RESIST ACINETO

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In vitro rapid diagnostic test for the detection of OXA-23, OXA-40, OXA-58 and NDM carbapenemases in bacterial culture

FOR IN VITRO DIAGNOSTIC USE **EN**
FOR PROFESSIONAL USE ONLY
References: K-15R13, 20 cassettes, buffer, 20 tubes and transfer pipets

I. INTRODUCTION

Acinetobacter baumannii is an important opportunistic and multidrug-resistant Gram-negative bacteria responsible for nosocomial infections in health facilities. If left untreated, this infection can lead to septicemia and death. The carbapenem-hydrolysing oxacillinases (OXAs) are the most commonly reported carbapenem-resistance determinants in *Acinetobacter* spp., particularly in *A. baumannii,* with OXA-23 as the most prevalent carbapenem-resistance determinant observed in *these* isolates. OXA-40 (=24) and OXA-58 are also often encountered while other OXAs are less frequent. Recently *Acinetobacter spp.* harbouring OXA's together with NDM have emerged, particularly because of mobile genetic elements co-harbouring NDM and OXA encoding genes. Mobile genetic elements (incl. plasmids) constitute reservoirs for horizontal transmission of these resistance factors. Detection of these resistance factors, not only in resistant species but also in carrier species, is therefore of paramount importance in the control of antibiotic resistance in the hospital.
Nowadays, definitive confirmation of OXA-23, OXA-40, OXA-58, other less frequent OXAs and NDM relies on molecular amplification analysis and DNA sequencing. These tests are expensive and can only be performed in dedicated environment and by skilled staff, hence limiting their more generalized usage.
The 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.
The RESIST ACINETO test aiming at a rapid identification of the OXA-23, OXA-40/58 and related OXAs and NDM carbapenemases ensures effective treatment of patients and prevention of spread of carbapenemases *Acinetobacter* spp. carrier, especially in hospitals.

II. PRINCIPLE OF THE TEST

This test is ready to use and is based on a membrane technology with colloidal gold nanoparticles. Our kit is aimed to detect and identify the carbapenemases from bacterial colony isolate of *Enterobacteriaceae* or NFGNB growing on agar plate.
A nitrocellulose membrane is sensitized with:
(1) a monoclonal antibody directed against NDM carbapenemase ("NDM" line)
(2) a monoclonal antibody directed against OXA-23 carbapenemase ("O23" line)
(3) a monoclonal antibody directed against OXA-40 and OXA-58 carbapenemases ("O40/58" line)
(4) a control capture reagent (upper "C" line).
There are different conjugates coupled to colloidal gold particles which are dried on a membrane: a conjugate directed against a second epitope of the NDM carbapenemase, a conjugate directed against a second epitope of the OXA-23 carbapenemase, a third conjugate specific to OXA-40 carbapenemase, a fourth conjugate specific to OXA-58 carbapenemase and a control conjugate to valid the test conditions.
This test is aimed at the detection of NDM, OXA-23, OXA-40 and OXA-58 carbapenemases (and related variants) on colonies of *Enterobacteriaceae* isolates growing on agar plate.
When the provided buffer containing the resuspended bacteria comes into contact with the membrane, 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 an NDM, OXA-23, OXA-40 or OXA-58 carbapenemase, the respective complexes made of the conjugates and their specific targets will remain bound to their respective specific lines (NDM: "NDM" line; OXA-23: "O23" line; OXA-40 or OXA-58: "O40/58" 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.
The result is visible within 15 minutes in the form of red lines on the strip.

III. REAGENTS AND MATERIALS

1. RESIST ACINETO (20)
20 sealed pouches containing one device and one desiccant. Each device contains one sensitized strip.
2. LY-D buffer vial (15 mL)
Tris-EDTA solution containing NaN3 (<0.1%) and a detergent.
3. Instruction for use (1)
4. Disposable collection tubes (20)
5. Disposable transfer pipettes (20)

Materials to be ordered separately:
- RESIST-BC (S-1001): reagents kit for use of blood culture
- ReSCape (S-1002): reagents kits for use of rectal swab

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with good laboratory practices.
- 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 or blue lines indicate immunoreagents adsorption sites. Green or blue colour disappears during the test.
- Reagents' quality 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 good laboratory practices.
- 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, perform the test immediately.
- Avoid freezing devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

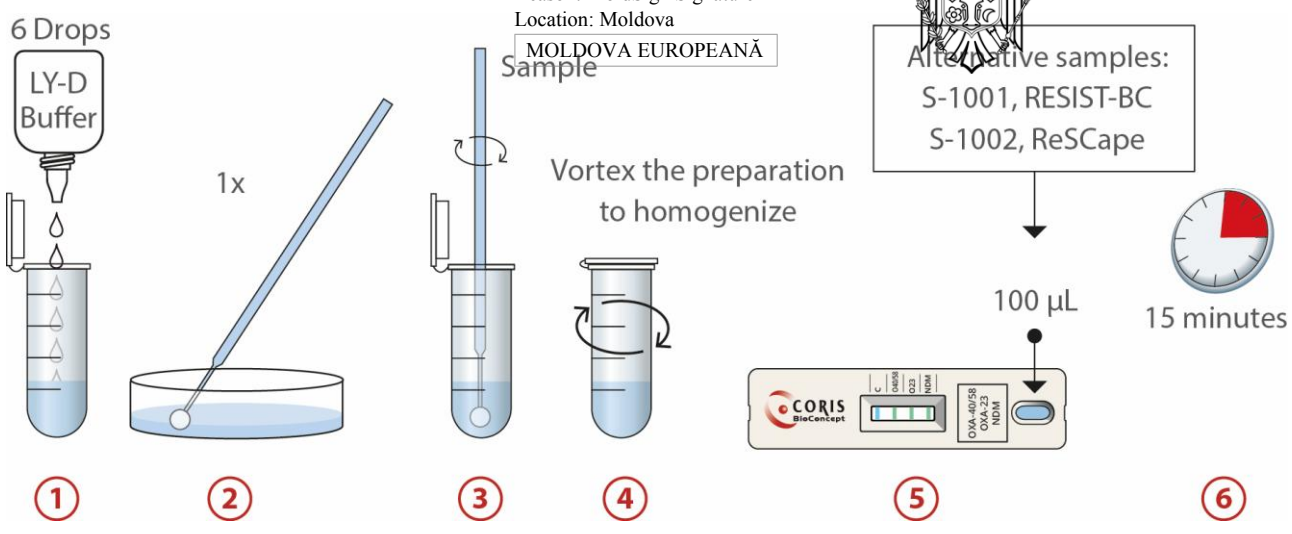
Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIT kits are listed on the website: https://www.corisbio.com/products/oknvi-resist-5/faq

VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens (in case the plate containing colony to be tested was kept at 4°C) to reach room temperature (15-30°C) before performing a test.
Open the pouch and remove the device. Once opened, perform 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 been established for rectal swabs and blood cultures.
With rectal swabs and blood cultures, the preparation procedure to be followed is that described in the respective kits (S-1002, ReSCape; S-1001, RESIST-BC).
With bacterial colonies, we recommend the use of fresh agar cultures for optimal test performance and as followed:

1. Prepare one tube and add **6 drops** of LY-D buffer in the tube.
2. Harvest bacteria by taking **1 colony** with a disposable bacteriological loop and dip the loop in the bottom of the collection tube containing the buffer.
3. Stir thoroughly before removing the loop.
4. Close the tube and vortex the preparation to homogenize.
5. Use transfer pipette and add 100 µL of diluted sample into the sample well of the cassette labelled NDM, OXA-23 and OXA-40/58 (**diluted sample must reach the black line indicated on the transfer pipette to accurately aspirate 100 µL**).
6. Allow to incubate for 15 min max and read the result.

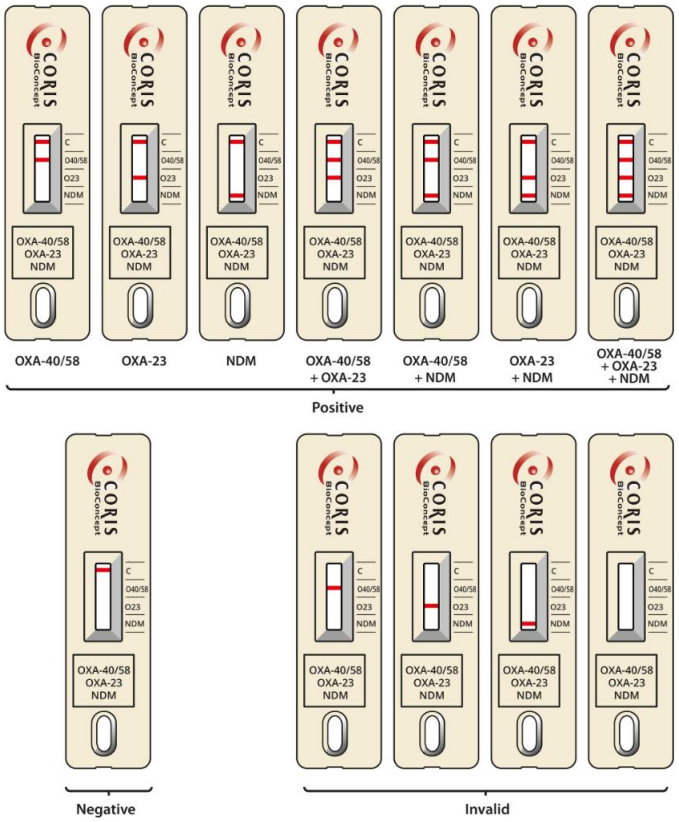


Positive results may be reported sooner the moment the test and control lines become visible.

Do not take the appearance of new lines into account after the reaction time is passed.

The result must be read on still wet strip.

IX. INTERPRETING RESULTS



The results are to be interpreted as follows:

Negative test result: a reddish-purple line appears across the central reading window at the Control line ("C") position. No other band 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 ("NDM" or "O23" or "O40/58") on cassette.

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 ("NDM", "O23" and "O40/58"), even weak, should be considered as a positive result.

If a positive test line appears beside of the "O40/58" mark, the sample contains OXA-40 or OXA-58 variants*. If it appears beside the "O23" mark, the sample contains OXA-23; beside the "NDM" mark, the sample contains NDM. Combinations of positive test lines can occur. In this case the sample contains several carbapenemases.

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 at the Test line positions. It should not be regarded as a positive result.

The RESIST ACINETO test can generate positive results on the OXA-40/58 line for other related families of OXA in *A. baumanii* as well as non-baumannii *Acinetobacter* species, a.o. *A. pittii*. This has been documented for OXA-143 or related variants (OXA-255, OXA-499 and like) or OXA-213. This positive signal will depend on homology between these OXA and the OXA-40 or OXA-58 and on the expression level in the strain. It is important to identify *Acinetobacter* at the species level in parallel of carrying out the RESIST ACINETO test and to perform antibiogram.Confirming an Acinetobacter species is important since OXA-23 and OXA-58 may be (rarely) present in *P. mirabilis* as well as in *E. coli*, without any therapeutic involvement.

X. PERFORMANCE

A. Detection Limit

The detection limit determined with purified recombinant proteins of OXA-23, OXA-40, OXA-58 and NDM have been evaluated at 1.5 ng/ml, 0.099 ng/ml, 0.104 ng/ml and 0.077 ng/ml respectively.

B. Validation on collection of reference strains

The RESIST ACINETO test was evaluated on a collection of 297 clinical isolates with fully characterized resistance mechanisms to beta-lactams antibiotics by phenotypic and molecular tests (Germany).

OXA-23 status / RESIST ACINETO	Positive	Negative	Total
Positive	189	0	189
Negative	2	106	108
Total	191	106	297

95 % Confidence Interval [H]

Sensitivity:	99 %	(95.9 to 99.8 %)
Specificity:	100 %	(95.6 to 100 %)
Positive Predictive value:	100 %	(97.5 to 100 %)
Negative predictive value:	98.1 %	(92.8 to 99.7 %)
Agreement:	99.3 %	(295/297)

OXA-40/58 status / RESIST ACINETO	Positive	Negative	Total
Positive	100	3	103
Negative	0	194	194
Total	100	197	297

95 % Confidence Interval [H]

Sensitivity:	100 %	(95.4 to 100 %)
Specificity:	98.5 %	(95.3 to 99.6 %)
Positive Predictive value:	97.1 %	(91.1 to 99.2 %)
Negative predictive value:	100 %	(97.6 to 100 %)
Agreement:	99 %	(294/297)

NDM status / RESIST ACINETO	Positive	Negative	Total
Positive	13	0	13
Negative	0	284	284
Total	13	284	297

95 % Confidence Interval [H]

Sensitivity:	100 %	(71.7 to 100 %)
Specificity:	100 %	(98.3 to 100 %)
Positive Predictive value:	100 %	(71.7 to 100 %)
Negative predictive value:	100 %	(98.3 to 100 %)
Agreement:	100 %	(297/297)

The RESIST ACINETO test was also validated with rectal swabs and blood cultures.

C. 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.

D. Variants Detected by the RESIST test

A summary of variants reported in publications as detected is listed in an FAQ on the Coris BioConcept website https://www.corisbio.com/faq/

This list is not exhaustive of all the enzymatic variants that can be detected by the RESIST test.

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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

XIII. BIBLIOGRAPHIC REFERENCES

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Last update: 03 APRIL 2025

REF	Catalogue number		Manufacturer
IVD	In vitro diagnostic medical device		Temperature limits
	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
UDI	Unique device identifier		