals NHS Foundation Trust.

Table 12a: Detection of carbapenemases in 248 Gram-negative bacteria with MAST ISOPLEX® *CRE-ART* kit

Isolate type/ target gene	Number tested	Number detected by MAST ISOPLEX® *CRE-ART* kit
Carbapenemase Producing Enterobacterales		
KPC	16	16
VIM	13	13
IMP	12	12
OXA-48 like	61	61
NDM	52	52
NCM-A	1	0
Carbapenemase Producing *Pseudomonas aeruginosa*		
VIM	2	2
NDM	2	2
Carbapenemase Producing *Acinetobacter* species		
OXA-23-like	23	23
OXA-24-like/ OXA-40-like	2	2
NDM	3	3
OXA-51-like	12	0
OXA-58-like	5	0
OXA-69-like	1	0
Isolates without carbapenemase	53	2

Note: *MAST ISOPLEX® CRE-ART kit does not target NCM-A, OXA-51like, OXA-58-like or OXA-69-like DNA and the results of test conducted on these strains are considered as true negative results.*

From the above data the diagnostic performance characteristics of MAST ISOPLEX® *CRE-ART* kit is shown in Table 12b

Table 12b: Diagnostic performance characteristics of MAST ISOPLEX® *CRE-ART* kit

Diagnostic characteristic	
Sensitivity	100%
Specificity	97%
Positive predictive value	99%
Negative predictive value	100%

Measuring interval

MAST ISOPLEX® *CRE-ART* is a qualitative test however the kit was tested for the purposes of determining the analytical sensitivity using a range of specific target DNA ranging from 100 fg to 1 pg per reaction.

Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Biological reference interval

MAST ISOPLEX® *CRE-ART* is a qualitative test and not quantitative therefore biological reference intervals are not relevant.

Limitations of the examination procedure

1. The results obtained from use of MAST ISOPLEX® *CRE-ART* should not be used for the sole diagnosis of a clinical condition but used in conjunction with other clinical factors.
2. MAST ISOPLEX® *CRE-ART* kit is validated for testing of an isolated bacteria colony cultured from a clinical specimen.
3. The amount of bacteria (refer to step 2 of Examination procedure) inoculated should be equivalent to ~0.2 McFarland or 10^8cfu/mL.
4. MAST ISOPLEX® *CRE-ART kit* has been validated on the instruments and microbiological media listed in this document. Use of alternative instruments or microbiological medium must be validated by the end user prior to diagnostic use.
5. The presence of DNAse or protease may cause invalid results, the user must ensure all equipment and accessories are DNAse free.
6. Extremely high levels of target DNA may cause a high background and accordingly a false negative result due to the amplification assay reaching the maximum threshold of the instrument. Adherence to the instructions for use, in particular the picking of a small quantity of colony, should prevent such an occurrence.
7. LAMP amplification can produce a positive result from very low level quantity of target DNA however, below the given limits of detection these may not be reproducible. Adherence to the instructions for use, in particular the picking of a small quantity of colony, should prevent such an occurrence.
8. MAST ISOPLEX® *CRE-ART* kit is designed to specifically amplify certain β-lactamase classes and subgroups. The test will amplify the classes/subgroups identified in Table 13.

Table 13: Target Family and Family members identified by MAST ISOPLEX® *CRE-ART*

Target	
Family	**Family members**
OXA-48	48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 252, 370, 405, 416, 438, 439, 484, 505, 514, 515, 517, 519, 538, 546, 547, 566, 567, 731, 788, 793, 833
OXA-23	23, 27, 49, 73, 103, 133, 146, 165, 166, 167, 168, 169, 170, 171, 225, 239, 366, 398, 422, 423, 435, 440, 482, 483, 565, 657, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818
OXA-24/OXA-40	24, 25, 26, 40, 72, 139, 160, 207, 437, 653
KPC	2, 3, 4, 5, 6, 7, 8 ,9, 10, 11, 12, 13, 14 ,15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,4,2, 43, 44, 45, 46,
VIM	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35,36,37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68
NDM	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,19, 20, 21, 22, 23, 24, 27, 28
IMP	1, 3, 4, 6, 10, 25, 26, 30, 34, 38, 40, 42, 52, 55, 61, 60

9. MAST ISOPLEX® *CRE-ART* may not amplify OXA-535, OXA-436, OXA-54, VIM-7, VIM-61, NDM-25 and NDM-26
10. User must always refer to the amplification plot (raw data) generated during testing to ensure a positive result is due to amplification of the specific target and not a consequence of ramping effect which may be seen on instruments whereby the background threshold cannot be manually altered.
11. Each CRE STRIP in the MAST ISOPLEX® *CRE-ART* kit is to be used on a single test sample. To ensure the validity of positive amplification the user should conduct a no template control CRE STRIP and a positive control CRE STRIP.

Literature references

Notomi T et al. Nucleic Acids Research (2000) 28 12, 63

IFU214 GB 08/20 V3