Dynamiker Biotechnology (Tianjin) Co., Ltd.

Carbapenem Resistant Enterobacteriaceae Test Kit (LFA)

Catalogue No.: DNK-2302-1

User Manual/ 40 tests

1. Intended Use

This product is used by medical institutions for in-vitro qualitative test of carbapenemase genotypes KPC, NDM, OXA-48, IMP, and VIM in bacterial colony samples obtained after culture, with an aim to rapidly detect the presence of one or more of the five carbapenemases in colonies. It is clinically applied to identify patients' resistance to carbapenem antibiotics[1] .

2. Test Principle

This product uses double antibody sandwich method colloidal gold immunochromatographyto in-vitro qualitative test carbapenemases KPC, NDM, OXA-48, IMP and VIM in bacterial colonies obtained from clinical specimens after culture, with an aim to rapidly identify the presence of one or more of the five carbapenemases in bacterial colonies. The product embeds five carbapenemase antibodies I labelled with colloidal gold on the conjugate release pads, and coats five carbapenemase antibodies II on the test lines (K, N, O, I, V) respectively. The quality control line (C) is coated with goat anti-mouse antibodies. If the sample to be tested contains carbapenemase antigen and the concentration of the antigen is higher than the minimum detection limit, the carbapenemase and colloidal gold-labeled carbapenemase antibody I bind to form a complex. The complex then moves forward along the paper strip under the action of chromatography and is captured by the pre-coated carbapenemase antibody II when it passes through the test line (K, N, O, I, V), thereby forming an immune complex and showing a red band, which indicates a positive result. On the contrary, if the sample to be tested does not contain carbapenemase antigen or the antigen concentration is lower than the minimum detection limit, no reaction band appears on the test line, and the result is negative at this time. The five carbapenemase antibodies labeled with colloidal gold are all monoclonal antibodies of mouse origin, which are captured by the goat anti-mouse antibody when they are chromatographed to the quality control line (C). On that account, a band is supposed to appear on the quality control line (C) when the sample is tested, and the red band shown therein serves as the criterion to determine whether the chromatographic process is normal, and also as the internal control standard of the reagent.

3. Main Components

Main Components	Composition	Specification
Test Cassette	It consists of an aluminum foil bag, a plastic case, a test strip, and desiccant. The main components of the test strip are sample pads, colloidal gold pads for embedding five carbapenemase antibodies I (0.05~0.20mg/mL) labeled with colloidal gold, nitrocellulose membranes for coating five carbapenemase antibodies II (0.5~2.0mg/mL) and goat anti-mouse antibodies (0.2~2.0mg/mL), absorbent pads, PVC bottom plates, etc.	40 tests/kit
Extraction Buffer	0.5%~2% potassium chloride solution containing surfactant	2×12mL/ bottle
Disposable Pipette	Disposable quantitative pipette	2×20 pcs /pack
EP Tube	EP tube (spec.: 1.5 mL)	2×20 pcs /pack

4. Materials required but not provided

4.1 Pipette, adjustable or fixed, to measure and dispense 300 μL.

4.2 Pipette tips

4.3 Inoculating loops

4.4 Vortex mixer

4.5 Timer

5. Storage and Stability

5.1 The kit shall be stored at 2~30°C for 18 months.

5.2 The test cassette packaged in the aluminum foil bag shall be used within 30 minutes after unpackaged, and the extraction buffer remains valid for 12 weeks after opening.

Please refer to the label for the date of manufacture and shelf life.

6. Sample Requirements

If the pure bacterial colonies obtained after culture are stored at 2~8°C, they shall be tested within 4 weeks; if they are frozen bacterial solutions, they then need to be re-cultured to obtain pure bacterial colonies for testing.

7. Test Procedure

7.1 Bring the kit to room temperature for at least 10 minutes before use.

7.2 Sample extraction

7.2.1 Pipette 300 μL extraction buffer into the EP tube.

7.2.2 Use a 1 μL inoculating loop to take a loop of bacterial samples and place them in an EP tube containing 300 μL of extraction buffer. Shake the inoculating loop to elute the bacterial samples with the extraction buffer.

7.2.3 Fasten the EP tube, use a vortex mixer to mix and shake for about 10 seconds to disperse the bacterial samples in the extraction buffer as evenly as possible (if the samples are viscous, the shaking time needs to be extended), and then let them stand at room temperature for 10 minutes.

7.3 Sample test

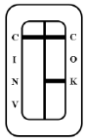
7.3.1 Open the aluminum foil bag, take out the test cassette, and place it on a flat and clean workbench.

7.3.2 Use a disposable pipette to suck the prepared bacterial extraction buffer, and slowly add about 200 μL (1 whole amount of disposable pipette) to the sampling hole of the test cassette.

7.3.3 After sampling, put the test cassette at room temperature for 15 minutes and observe the displayed results, which become unreliable after 30 minutes.

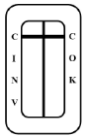
8. Result Interpretation

The left part of this test cassette detects IMP (I), NDM (N), and VIM (V) carbapenemases, and the right part detects OXA-48 (O) and KPC (K) carbapenemases. The following legends all take type KPC (K) as an example.



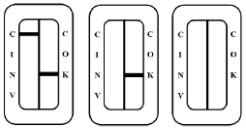
Positive result

If a red band appears on the quality control line (C), and one or more red bands appear on the test line (K, N, O, I, V), it indicates that the sample to be tested contains one or more carbapenemases.



Negative result

If a red band appears only on the quality control line (C), and no red band appears on the test line (K, N, O, I, V), it means that the sample to be tested does not contain carbapenemases or the carbapenemase concentration is lower than the limit of detection.



Invalid result

Absence of red band on any of the quality control lines (C) on the test cassette may be a result of incorrect operation or reagent expiry. The test shall be re-performed under any circumstances. If the problem persists, stop using the products of this lot number immediately and contact the local supplier for assistance.

9. Limitations of Methodology

9.1 This test kit performs a qualitative test and therefore cannot produce any quantitative results.

9.2 Improper sample treatment and reagent use will affect the test results of the sample.

9.3 This reagent is used to qualitatively detect the five carbapenemases KPC, NDM, OXA-48, IMP and VIM in cultured bacterial samples. A positive or negative test result does not rule out the existence of other antibiotic resistance mechanisms.

9.4 Currently no interfering substances and cross-reacting substances related to this reagent have been found.

10. Product Performance

10.1 Negative coincidence rate: Test the negative reference products from the internal reference products. The test results shall be all negative.

10.2 Positive coincidence rate: Test the positive reference products from the internal reference products. The test results shall be all positive.

10.3 Limit of detection: Test three limit of detection reference products L1~L3 respectively. The test result of L1 shall be negative and the test results of L2 and L3 shall be positive. Minimum detection limit concentration: KPC, 0.3ng/mL; NDM, 0.1ng/mL; OXA-48, 0.1ng/mL; IMP, 0.1ng/mL; VIM, 0.3ng/mL.

10.4 Repeatability: Take the same batch of reagents, and continuously repeat the determination of the repeatable reference products in internal reference products 10 times. The test results shall be all positive and the color rendering shall be uniform.

10.5 HOOK effect: When the concentration of the KPC, NDM, OXA-48, IMP and VIM carbapenemases is 1000 ng/mL, there is no HOOK effect. No validation is carried out for higher concentration.

11. Precautions

11.1 This product is for in-vitro diagnosis only. The test result shall not be used as the only index for evaluating the patient's condition, and the patient's clinical manifestation and other laboratory tests must be combined to conduct a comprehensive analysis of the condition.

11.2 Please check whether the product is within the validity period and whether the package is well sealed before use. Read the IFU carefully and perform the test in strict accordance with the requirements indicated therein.

11.3 During sample treatment, pay attention to selecting a single colony to avoid contamination by miscellaneous bacteria.

11.4 The reagent can be stored at room temperature, but it shall be kept away from moisture and shall not be frozen. If it is stored at low temperature, it shall reach room temperature before use.

11.5 The testing shall be carried out as soon as possible after the reagent is taken out of the package. Since if it is left in the air for too long, it will be damaged by moisture.

11.6 Too much sampling volume may lead to sample reflux, resulting in false positive result.

11.7 The test sensitivity cannot be guaranteed if the ambient temperature is lower than 10°C or higher than 40°C or the relative humidity is higher than 80%.

12. References

[1] YU Hua, XU Xuesong, LI Min, et al. Consensus statement on laboratory detection and clinical report of carbapenemases among Enterobacterales [J]. Chinese Journal of Infection and Chemotherapy, 2020(6).

13. Manufacturer

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EC REP

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[Symbols]

Symbol	Interpretation
	Expiry date
LOT	Batch number
	Production date
	Avoid to sunlight
	Manufacturer
	Temperature limit
IVD	In vitro diagnostic medical device
EC REP	European Authorized Representative
CE	CE Mark

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