

EZER™ Carbapenemase Rapid Test

INTENDED USE

The **EZER™** Carbapenemase Rapid Test is an immunochromatographic assay (ICA) for the qualitative detection and differentiation of five common carbapenemases (NDM, KPC, OXA-48-like, IMP and VIM) from carbapenem non-susceptible pure bacterial colonies when grown on the blood agar or MacConkey agar (18-24 hours).

The **EZER™** Carbapenemase Rapid Test is intended as an aid for infection control in the detection of carbapenemase-producing Enterobacterales and *Pseudomonas aeruginosa* in healthcare settings. The **EZER™** Carbapenemase Rapid Test is not intended to guide or monitor treatment for carbapenem non-susceptible bacterial infections. A positive or negative Carbapenemase Rapid Test result does not rule out the presence of other mechanisms of antibiotic resistance. The **EZER™** Carbapenemase Rapid Test should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.

BACKGROUND

Carbapenemase-producing Organisms (CPO), and more specifically, Carbapenem-resistant Enterobacterales (CRE) represent a major public health concern worldwide due to their broad spectrum of resistance to antibiotics including, besides carbapenems, most classes of antimicrobial agents, and thus leaving very few options for the management of infected patients. Besides CREs, CPOs also include nonfermenting Gram-negative bacilli (NFGNB), such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that exhibit resistance not only to beta lactam and other groups of antibiotics, but also to carbapenems. Before the 1940s, most of the resistance to antibiotics was associated with the production of Extended-Spectrum β -Lactamases (ESBLs) belonging to classes A, B, C, and D of Ambler's classification. Since then, studies have shown an increase in the production of carbapenemases among the Enterobacterales family. Among those carbapenemases, the β -lactamase KPC (Class A) has spread worldwide in the 2000s. In addition, the metallo β -lactams (MBL) of IMP type, VIM and NDM (Class B) as well as OXA-48-like (Class D) have also been expanded. These carbapenemases are mainly detected in hospital settings and are responsible for most of the nosocomial infections, raising major global health problems since their presence can be difficult to detect. Carbapenemase Rapid Test is an in vitro rapid and visual multiplex immunochromatographic assay that detects one or more of the five common types of carbapenemase enzymes (NDM (N), KPC (K), OXA-48-like (O), IMP (I), VIM (V)) in bacterial colonies.

PRINCIPLE

The test is ready to use and is based on a membrane technology with colloidal gold nanoparticles. Our kit is aimed to the detection of carbapenemases from a single bacterial colony isolate of Enterobacterales or *Pseudomonas aeruginosa* on agar plate. Each pouch contains: 2 lateral-flow strips for the identification of NDM, KPC, OXA-48-like, IMP and VIM.

Identification of NDM, KPC and OXA-48-like.

Nitrocellulose membrane is sensitized with NDM monoclonal antibody ("N" line), KPC monoclonal antibody ("K" line), OXA-48-like monoclonal antibody ("O" line) and control capture reagent ("C" line).

Monoclonal antibodies that individually recognize each of the three carbapenemases label with colloidal gold and dried on a membrane.

Identification of IMP and VIM.

Nitrocellulose membrane is sensitized with IMP monoclonal antibody ("I" line), VIM monoclonal antibody ("V" line) and control capture reagent ("C" line).

Monoclonal antibodies that individually recognize IMP and VIM label with colloidal gold and dried on a membrane.

When the provided buffer containing the resuspended bacteria comes into contact with the strip, the solubilized antibody-colloidal gold conjugates migrate with the sample by passive diffusion, while conjugates and sample material come into contact with the immobilized respective antibodies that are adsorbed onto the nitrocellulose strip. If the sample contains an NDM, KPC, OXA-48-like, IMP and VIM carbapenemase, the respective complexes made of the conjugates and either carbapenemase will remain bound to their respective specific lines. The migration continues by passive diffusion and both conjugates and sample material come into contact with the (upper) line control reagent that binds a control conjugate ("C" line), thereby producing a red line. The result is visible within 15 minutes in the form of red lines on the strip.

CONTENTS

- Test Device (20 Pcs)
- Extraction Tube with Buffer (20 Pcs)
- Package Insert (1 Pc)

WARNINGS AND PRECAUTIONS

1. For *in vitro* Diagnostic Use.
2. All the operations must be carried out according to good laboratory practices.

- Do not use kit components beyond the expiration date.
- The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
- Please do not touch the sample drop and the judgment part of the test board directly by hand.
- Do not reuse the device.
- The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid as long as a red line is present.
- If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
- After use, discard the device in an infectious waste container.

STORAGE CONDITIONS

Test devices must be stored at 2–30°C. **DO NOT FREEZE.**

CULTURE AND SAMPLING

Samples to be tested should be obtained and handled by standard microbiological procedures.

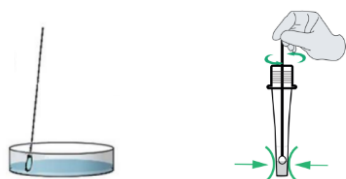
PROCEDURE

Bring the kit components to room temperature.

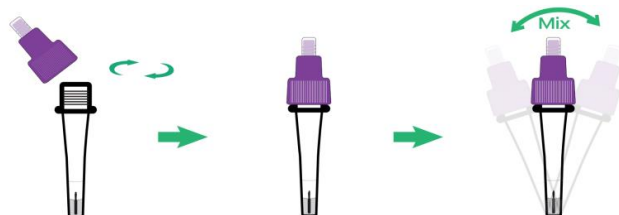
With bacterial colonies, we recommend the use of fresh bacterial colonies for optimal test performance.

1. Sample Extraction

- From the agar culture, touch 3 colonies with a 1 µL loop, and then suspend it in the extraction tube containing the buffer. The entire bacterial colony must be suspended into the buffer.



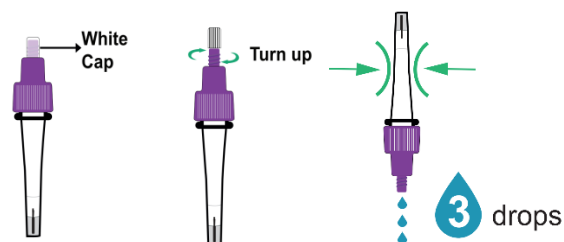
- Screw the cap on the extraction tube.
- Vortex to homogenize the mixture before use.



2. Test Reaction

- Open the pouch, and take out the device. Once opened, use the test immediately.
- Open the top cap of the extraction tube, invert

extraction tube and add 3 drops (80µl) of test sample into the sample wells by gently squeezing extraction tube.

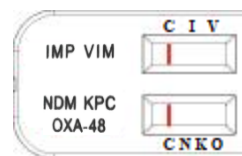


- Read results at 15 minutes and disregard after 30 minutes. A positive result may be visible at 3 minutes. However, the complete reaction time of 15 minutes is required to confirm a negative result.

INTERPRETATION OF RESULTS

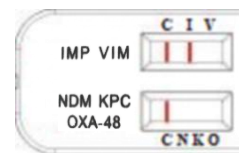
Allow the samples to react according to the procedure and read the red lines that appear in the reading area.

The results are to be interpreted as follows for each of the two strips:



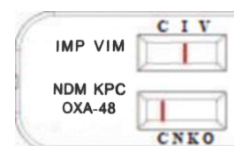
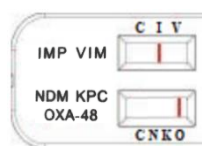
Negative

Negative test result: If a red line appears at the quality control line (C) on both sides of the plate. No other line is present.



Positive

Positive test result: If a red line appears at the quality control line on both sides of the plate and one or more visible red lines appears in the test regions N, K, O, I, V, the sample contains one or several carbapenemase and must be interpreted as a positive result.



Invalid

Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat the test with a new test device.

QUALITY CONTROL

Each EZER™ Carbapenemase Rapid Test contains internal/procedural controls. The appearance of a control line at the Control “C” position validates the proper reagent function and assures that the correct test procedure was followed.

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit was determined using purified recombinant enzymes:

NDM 0.1ng/mL, KPC 0.5ng/mL,
OXA-48-like 0.125ng/mL,
IMP 0.125ng/mL, VIM 0.05ng/mL.

Cross-reactivity

Cross-reactivity confirmed that *EZER*TM Carbapenemase Rapid Test does not react with the organisms that exhibit antibiotic resistance mechanisms other than the target (NDM, KPC, OXA-48-like, IMP and VIM), as described below for specific resistant mechanisms evaluated.

TEM-1, SHV-1, SHV-11, SHV-53, SHV-51, CTX-M-55, CTX-M-65, CTX-M-15, AmpH, aac(6')-Iai, aac(3)-IIId, rmtB, aadA3, aadA2, aadA16, arr-3, gyrA, parC, marA, acrA, acrB, acrD, msbA, mdtB, mdtC, baeR, mdfA, CBP-1, tet(A), oqxA, oqxB, sul1, sul2, dfrA14, dfrA27, qnrS1, qnrB6, qnrA1, PmrF, FosA6, FosA5, fosA3, catII, mcr-1.1, mecA, VanA, mph(A), OmpK37, KpnG, KpnH, KpnE, KpnF.

Clinical Evaluation

Performance of *EZER*TM Carbapenemase Rapid Test was evaluated at hospital with prospectively-collected and stock bacterial isolates. The identification of carbapenemase production on *EZER*TM Carbapenemase Rapid Test was compared to Xpert Carba-R by Cepheid. A total of 265 organisms isolates were tested on *EZER*TM Carbapenemase Rapid Test with concordant results obtained by phenotypic testing paired with Xpert Carba-R results.

		molecular method		
		Positive	Negative	Total
Carbapenemase Rapid Test	Positive	194	0	194
	Negative	0	71	71
	Total	194	71	265

Relative Sensitivity: 100.00%

Relative Specificity: 100.00%

Accuracy: 100.00%

LIMITATIONS OF THE PROCEDURE

1. The performance of *EZER*TM Carbapenemase Rapid Test was established with colonies from blood agar, MacConkey agar. Performance with other culture media has not been evaluated and is therefore unknown.
2. The performance of *EZER*TM Carbapenemase Rapid Test with bacteria other than Enterobacterales and *Pseudomonas aeruginosa* has not been evaluated.
3. A negative result does not preclude the presence of carbapenemase producing organisms (example: SME, GES, IMI).
4. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance.
5. False negative results may occur with multiple

subcultures of a bacterial isolate without any selective pressure.

6. This test is a qualitative assay and will not yield any quantitative result.
7. This test should be used as an aid for the rapid identification of patients bearing a resistance to carbapenem antibiotics.
8. The device is intended for Carbapenemase testing on single colonies only. For positive blood culture samples, please use the *EZER*TM BloodPrime for pre-treatment.

AVAILABILITY

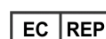
Product	Cat. No.	Contents
<i>EZER</i> TM Carbapenemase Rapid Test	P261102	20 Tests

REFERENCES

1. Brolund A et al. Worsening epidemiological situation of carbapenemase-producing Enterobacterales in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019 Feb; 24 (9) 1560-7917.
2. Boutal H et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacterales. J Antimicrob Chemother. 2018.

Index of Symbols

 Attention, see instructions for use	 Tests per kit	 Authorized Representative
 For in vitro diagnostic use only	 Use by	 Do not reuse
 Store between 2~30°C	 Lot Number	 Catalog #



CMC Medical Devices & Drugs S.L.
C/ Horacio Lengo no 18, CP 29006,
Málaga, Spain



**Hangzhou Genesis
Biodetection and Biocontrol Co., Ltd.**

ADD : NO.139, St.10th (East), Hangzhou Economic &
Technological Development Zone. Hangzhou,
Zhejiang Province, China, 310018

TEL : +86-571-8781-8163

FAX : +86-571-8782-4695

Web : www.genesis-ivd.com