MultNAT Carba-R Assay
Instructions for Use

IVD CE

[PRODUCT NAME]

MultNAT® Carba-R Assay

[SPECIFICATIONS]

Σ 20

[INTENDED USE]

The assay is intended for qualitative in vitro detection of carbapenem resistance genes (blaKPC, blaNDM, blaVIM, blaOXA, and blaIMP) in rectal swab specimens. It is used as an aid in the diagnosis of carbapenem resistance.

Bacterial drug resistance is now a global public health problem of great concern, with carbapenem-resistant Enterobacterales (CRE) causing the most serious infections. Carbapenems, including imipenem, meropenem and ertapenem etc., are among the most effective antibacterial drugs for the treatment of infections caused by multi-drug resistant Gram-negative bacilli. However, with the massive use of carbapenems, cases of bacteria non-susceptible to carbapenems and even drug-resistant bacteria have been reported in China and abroad, greatly limiting the use of carbapenems. This is due to the fact that some strains have obtained the genes that produce carbapenemase.

The presence and prevalence of carbapenem-resistant bacteria has caused great difficulties in clinical anti-infective treatment and nosocomial infection control. The detection of drug resistance genes in clinical samples using PCR technology provides a scientific basis for infection prevention and control measures, and enables timely diagnosis and treatment of clinical infectious diseases.

[MATERIALS PROVIDED]

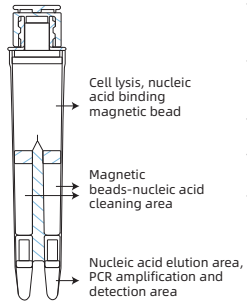
No.	Material	Specification	Quantity	Main components
1	CRE-Cartridge	1mL/tube	20 cartridges	Specific primers and probe for OXA, KPC, VIM IMP, NDM and internal control gene, deoxyribonucleoside triphosphate (dNTP), DNA polymerase and Uracil-DNA glycosylase (UDG)
2	CRE-DNA Extraction Solution	1mL/tube	20 tubes	Guanidine salt, magnetic beads and internal control-specific fragment
3	CRE-Positive Control	1.2mL/tube	1 tube	Containing the target sequence
4	CRE-Negative Control	1.2mL/tube	1 tube	Sample preservation solution

Note: Do not mix components from different kit lots.

[TEST PRINCIPLE]

IIn this assay, the cartridge is equipped with multiple hydrophobic separation layers to isolate the lysis solution, wash solution and reaction solution. The specimens are chemically lysed in extraction solution at high temperature to release the nucleic acids under the heating control of the external instrument. An external magnetic field provided by the instrument allows the nucleic acid samples to pass through different layers. Finally, the nucleic acids are eluted in the cartridge legs where the amplification reaction occurs. Thus, it enables fully automatic "All-in-One" nucleic acid analysis in which the lysis, binding, washing, elution and amplification reaction are completed in a closed cartridge.

The assay adopts real-time fluorescent PCR technology, in which specific primers and fluorescent probes are designed based on the target sequences in the conserved regions of carbapenem resistance genes (IMP, OXA, VIM, KPC, NDM). Different fluorescent signals enable the detection and identification of multiple genes in one cartridge.



UDG and dUTP are used as anti-pollution components of the PCR reaction system to prevent potential carryover of amplified product and avoid false positive results.

The kit also provides internal control (IC), which consists of a system for the specific detection of Bacillus subtilis gene fragment to monitor the effectiveness of extraction, purification, and amplification reactions and avoid false negative results.

Before starting the test, the operator only needs to add CRE-DNA Extraction Solution and the specimen into the cartridge, then the nucleic acids in the specimen can be purified automatically under the control of the applicable instrument. The purified nucleic acids and PCR reaction reagent are mixed and heated by the applicable instrument for amplification. Meanwhile, the fluorescence probe will specifically bind to the target sequence and produce fluorescence signal. The instrument collects real-time fluorescence signal and automatically reports test results by analyzing the change of the signal.

[STORAGE AND STABILITY]

1.Storage condition: store at 2℃-8℃. Out of light.

2.Validity period: 6 months (provisional). For production and expiry date, please refer to the label.

3.Transport at ambient temperature (-25℃-30℃) within 15 days does not affect kit performance.

[SAMPLE COLLECTION AND HANDLING]

Sample type: rectal swab samples

1.Sample collection

Before initiating collection, prepare sterile sampling tubes, put on disposable gloves, and peel off the swab packaging. Carefully insert both swab tips approximately 3-5cm into the anus and gently rotate against the colorectal walls to collect mucus or feces, then remove the swabs. Place the two swabs into two sampling tubes containing the sample preservation solution. Seal and send it for testing.

2.Sample preservation

Collected sample should be sent for testing as soon as possible. For anticipated delay in delivery, samples should be refrigerated at 2~8℃ for not more than 72 hours. Received samples should be tested as soon as possible in the laboratory. Samples can be stored at -25℃±5℃ for at least 6 months, or below -70℃ for at least 12 months. Avoid repeated freezing and thawing. 6 freeze-thaw cycles do not affect kit performance.

3.Transportation

Transport in a sealed pot or foam box filled with ice.

[APPLICABLE INSTRUMENT]

Molecular Diagnostic PCR System (UP0102/UP0104) produced by Ustar Biotechnologies (Hangzhou) Ltd.

[TEST PROCEDURE]

Please test in accordance with this IFU.

1.1 Sample loading

Fully mix the CRE-DNA Extraction Solution until there are no visible brown sediments or agglomerates in the tube. Transfer all the solution into the CRE-Cartridge. Add 500μL sample to the cartridge, reset the cartridge cap, and shake to mix well (paraffin may float but it will not influence following steps). The cartridge is ready to be tested.

1.2 Testing

Note: this section lists the basic steps of running the test. For more details, please refer to the instructions of the applicable instrument.

1.2.1 Input cartridge information

Put the QR code of the cartridge (located on the cartridge cap) prepared in [*TEST PROCEDURE*] 1.1 in the scanning area of the instrument, and the system will automatically input the cartridge information and testing program. If the scanning fails, click the [*Scan QR code on the cartridge*] button on the touch screen to input it manually.

1.2.2 Input sample information

Put the sample barcode corresponding to the cartridge in 1.2.1 in the scanning area of the same instrument, and the instrument will automatically input the sample information. If the scanning fails, click the [*Scan the sample barcode*] button on the touch screen to manually input it.

1.2.3 Start the test

Put the cartridge into the module of applicable instrument, close the module cover, and click [START] on the touch screen. The instrument will start the test procedure.

1.3 Result viewing

At the end of the test, the results will be displayed on and saved by the applicable instrument automatically. Please refer to the [*INTERPRETATION OF RESULTS*] for details.

2. External Quality Control Testing

2.1 Fully mix the CRE-DNA Extraction Solution and transfer all the solution into the CRE-Cartridge.

2.2 Take 500μL positive or negative control and add it into the cartridge prepared in step 2.1, and close the cartridge cap.

2.3 Follow the steps as described in [*TEST PROCEDURE*] 1.2-1.3.

[CUT-OFF VALUE]

Upon completion of a test, the instrument will automatically report the result of each target. The instrument automatically calculates the Tt values of each target and IC and reports negative/positive result according to the following criteria:

The result of OXA should show a typical S-type amplification curve (including the S-curve before the plateau). When OXA Tt ≤ 38, OXA is positive; otherwise, OXA is negative. The result of KPC should show a typical S-type amplification curve (including the S-curve before the plateau). When KPC Tt ≤ 38, KPC is positive; otherwise, KPC is negative. The result of VIM should show a typical S-type amplification curve (including the S-curve before the plateau). When VIM Tt ≤ 38, VIM is positive; otherwise, VIM is negative. The result of IMP should show a typical S-type amplification curve (including the S-curve before the plateau). When IMP Tt ≤ 38, IMP is positive; otherwise, IMP is negative. The result of NDM should show a typical S-type amplification curve (including the S-curve before the plateau). When NDM Tt ≤ 38, NDM is positive; otherwise, NDM is negative. The result of IC should show a typical S-type amplification curve (including the S-curve before the plateau). When IC Tt ≤ 38, IC is positive; otherwise, IC is negative.

[INTERPRETATION OF RESULTS]

Results	Interpretations	Results	Interpretations
OXA **positive** KPC negative VIM negative IMP negative NDM negative	• DNA of the OXA gene is detected in the sample. • DNA of KPC, VIM, IMP and NDM genes are not detected in the sample and the internal control meets the acceptance criteria.	OXA **positive** KPC **positive** VIM negative IMP **positive** NDM **positive**	• DNA of OXA, KPC, IMP and NDM genes are detected in the sample. • DNA of VIM is not detected in the sample and the internal control meets the acceptance criteria.
OXA negative KPC negative VIM **positive** IMP negative NDM negative	• DNA of the VIM gene is detected in the sample. • DNA of OXA, KPC, IMP and NDM genes are not detected in the sample and the internal control meets the acceptance criteria.	OXA **positive** KPC **positive** VIM **positive** IMP **positive** NDM **positive**	• DNA of OXA, KPC, VIM, IMP and NDM genes are detected in the sample.
OXA negative KPC negative VIM negative IMP negative NDM **positive**	• DNA of the NDM gene is detected in the sample. • DNA of OXA, KPC, VIM, IMP genes are not detected in the sample and the internal control meets the acceptance criteria.	OXA negative KPC negative VIM negative IMP negative NDM negative	• DNA of OXA, KPC, VIM, IMP and NDM genes are not detected in the sample and the internal control meets the acceptance criteria.
OXA **positive** KPC negative VIM **positive** IMP negative NDM negative	• DNA of the OXA and VIM genes are detected in the sample. • DNA of KPC, IMP and NDM genes are not detected in the sample and the internal control meets the acceptance criteria.	OXA negative KPC negative VIM negative IMP invalid NDM invalid	• DNA of OXA, KPC and VIM genes are not detected in the sample and the internal control meets the acceptance criteria. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of IMP and NDM are present in the sample.
OXA **positive** KPC **positive** VIM **positive** IMP negative NDM negative	• DNA of the OXA, KPC and VIM genes are detected in the sample. • DNA of IMP and NDM genes are not detected in the sample and the internal control meets the acceptance criteria.	OXA **positive** KPC **positive** VIM **positive** IMP invalid NDM invalid	• DNA of OXA, KPC and VIM genes are detected in the sample. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of IMP and NDM are present in the sample.
OXA invalid KPC invalid VIM invalid IMP **positive** NDM **positive**	• DNA of IMP and NDM genes are detected in the sample. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of OXA, KPC and VIM are present in the sample.	OXA negative KPC **positive** VIM negative IMP invalid NDM invalid	• DNA of OXA and VIM genes are not detected in the sample and the internal control meets the acceptance criteria. • DNA of KPC is detected in the sample. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of IMP and NDM are present in the sample.
OXA invalid KPC invalid VIM invalid IMP **positive** NDM negative	• DNA of IMP gene is detected in the sample. • DNA of NDM gene is not detected in the sample and the internal control meets the acceptance criteria. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of OXA, KPC and VIM are present in the sample.	OXA invalid KPC invalid VIM invalid IMP negative NDM negative	• DNA of IMP and NDM are not detected in the sample and the internal control meets the acceptance criteria. • The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of OXA, KPC and VIM are present in the sample.
OXA invalid KPC invalid VIM invalid IMP invalid NDM invalid	• The internal control fails to meet the acceptance criteria and it is uncertain that whether the DNA of OXA, KPC, VIM, IMP and NDM are present in the sample.	No result	• Incomplete data is collected.

1.Conditions that require retesting

If any of the following results occur, please retest the remaining samples with a new CRE-Cartridge.

• Invalid: e.g. improper sample handling, inhibition of PCR reagent or kit failure.

• No result: e.g. the operator terminates the test midway.

• Abnormal results of external quality control (CRE-Positive Control and CRE-Negative Control): e.g. if the CRE-Negative Control result is positive, there may be contamination.

2. Quality control

The assay provides internal quality control and external quality control.

2.1 Internal control

The internal quality control (IC) system is used to monitor failures in sample processing and reagents, and malfunction of applicable instrument.

2.2 External control

The CRE-Positive Control is used to monitor the failure of amplification reagent or malfunction of the instrument. CRE-Negative Control is used to monitor the contamination of the reagent or in the environment.

The test result of CRE-Positive Control should be "OXA positive, KPC positive, VIM positive, IMP positive, NDM positive", and that of CRE-Negative Control should be "OXA negative, KPC negative, VIM negative, IMP negative, NDM negative".

[LIMITATIONS]

1.Result from this assay is for clinical reference only. The clinical diagnosis and treatment of patients
should be considered in combination with their symptoms, signs, medical history, other laboratory tests and therapeutic effect.
2.The test results cannot be used to guide or monitor the treatment of bacterial infections not susceptible to carbapenems and a negative result does not exclude the presence of other drug resistance mechanisms.
3.Inappropriate sample collection, transportation and treatment or low pathogen load in samples may lead to false negative results.
4.The variation of the target nucleic acid to be tested or the sequence change caused by other reasons may lead to false negative results.
5.Other unverified interference or amplification inhibitors may lead to false negative results.

[PERFORMANCE CHARACTERISTICS]

1.Limit of detection (LoD):

Bacteria	LoD of drug resistance gene
IMP-1 (*Escherichia coli*)	50 CFU/mL
IMP-8 (*Klebsiella pneumoniae*)	100 CFU/mL
VIM-4 (*Escherichia coli*)	50 CFU/mL
VIM-1 (*Klebsiella pneumoniae*)	50 CFU/mL
NDM-1 (*Klebsiella pneumoniae*)	200 CFU/mL
NDM-5 (*Escherichia coli*)	50 CFU/mL
KPC-2 (*Klebsiella pneumoniae*)	50 CFU/mL
KPC-3 (*Klebsiella pneumoniae*)	100 CFU/mL
OXA-48 (*Klebsiella pneumoniae*)	100 CFU/mL
OXA-48 (*Escherichia coli*)	50 CFU/mL

2.Agreement with commercial reference panels
2.1Positive reference panels: P1-P10 are positive for corresponding drug resistance gene.
2.2Negative reference panels: N1-N12 are all negative.
2.3 Medium-positive precision reference panels R1-R10, weak-positive precision reference panels S1-S10: R1-R10 and S1-S10 are positive for all drug resistance genes.
2.4 LoD reference panels: A2-E2 are detected, the test results of A3-E3 are not required.
3.Analytical specificity
3.1Cross reactivity
No cross reactivity was seen with E*scherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Clostridium difficile*, *Helicobacter pylori*, *Candida albicans*, *Enterobacter aerogenes* and *Enterobacter cloacae* and test results of these microorganism were all negative.
3.2 Interfering substances
Feces, mucus, blood and the commonly used drugs, such as cephalexin, ciprofloxacin, meropenem, cholesterol, neomycin, famotidine, borneol, omeprazole and bismuth subsalicylate do not interfere with the test results of this kit.

[PRECAUTIONS]

1.This assay is for professional use only. Please read this IFU carefully before use. Ustar accept no responsibility for the false results and corresponding consequences due to improper handling of the assay or any problems not derived from the performance defects of the assay.
2.The assay is only used for in vitro diagnosis of carbapenem resistance genes (IMP, OXA, VIM, KPC, NDM) and the results shall not be used as the sole standard for definite diagnosis. The diagnosis should integrate other test results and clinical manifestations for comprehensive consideration.
3. Testing
1) The assay is a disposable product. The worktable and necessary articles should be regularly disinfected with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.
2) The laboratory should carry out corresponding laboratory quality control according to the relevant national regulations to avoid the false positive results caused by laboratory contamination.
3) The protective facilities used by operators, such as gloves, laboratory clothes, etc. should be managed in different areas to avoid the introduction of pollution, which will lead to false test result.
4. View the result
After the test, the system of the instrument will automatically save the test results, and the previous test results can be queried in the [View] interface of the instrument.
5. Operation
1) The samples are regarded as infectious materials. Be careful when operating and take necessary biological hazard prevention measures.
2) If the reagent enters the eyes or mouth by mistake, or contaminates the skin, rinse it with plenty of water immediately, and seek for medical help if necessary.
3) The kit components should be completely thawed, shaken to mix well and then centrifuged for a few seconds before use. Avoid repeated freezing and thawing.
4) It is recommended that the cartridge be subjected to PCR amplification immediately after nucleic acid extraction, otherwise please keep the cartridge tightly capped and stored below -18℃ until use.
6. Storage
1) The assay must be stored in accordance with the storage conditions specified in this IFU. Do not freeze the assay.
2) To prevent reagent deterioration, only take out the required amount for testing and store the remaining reagents under specified condition.
3) Please do not use the CRE-Positive Control of the assay for purposes not described in this IFU (such as dilution or addition to samples) to avoid contamination of the test environment.
4) Do not use expired reagents.
5) Do not mix components from different assay lots; do not refill the reagent.
7. Disposal
All samples and other materials should be disposed of in accordance with the [*Medical Waste Management Regulations*] after use.

[REFERENCE]

1.Xu X, Qu F. Progress in the diagnosis of carbapenem-resistant Enterobacteriaceae. Chinese Journal of Biotechnology, 2018, 34(8): 1338-1345.
2.Kallen A, Ouh A. United States Centers for Disease Control and Prevention issue updated guidance for tackling carbapenem-resistant Enterobacteriaceae. Euro Surveill. 2012, 17(26): pii: 20207.
3.Clinical and Laboratory Standards Institute. M100-S27: Performanee standards for antimiembial susceptibility testing:Twenty seventh informational supplement[S]. Wayne, Pa:Clinical and Laboratory Standards Institute, 2017.

[EXPLANATION OF SYMBOLS]

IVD	*In vitro* diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
EC REP	Authorized representative in the European Community		Keep dry
	Keep away from sunlight		Do not use if package is damaged
	Date of manufacture		Biological risks
	Contains sufficient for <*n*> tests	REF	Catalogue number
CE	The product meets the basic requirements of European *in vitro* diagnostic medical devices directive 98/79/EC.		

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[INSTRUCTION VERSION AND MODIFICATION DATE]