RESIST-5 O.O.K.N.V.

Manufacturer:

Coris BioConcept
Science Park CREALYS
Rue Jean Sonet 4A
B – 5032 GEMBLOUX
BELGIUM
Tel.: +32(0)81.719.917
Fax: +32(0)81.719.919
info@corisbio.com

Produced in BELGIUM

www.corisbio.com
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***In vitro* rapid diagnostic test for the detection of OXA-163, OXA-48, KPC, NDM and VIM carbapenemases in bacterial culture**

FOR IN VITRO DIAGNOSTIC USE ***EN***
FOR PROFESSIONAL USE ONLY
References: K-15R9, 2x20 cassettes, buffer, 20 tubes and droppers

I. INTRODUCTION

Carbapenemase-producing Organisms (CPO), and more specifically, Carbapenem-resistant Enterobacteriaceae (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 non fermenting 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. The rapid spread of CPOs and genes encoding these resistances has led to nosocomial outbreaks and endemic situations in several countries in Europe as well as elsewhere worldwide.
Development of new rapid diagnostic tests to track antimicrobial resistance patterns is considered as one of the priority core actions by international experts and health authorities. NDM and KPC represent two of the most increasing and prevalent carbapenemases in many countries. On the other hand, class D OXA-48 type carbapenemases are the most challenging resistance mechanisms to be detected by clinical laboratories. Particularly, the OXA-163 variant is a difficult-to-identify enzyme. Indeed, although OXA-163 shows weaker carbapenemase activity as compared to OXA-48, it also shows an increased activity of Expanded-spectrum Cephalosporins (ESC), which represent another challenge for rapid identification. VIM is not only present in Enterobacteriaceae but is also highly prevalent in non-fermenting bacteria. Rapid identification of those carbapenemases is of upmost importance to improve patient therapy and control of the spread of antibiotic resistance in hospitals.
Confirmatory phenotyping tests using combination disks with specific inhibitors already exist for detection of selected types of carbapenemases including class A (KPC) and class B (VIM, IMP, NDM) carbapenemases; however, these tests are time-consuming and require an extra additional day following antimicrobial susceptibility testing results. Moreover, phenotypic colourimetric assays are often not sensitive enough for the detection of low-activity carbapenemases such as OXA-48 and closely-related variants (so-called "OXA-48-like") and for the OXA-163 sub-family which exhibits very low carbapenemase activity. Several molecular and gene sequencing assays also allow detection of carbapenemases, especially for definitive confirmation of OXA-48 and OXA-163. These tests are expensive, time-consuming and can only be performed in a dedicated environment and by skilled laboratory staff, hence limiting their generalised use.

II. PRINCIPLE OF THE TESTS

These tests are ready to use and are 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 Enterobacteriaceae or NFGNB growing on agar plate. Each pouch contains: 2 lateral-flow cassettes for the identification of (i) KPC, OXA-163 and OXA-48 and (ii) NDM and VIM. These two devices are aimed at the detection of KPC, OXA-163, OXA-48, NDM and VIM carbapenemases from colonies of bacterial isolates growing on an agar plate and resuspended in the provided buffer.
Identification of KPC OXA-163 and OXA-48. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against KPC carbapenemases (“KPC” line)
(2) a monoclonal antibody directed against a first epitope of OXA-48 carbapenemases and variants (but not the OXA-163 variant) (“48” line)
(3) a monoclonal antibody directed against a second epitope of OXA-48 carbapenemases and variants including OXA-163 (“163” line)
(3) a control capture reagent (upper “C” line).
Three different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against a second epitope of KPC carbapenemases, a conjugate directed against a third epitope of OXA-48 carbapenemases and variants including OXA-163 and a control conjugate.
Identification of NDM and VIM. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against NDM carbapenemases (bottom “N” line),
(2) a monoclonal antibody directed against VIM carbapenemases (middle “V” line),
(3) a control capture reagent (upper “C” line).
Three different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against NDM carbapenemases, a conjugate directed against VIM carbapenemases, and a control conjugate.
When the provided buffer containing the resuspended bacteria comes into contact with the strip, the solubilised conjugates migrate with the sample by passive diffusion, while conjugates and sample material come into contact with the immobilised respective antibodies that are adsorbed onto the nitrocellulose strip. If the sample contains a KPC, OXA-163, OXA-48, NDM or VIM carbapenemase, the respective complexes made of the conjugates and either KPC, or OXA-163, or OXA-48, or NDM or VIM will remain bound to their respective specific lines (KPC : “KPC” line; OXA-48 : “48” line, OXA-163* : “163” line ; NDM : “N” line, VIM : “V” line). 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.
*As the second conjugate is directed against all OXA-163 and all OXA-48 variants, if the sample contains a very high amount of OXA-48, the second line (labelled as "48" on the cassette) will be strongly positive; whereas the third line (labelled as "163" on the cassette) may show a faint signal because some OXA-48 in excess would not be captured by the specific OXA-48 line (second line) but by the OXA-163 line (third line).
The result is visible within 15 minutes in the form of red lines on the strip.

III. REAGENTS AND MATERIALS

1. RESIST-5 O.O.K.N.V. (2x20 cassettes)
20 sealed pouches containing two lateral-flow cassettes and one desiccant.
Each device contains one sensitised strip.
2. LY-A buffer vial (15 mL)
Saline solution buffered to pH 7.5 containing TRIS, NaN_3 (<0.1%) and a detergent.
3. Instruction for use (1)
4. Semi-rigid disposable collection tubes with droppers (20)

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- The quality of the reagents cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.

V. WASTE DISPOSAL

- Dispose of gloves, swabs, test tubes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.
- Avoid freezing devices and buffer.

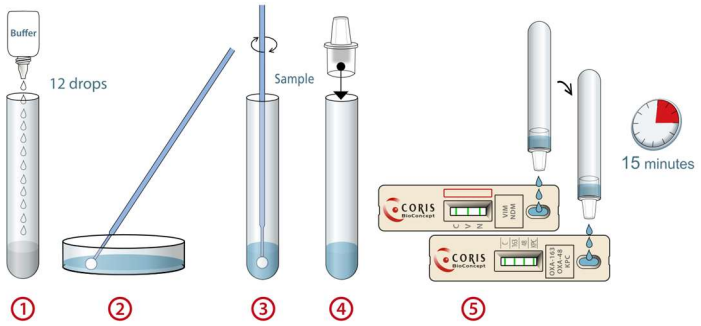
VII. SPECIMEN HANDLING AND COLLECTION

Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIT kits are listed on the website: https://www.corisbio.com/Products/Human-Field/RESIST-5-OOKNV.php

VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens (in the event that the plate containing colony to be tested was kept at 4°C) to equilibrate at room temperature (15-30°C) before performing a test.
Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient’s name or specimen number on the device (one device per sample).
SPECIMEN PREPARATION PROCEDURE:
Performance claims with regard to sample types other than bacterial colonies have not been established. We recommend the use of fresh bacterial colonies for optimal test performance.

1. Prepare one semi-rigid tube and add **12** drops of LY-A buffer in the tube.
2. Harvest bacteria by taking one colony with a disposable bacteriological loop and dip the loop in the bottom of the semi-rigid tube containing the buffer.
3. Stir thoroughly before removing the loop
4. Insert tightly the dropper on the semi-rigid tube.
5. Vortex the preparation to homogenize. The entire bacterial colony must be suspended into the buffer.
6. Invert the test tube and add slowly **3** drops of diluted sample into the sample well of each of the two cassettes labelled (i) KPC, OXA-48 and OXA-163 and (ii) NDM and VIM. Alternatively, add 100µl with a micropipette to both cassettes
7. Allow to react for 15 min max and read the result.

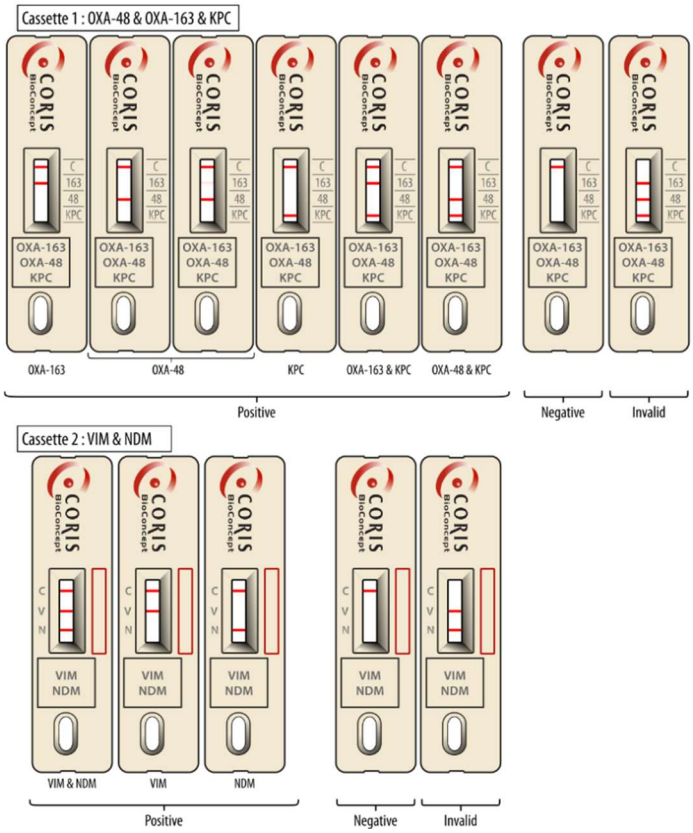


Positive results may be reported as soon as the test and control lines become visible.
Do not take the appearance of new lines into account after the reaction time has passed.
The result must be read on still wet strip.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows for each of the two cassettes:
Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other line is present.
Positive test result: in addition to a reddish-purple line at the Control line (C), a visible reddish-purple line appears at one of the Test lines position (“KPC” or ”163” or “48”) on cassette labelled (i) KPC, OXA-48, OXA-163, or at one of the Test lines position (“V” or “N”) on cassette labelled (ii) NDM and VIM. Intensity of the test line may vary according to the quantity of antigens as well as of the variant type present in the sample. Any reddish-purple test line (KPC, OXA-48, OXA-163 NDM and VIM), even weak, should be considered as a positive result.
If a positive test line appears beside of the “KPC” mark, the sample contains KPC variants. If it appears beside the “48” mark, the sample contains OXA-48 or OXA-48-like variants. If it is beside the “163” mark, the sample contains OXA-163 variant or closely related variants as OXA-247, 405 or 438; beside the “N” mark, the sample contains NDM; and beside of the “V” mark, VIM is present in the sample. Combinations of positive test lines can occur.
In this case the sample contains the combination of several carbapenemases.
Nevertheless, in case of a highly positive OXA-48 test line, a faint signal may appear on the OXA-163 test line. In this case, the test should be interpreted as an OXA-48 positive and OXA-163 negative result.
Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.

Note: during the drying process, a very faint shadow may appear in the Test line positions. It should not be regarded as a positive result.



X. PERFORMANCE

A. Detection Limit

The detection limit determined with purified recombinant proteins of OXA-48, OXA-163, KPC, NDM and VIM have been evaluated at 0.125 ng/mL, 0.49 ng/mL, 0.625 ng/mL, 0.25 ng/mL and 0.23 ng/mlL, respectively.

B. Prospective study (based on RESIST-3 O.K.N. *K*-SeT kit)

The OXA-48 and KPC cassette test was validated by comparison with a reference molecular method (validated multiplex PCR, including sequencing) in the National Reference Laboratory for Multidrug-Resistant Gram Negative Bacilli (Belgium) in a prospective study performed on 173 non duplicated, consecutive suspected CPE clinical isolates referred from July to September 2016.

OXA-48 test	Positive	Negative	Total
Positive	69	0	69
Negative	0	104	104
Total	69	104	173

		95 % Confidence Interval [1]
Sensitivity:	100 %	(95.7 to 100 %)
Specificity:	100 %	(97.2 to 100 %)
Positive Predictive value:	100 %	(95.7 to 100 %)
Negative predictive value:	100 %	(97.2 to 100 %)
Agreement:	100 %	(173/173)

KPC test	Positive	Negative	Total
Positive	9	0	9
Negative	0	164	164
Total	9	164	173

		95 % Confidence Interval [1]
Sensitivity:	100 %	(68.4 to 100 %)
Specificity:	100 %	(98.2 to 100 %)
Positive Predictive value:	100 %	(68.4 to 100 %)
Negative predictive value:	100 %	(98.2 to 100 %)
Agreement:	100 %	(173/173)

C. Validation on collection of reference strains

The OXA-48 and OXA-163-type Carbapenemases of *K*-SeT test was evaluated on a collection of 75 fully-characterised clinical strains in the National Reference Laboratory for antimicrobials (Argentina).

75 strains	**50 strains** tested positive for OXA-48 and OXA-163	**17 strains** carrying OXA-48 and OXA-48 –like type Carbapenemase	OXA-48, OXA-162, OXA-181, OXA-232, OXA-244 from *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae*
		33 strains carrying OXA-163 variants of Carbapenemase	OXA-163, OXA-247, OXA-438 from *Citrobacter freundii, Enterobacter cloacae, Providencia stuartii, Enterobacter kobei, Escherichia coli, Klebsiella ozoneae, Klebsiella pneumoniae, Kluyvera georgiana*
	25 strains tested negative for OXA-48 and OXA-163	**14 strains** carrying a non-OXA-48 Carbapenemase	GES-5, IMP-8, KPC-2, KPC-3, NDM-1, VIM-1, VIM-2, SPM-1, OXA-23, OXA-58, OXA-72, OXA-143, Sme, IMI
		11 strains nonproducers of Carbapenemase	Including OXA-1, -3, -4, -5, -6, -7, -9, CMY-2, GES-1 + OXA-2, AmpC + porins, CTX-M + porins

D. Retrospective study

The VIM and NDM cassette test was validated by comparison with a reference molecular method in the National Reference Laboratory for Multidrug-Resistant Gram Negative *Bacilli* (Belgium) in a retrospective study on a collection of reference strains.

NDM test	Positive	Negative	Total
Positive	24	0	24
Negative	0	95	95
Total	24	95	119

		95 % Confidence Interval [1]
Sensitivity:	100 %	(82.8 to 100 %)
Specificity:	100 %	(95.2 to 100 %)
Positive Predictive value:	100 %	(82.8 to 100 %)
Negative predictive value:	100 %	(95.2 to 100 %)
Agreement:	100 %	(119/119)

VIM test	Positive	Negative	Total
Positive	38	0	38
Negative	1*	80	81
Total	39	80	119

*: the false-negative result is a ***P. aeruginosa*** colony harbouring VIM-5 and NDM-1 genes. This colony was detected as NDM-positive but VIM-negative. The production of VIM-5 was not assessed.

		95 % Confidence Interval [1]
Sensitivity:	97.4 %	(84.9 to 99.9 %)
Specificity:	100 %	(94.3 to 100 %)
Positive Predictive value:	100 %	(88.6 to 100 %)
Negative predictive value:	98.8 %	(92.4 to 99.9 %)
Agreement:	99.2 %	(118/119)

E. Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.

To check inter-batch accuracy (reproducibility), some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

XI. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis. A positive test does not rule out the possibility that other antibiotic resistance mechanisms may be present.

XII. TECHNICAL PROBLEMS / COMPLAINTS

If you face a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the lot number of the kit concerned.
2. If possible, keep the sample in the appropriate storage condition during the complaint management.
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor.

XIII. BIBLIOGRAPHIC REFERENCES

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Last update 07 AUGUST 2019

REF	Catalogue number		Manufacturer
IVD	In vitro diagnostic medical device		Temperature limits
Σ	Contains sufficient for <n> tests	LOT	Batch code
	Consult instructions for use		Do not reuse
	Keep dry		Use by
DIL SPE	Diluent specimen	CONT NaN_3	Contains Sodium azide

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).