Carbapenem Resistance Diagnostic Kit (PCR-Fluorescence)

REF 106-0102-01

Product Name
Carbapenem Resistance Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
This kit uses real-time multiplex fluorescent PCR technology, combined with the Automated Fully Enclosed qPCR Instrument, for the in vitro qualitative detection of carbapenem resistance genes KPC, NDM, VIM, IMP and OXA-48 in human sputum samples, the detection results can be used to assist in the treatment of carbapenem antibiotics during use.

Rapid global spread of carbapenem-resistant Enterobacteriaceae (CRE) poses a threat to public safety systems. The resistance mechanisms of Enterobacteriaceae to carbapenem antibiotics mainly include carbapenemase -producing Enterobacteriaceae , outer membrane protein deletion or mutation, penicillin-binding protein variation and over expression of efflux pump, among which the most important is carbapenemase -producing Enterobacteriaceae. The common carbapenemase -producing Enterobacteriaceae include Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Imipenemase (IMP), Verona integron-mediated metallo-beta-lactamase (VIM), and Oxaciuinase-48-type carbapenemases (OXA-48). KPC is CLASS A enzymes and is the most prevalent carbapenemase in Enterobacteriaceae bacteria, especially in Klebsiella pneumoniae, and also exists in bacteria such as Enterobacteriaceae, Acinetobacter and Pseudomonas aeruginosa. NDM, IMP, and VIM are class B, also known as metallo-β-lactamases. NDM is the most common metalloenzyme in Enterobacteriaceae bacteria such as Escherichia coli and Enterobacter cloacae, and it does not hydrolyze altreonam. Mostly otherβ-lactam antibiotics are resistant. VIM has a strong ability to hydrolyze carbapenem antibiotics. IMP hydrolysis carbapenemase ability is not strong, but often combined with outer membrane protein deletion and other resistance mechanisms, showing a high degree of resistance to carbapenems. OXA-48 is class D enzymes, hydrolyze only penicillins and carbapenems, but not super broad-spectrum cephalosporins, often showing low-level resistance to carbapenem antibiotics. Therefore, it is very important to monitor and prevent carbapenem-resistant bacteria in clinical treatment.

The kit uses molecular biology methods to detect. The operators should have received professional training in gene amplification or molecular biological methods and have relevant experimental operating qualifications. The laboratory should have reasonable biological safety facilities and protective procedures.

Test Principle
The kit uses iCassette technology in combination with supporting instruments to automatically perform nucleic acid extraction and nucleic acid amplification by the instrument throughout the entire process, reducing direct cross-contamination of samples. At the same time, a built-in QR code scanner can automatically identify the execution process corresponding to this kit. A uniquely designed software of the kit is used to perform nucleic acid extraction, whole-process PCR, result display and analysis. The Kit contains twelve sets of Carba-R iCassette, which includes nucleic acid extraction reagents and PCR reagents. For a complete description of the instrument, please refer to the instruction for use of the corresponding instrument.

This kit uses real-time multiplex fluorescent PCR technology to design specific primers for KPC gene (FAM marker), OXA-48 (HEX), IMP gene (TEXAS RED marker), VIM (CY5 marker) and NDM gene (CY5.5 marker) and fluorescent probes for PCR. and also designs primers and probes for internal control (TAMRA marker). Through the detection of the quality control sample, confirm whether the target virus is fully processed, and detect whether there are PCR reaction inhibitors.

Components

Kit Components			Content	Quantity
Carba-R iCassette (12 pcs)	Nucleic acid extraction reagent	Carba-R lyophilization A	Proteinase K	1 pc/iCassette
		Carba-R lyophilization B	lyophilization containing internal control fragments	1 pc/iCassette
		Sample lysate solution	Guanidinium isothiocyanate	450μL/iCassette
		Sample binding solution	Guanidinium isothiocyanate	175μL/iCassette
		Sample washing solution	Sodium Chloride	900μL/iCassette
		Sample Virus eluent	Tris-HCl	90μL/iCassette
		Magnetic beads	Magnetic Microspheres	11 μL/iCassette
	Carba-R PCR reagent	Carba-R PCR cosolvent solution	PCR Buffer, $MgCl_2$	35 μL/iCassette
		Carba-R lyophilization	Specific primer, probe, dNTP, enzyme	1 pc/iCassette
Control	Carba-R Postive Control		Pseudovirus with target fragments	1 tube (200μL)
	Carba-R Negative Control		Sterilized PW	1 tube (200μL)
Pretreatment solution			NaOH	28 mLx2 bottles
Enhancer			/	1 tube (1mL)

Note:Components in kits with different batch numbers are not interchangeable

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette lid before adding the sample. If you open the iCassette lid, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Nano, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Timer.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1. Sample types: Sputum and rectal swab.
2. Sample collection

Sputum: Before collecting, subjects should be asked to brush their teeth and rinse their mouths to remove most of the bacteria in the oral cavity. Use a sterile 5mL glass tube to collect 1-3 mL of sputum coughed up from the deep lung, and immediately seal it and send it for inspection.

Rectal swab:Wash around the anus with soapy water, gently insert 3-5cm into the anus with a sterilized cotton swab, then gently rotate and pull it out, and immediately put it into a sampling tube with outer screw cap containing 3 ml of preservation solution for storage.

3. Sample storage and transportation

Samples that can be tested within 12 hours can be stored at 2-8°C, or placed at -20±5°C as soon as possible, but no longer than 1 month.

4. Principles of Bio-safety Protection

All operations should comply with relevant local laws and regulations.

Test Method and Operation
1. Prepare Carba-R iCassette

1.1 Process the samples:

A: Pre-process the Sample:

For sputum: add 2 times the sample volume of pretreatment liquid, then add the appropriate amount of enhancer in the proportion indicated in Table 1, shake vigorously for 1 minute, and leave for 15 minutes at room temperature to liquify, shake for 10 seconds every 5 minutes. The sample should be fully liquified and free of tiny lumps of unliquified sputum.

Table 1 Proportion of Added Enhancer

Sputum specimen	Pretreatment liquid	Total volume	Enhancer
1mL	2 mL	3mL	30μL
2mL	4 mL	6mL	60μL

For rectal swab:shake vigorously the sample collection tube to mix it well for testing.

B. Open the package of Carba-R lyophilization, observe whether the Carba-R lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the Carba-R lyophilization, and make sure the PCR tube is screwed up.

C. Open the lid of the iCassette, and pipette 200 μl of sample to the Carba-R iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)

D. Place the iCassette into the instrument.

1.2 Process negative and positive controls

A.Open the package of Carba-R lyophilization, observe whether the Carba-R lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the Carba-R lyophilization, and make sure the PCR tube is screwed up.

B.Pipette 200 μL of positive control or negative control into Carba-R iCassette sample compartment as shown in Figure1, close the lid tightly.

C. Place the iCassette into the instrument.

Figure 1 Carba-R iCassette (Lateral View)

2. Test Operation

2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.

2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.

2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.

2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)

2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.

2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.

2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.

2.8 After the amplification is completed, the compartment door will open automatically.

For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis

After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Internal control: Within the effective range of Ct ≤ 38 for the TAMRA channel , which is qualified for internal control.

Probe Quality Control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.

The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be conducted again.

Result Judgment
Test results of positive control: FAM channel, HEX channel, TEXAS RED channel ,CY5 channel and CY5.5 channel are all positive;

Test results of negative control: FAM channel, HEX channel, TEXAS RED channel, CY5 channel and CY5.5 channel are all negative; TAMRA channel is positive;

Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this reagent kit is 38.

Interpretation of Test Results
The sample to be tested will be determined according to the standards in the table below:

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FAM Channel	HEX Channel	TAMRA Channel	TEXAS RED Channel	CY 5 Channel	CY5.5 Channel	Results
√	×	√	×	×	×	KPC Positive (Figure 2)
×	√	√	×	×	×	OXA-48 Positive (Figure 3)
×	×	√	√	×	×	IMP Positive(Figure 4)
×	×	√	×	√	×	VIM Positive (Figure 5)
×	×	√	×	×	√	NDM Positive (Figure 6)
×	×	√	×	×	×	Negative (Figure 7)
×	×	×	×	×	×	Invalid

Note:"√" means that the result "has an obvious logarithmic amplification curve, and Ct≤38"; "×" indicates the result has "no logarithmic amplification curve or Ct＞38". The TAMRA channel is an internal control channel, due to the specific competition with the sample, when the test sample is positive, it may be tested negative

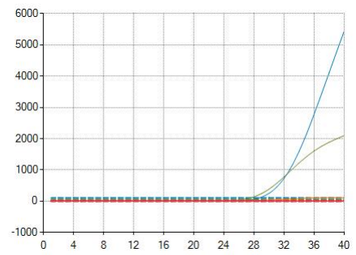

Figure 2. KPC Positive

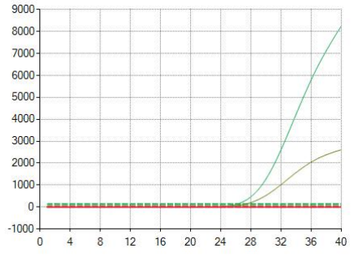

Figure 3. OXA- 48 Positive

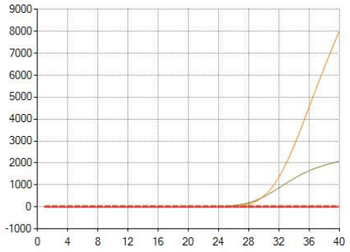

Figure 4. IMP Positive

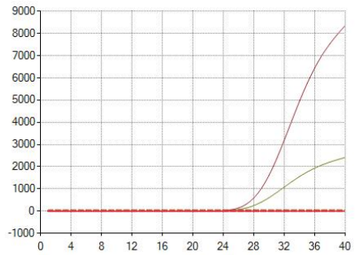

Figure 5. VIM Postive

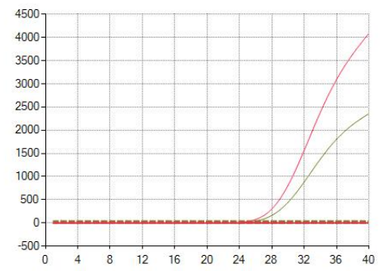

Figure 6. NDM Positive

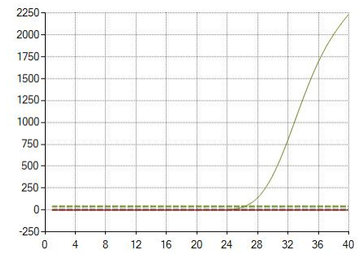

Figure 7. Negative Sample

Limitations of Test Method

1.The test results of this kit are for clinical reference only, and the clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests, and treatment responses.

2. Improper sample collection, transportation and processing, too low virus content in the sample may lead to false negative results.

Product Performance Index

1. The lower limit of detection of the Kit:

KPC	100CFU/mL
VIM	100CFU/mL
NDM	100CFU/mL
OXA-48	1000CFU/mL
IMP	1000CFU/mL

2. Analysis specificity

The specificity test results show that there is no cross reaction with KPC, NDM, IMP,VIM and OXA-48 enzyme bacteria, such as Klebsiella pneumoniae, Streptococcus pneumoniae, Serratia marcescens, Escherichia coli, Staphylococcus superficialis, Acinetobacter baumannii, Enterococcus faecium, Enterococcus faecalis, Legionella pneumophila, Enterobacter cloacae, Aeruginosa Pseudomonas.

3. Potential interfering substances

Endogenous substances: Blood (5%) and mucin (2mg/mL) do not interfere with the detection of the kit.

Exogenous substances: The samples may contain peak drug concentrations of vancomycin (30mg/L), imipenem (40mg/L), meropenem (10mg/L), cefepime (35mg/L), levofloxacin (3mg/L), ciprofloxacin (3mg/L), amikacin (40mg/L), aminotrans (50mg/L), ceftriaxone (10mg/L), ampicillin (10mg/L), rifampicin (9mg/L), tazobactam (1.5mg/L), sulbactam (45mg/L), polymyxin (8mg/L).

4. Precision:

The coefficient of variation of intra-batch precision is ≤ 5%.

Precautions

1. If the iCassette is leaked after adding the sample, do not use the iCassette.
2. Each iCassette is a single-used, please do not reuse it.
3. In order to avoid cross-contamination, traditional PCR tests must be carried out in the reagent preparation area, sample preparation area, amplification and product analysis area during the experiment. This iCassette is fully enclosed, and it is sufficient to add samples in the sample preparation area. The subsequent nucleic acid extraction, amplification detection and result analysis processes are all automated with the instrument, avoiding cross-contamination to the greatest extent.The tips used in the experiment should have reasonable cleaning and quality inspection procedures to avoid cross- contamination or false negative results caused amplification reaction inhibitors.
4. Sample preparation should be carried out in a biological safety cabinet. During the experiment, wear overalls, wear disposable gloves, and use a self-unloading pipette.
5. The used tips for sample preparation should be put into a container filled with disinfectant. After the preparation experiment is completed, they can be discarded after being sterilized together with the iCassette.
6. At the end of the experiment, the bench and pipette were treated with 10% hypochlorous acid or 75% alcohol and then irradiated with UV light for 30 minutes.
7. This kit uses the method of molecular biology, and requires that the laboratory operator should have received professional training in gene amplification or molecular biology, and have the relevant experimental qualifications, and the laboratory should have reasonable bio-safety preparedness facilities and protection procedures.

References

1. Expert Consensus on Diagnosis, Treatment, and Prevention of Infections by Carbapenem-Resistant Enterobacteriaceae in China, Expert Consensus Writing Group of China, Chinese Association of Medical Education Infectious Diseases Committee, Bacterial Infection and Antimicrobial Resistance Control Committee of the Chinese Medical Association. Expert Consensus on Diagnosis, Treatment, and Prevention of Infections by Carbapenem-Resistant Enterobacteriaceae in China [J]. Chinese Medical Journal, 2021, 101(36): 2850-2860 DOI:10.3760/cma.j.cn112137-20210219-00438.
2. Daniel Wei, Engelmann I, Braun S D, et al. A multiplex real-time PCR for the direct, fast, economical and simultaneous detection of the carbapenemase genes blaKPC, blaNDM, blaVIM, and blaOXA-48 [1]. Journal of Microbiological Methods, 2017, 142: 20-26.
3. Evans B A, Amyes SGB. OXA β -Lactamases [J]. Clinical Microbiology Reviews, 2014, 27(2): 241.

Instruction Version

Version: A/1
Date of Issue: May,2022
Last revised: February, 2024

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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