# OXA-23 *K*-SeT

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
**IFU-58R7/EN/02**

***In vitro* rapid diagnostic test for the detection of OXA-23 carbapenemase in bacterial culture**

***FOR IN VITRO DIAGNOSTIC USE*** **EN**
**FOR PROFESSIONAL USE ONLY**
**References: K-15R7, 20 cassettes, buffer, 20 tubes and droppers**

## I. INTRODUCTION

*Acinetobacter baumannii* is an important opportunistic and multidrug-resistant Gram-negative bacterium 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*. Among the OXAs, OXA-23 is the most prevalent carbapenem-resistance determinant in *A. baumannii* isolates.
OXA-23 has been detected in other bacterial species as chromosomal (*P. mirabilis,* Bonnet et al 2002 and Osterblad et al 2016; *A. radioresistans*) or plasmidic gene (*E. coli*, La et al, 2014), which can constitute reservoirs for horizontal transmission of this resistance factor (Poirel et al 2016). The detection of this resistance factor OXA-23, 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 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 action by international experts and health authorities.
The OXA-23 K-SeT test aimed at a rapid identification of the OXA-23 carbapenemase (and variants of the OXA-23 group) ensures effective treatment of patients and prevention of spread of OXA-23 *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. A nitrocellulose membrane is sensitized with a monoclonal antibody directed against one epitope of the OXA-23 carbapenemase. Another monoclonal antibody directed against a second epitope of the OXA-23 carbapenemase is conjugated to colloidal gold particles. This conjugate is dried on a membrane.
This test is aimed at the detection of OXA-23 like carbapenemases in a single bacterial colony growing on agar plate. The sample must be diluted in the dilution buffer supplied with the test. When the provided buffer containing the resuspended bacteria comes into contact with the strip, the solubilized conjugate migrates with the sample by passive diffusion and both the conjugate and sample material come into contact with the anti-OXA-23 antibody that it is adsorbed onto the nitrocellulose strip. If the sample contains the OXA-23 carbapenemase, the conjugate–OXA-23 complex will remain bound to the anti-OXA-23 antibody adsorbed onto the nitrocellulose and a red line will develop. Solution continues to migrate to reach a second reagent (control reagent) that binds the migration control conjugate, thereby producing a red control line that confirms that the test is valid. Result is visible within 15 minutes.

## III. REAGENTS AND MATERIALS

1. **OXA-23 *K*-SeT (20)**

20 sealed pouches containing one device and one desiccant. Each device contains one sensitized 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)**
5.

## 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.
- 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 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.

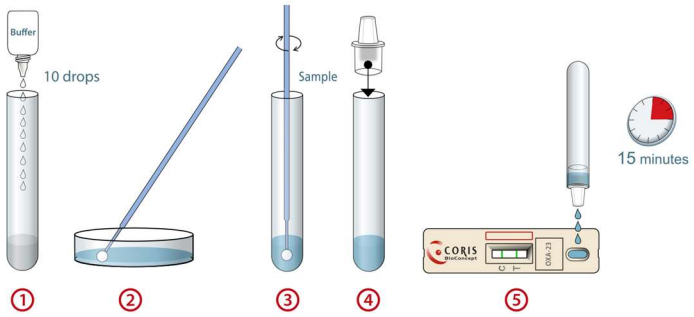
## VII. SPECIMEN HANDLING AND COLLECTION

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/OXA-23/FAQ.php

## 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, run the test immediately. Indicate the patient's name or specimen number on the device (one device per sample).
SPECIMEN PREPARATION PROCEDURE:
We recommend the use of fresh bacterial colonies for optimal test performance.

1. Prepare one semi-rigid tube provided in the kit and add **10** 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 the cassette. Alternatively, add 100µl with a micropipette into the sample well of the cassette.
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 is passed.**
**The result must be read on still wet strip.**

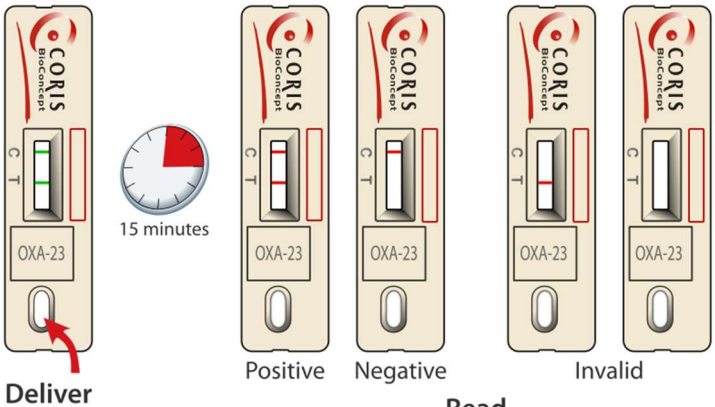
## IX. INTERPRETING RESULTS

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 band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the test line may vary according to the quantity of antigens present in the sample. Any reddish-purple line (T), even weak, should be considered as a positive 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 at the Test line position. It should not be regarded as a positive result.

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## X. PERFORMANCE

### A. Detection Limit

The detection limit was determined with a purified recombinant OXA-23 protein and has been evaluated at 0,156 ng/mL.

### B. Validation on collection of reference strains

The OXA-23 *K*-SeT was evaluated on a collection of 108 clinical isolates of carbapenem-resistant *Acinetobacter* spp. fully characterized resistance mechanisms to beta-lactams by phenotypic and molecular tests (Germany).

| | | | |
|---|---|---|---|
| **108 strains** | **35 strains** tested positive with the OXA-23 *K*-SeT | **35 strains** carrying OXA-23 carbapenemase | *Acinetobacter baumannii, Acinetobacter pittii, Acinetobacter nosocomialis, Acinetobacter radioresistens* |
| | **73 strains** tested negative with the OXA-23 *K*-SeT | **68 strains** carrying a non-OXA-23 carbapenemase | OXA-40, OXA-51, OXA-58, OXA-143, OXA-235 |
| | | **5 strains** carrying class B carbapenemases | Including VIM-2, NDM-1, NDM-2 |

A second evaluation was retrospectively performed on 448 clinical strains of *Acinetobacter spp*. and 14 oxacillinase-producing Gram-negative bacteria collected in Belgium and in Italy between 2008 and 2018 with an agreement of 100 % versus real-time PCR and molecular sequencing. see Riccobono, 2019

| | Italy | Belgium | Total | Test OXA-23 K-SeT |
|---|---|---|---|---|
| $bla_{OXA\text{-}23\text{-}like}$ | 170 | 137 | 307 | 307 + |
| $bla_{OXA\text{-}24\text{-}like}$ | 5 | 25 | 30 | negative |
| $bla_{OXA\text{-}58\text{-}like}$ | 1 | 30 | 31 | negative |
| IS*Aba1* $bla_{OXA\text{-}51\text{-}like}$ | 11 | 0 | 11 | negative |
| $bla_{OXA\text{-}23\text{-}like}$ + $bla_{OXA\text{-}58\text{-}like}$ | 5 | 2 | 7 | 7 + |
| $bla_{OXA\text{-}23\text{-}like}$ + IS*Aba1* $bla_{OXA\text{-}51\text{-}like}$ | 4 | 0 | 4 | 4 + |
| $bla_{OXA\text{-}23\text{-}like}$ + $bla_{NDM}$ | 0 | 3 | 3 | 3 + |
| $bla_{OXA\text{-}58\text{-}like}$ + $bla_{VIM}$ | 0 | 1 | 1 | negative |
| $bla_{NDM}$ | 0 | 13 | 13 | negative |
| $bla_{OXA\text{-}143\text{-}like}$ | 0 | 1 | 1 | negative |
| $bla_{IMP}$ | 0 | 3 | 3 | negative |
| $bla_{VIM}$ | 0 | 1 | 1 | negative |
| $bla_{GES}$ | 0 | 1 | 1 | negative |
| $bla_{OXA\text{-}48\text{-}like}$ | 0 | 2 | 2 | negative |
| $bla_{OXA\text{-}198\text{-}like}$ | 0 | 1 | 1 | negative |
| *non-carbapenemase producer* | 0 | 46 | 46 | negative |
| **Total** | 196 | 266 | 462 | 321 + |

### 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.

## 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 encounter a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the kit batch number
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


A. **E. Riccobono, P. Bogaerts, A. Antonelli, S. Evrard, T. Giani, G. M. Rossolini and Y. Glupczynski.** Evaluation of the OXA-23 K-SeT immunochromatographic assay for the rapid detection of OXA-23-like carbapenemase-producing Acinetobacter spp. **J Antimicrob Chemother. 2019 Jan 25. doi: 10.1093**

B. **DW. Wareham, LM. Phee, MHF. Abdul Momin.** Direct detection of carbapenem resistance determinants in clinical specimens using immunochromatographic lateral flow devices. J Antimicrob Chemother. 2018 Mar 22. doi: 10.1093

C. **A. Saleh, S. Göttig and A. Hamprecht.** Multiplex immunochromatographic detection of OXA-48, KPC and NDM carbapenemases: impact of the inoculum, antibiotics and agar. J Clin Microbiol. 2018 Feb 14. pii: JCM.00050-18.

D. **G. L. Vanstone, S. Woodhead, K. Roulston, H. Sharma, E. Wey, E. R. Smith, D. Mack and I. Balakrishna.** Improving the detection of carbapenemase-producing organisms (CPO) in a low-prevalence setting: evaluation of four commercial methods and implementation of an algorithm of testing. Journal of Medical Microbiology 2018;67: 208–214

E. **P. Bogaerts, S. Evrard, L. Denorme, Q. Gilleman, P. Mertens, T.-D. Huang, and Y. Glupczynski**. Evaluation of a new lateral flow assay for the detection of VIM-producing bacteria. 37th RICAI, Paris, December, 18-19, 2017, Abstract # P066.

F. **Y. Glupczynski, A. Jousset, S. Evrard, R. Bonnin, T. Huang, L. Dortet, P. Bogaerts and T. Naas.** Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48-like, KPC and NDM carbapenemases. J Antimicrob Chemother. 2017 Jul 1 ;72(7):1955-1960

G. **CS. Nodari, AC. Gales, AL. Barth, CM. Magagnin, AP. Zavascki, CG. Carvalhaes.** Detection of OXA-370 Directly from Rectal Swabs and Blood Culture Vials Using an Immunochromatographic Assay. J Microbiol Methods. 2017 May 5. pii: S0167-7012 : 30113-6

H. **Y. Glupczynski, A. Jousset, S. Evrard, R. Bonnin, TD Huang, L. Dortet, P. Bogaerts and T. Naas.** Evaluation of a new multiplex immunochromatographic assay OKN K-SeT for the rapid detection of OXA-48, KPC and NDM carbapenemases from cultured bacteria. 27th European Congress of Clinical Microbiology and Infectious Diseases, Infectious Diseases April 22 – 25, 2017

I. **C. Trouvé, R. De Smedt and E. De Laer.** Evaluation of five commercial confirmation tests for Carbapenemase-Producing Enterobacteriaceaein an OXA-48 endemic geographic region. 27th European Congress of Clinical Microbiology and Infectious Diseases, Infectious Diseases April 22 – 25, 2017

J. **E. Riccobono, A. Antonelli, P. Pecile, P. Bogaerts, MM. D'Andrea, GM. Rossolini.** Evaluation of the KPC K-SeTVR immunochromatographic assay for the rapid detection of KPC carbapenemase producers from positive blood cultures. J Antimicrob Chemother. 2017 Nov 8. doi: 10.109

K. **AC. Ramos, AC. Gales, J. Monteiro, S. Silbert, T. Chagas-Neto, AMO. Machado, CG. Carvalhaes.** Evaluation of a rapid immunochromatographic test for detection of distinct variants of Klebsiella pneumoniae carbapenemase (KPC) in Enterobacteriaceae. J Microbiol Methods. 2017 Nov;142:1-3

L. **F. Erdem, A. Abulaila, Z. Aktas, O. Oncul.** Comparison of the Novel Oxa-48 and Kpc K-SeT Assay, and Blue-Carba Test for the Detection of Carbapenemase-Producing Enterobacteriaceae Using PCR as a Reference Method. Clin Lab. 2017 Mar 1;63(3):515-522.

M. **DW Wareham and MH Abdul Momin.** Rapid Detection of Carbapenemases in Enterobacteriaceae: Evaluation of the RESIST-3 O.K.N (OXA-48, KPC, NDM) Multiplexed Lateral Flow Assay. J Clin Microbiol. 2017 Feb 1. pii: JCM.02471-16

N. **E Rubio, Y Zboromyrska, C Pitart, I Campo, I Alejo-Cancho, A Fasanella, A Vergara, F Marco, J Vila.** Evaluation of a rapid immunochromatographic test for the detection of OXA-48 carbapenemase. Diagn Microbiol Infect Dis. 2017 Mar;87(3):266-267

O. **F Koroska, S Göttig, M Kaase, J Steinmann, S Gatermann, J Sommer, T Wille, G Plum, A Hamprecht.** Comparison of phenotypic tests and an immunochromatographic assay and development of a new algorithm for OXA-48-like detection. J Clin Microbiol. 2016 Dec 28. Pii: JCM.01929-16

P. **F Pasteran, L Denorme, I Ote, S Gomez, D De Belder, Y Glupczynski, P Bogaerts, B Ghiglione, P Power, P Mertens, A Corso.** Rapid identification of OXA-48 and OXA-163 subfamily in carbapenem resistant gram-negative bacilli with a novel immunochromatographic lateral flow assay. J Clin Microbiol. 2016 Aug; 54(11):2832-2836

Q. **D. Meunier, A. Vickers, R. Pike, R.L. Hill, N. Woodford and K.L. Hopkins**. Evaluation of the K-SeT R.E.S.I.S.T. immunochromatographic assay for the rapid detection of KPC and OXA-48-like carbapenemases. J Antimicrob Chemother. 2016 Aug; 71 (8):2357-9

R. **Wareham DW, Shah R, Betts JW, Phee LM, Abdul Momin MH.** Evaluation of an Immunochromatographic Lateral Flow Assay (OXA-48 K-SeT) for the Rapid Detection of OXA-48-like Carbapenemases in Enterobacteriaceae. . J Clin Microbiol. 2016 Feb;54 (2):471-3

S. **Fernández J, Fleites A, Rodcio MR, Vazquez F.** Evaluation of OXA-48 K-Se T: an immunochromatographic assay for rapid detection of OXA-48-producing Enterobacteriaceae. Diagn Microbiol Infect Dis. 2016 May;85 (1):12-5

T. **Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T.** Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. J Antimicrob Chemother. 2016 Jul;71 (7):1834-40

U. **Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, Bogaerts P.** Evaluation of two new commercial immunochromatographic assays for the rapid detection of OXA-48 and KPC carbapenemases from cultured bacteria. J Antimicrob Chemother. 2016 May;71(5):1217-22

V. **B.M. Willey, X. Trimi, R. Ioboni, D.A. Boyd, G. Ricci, D.N. Grohn, D. Terenzi, A. Mazzulli, L. Mataseje, M. Mulvey, P. Lo, T. Mazzulli, S.M. Poutanen**. The Coris BioConcept OXA48 K-SeT Immuno-Chromatographic Assay Detects OXA48-type Carbapenemases with High Sensitivity and Specificity. 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam April 9-12, 2016

W. **P. Bogaerts, S. Evrard, G. Cuzon, TD. Huang, T. Naas and Y. Glupczynski** Specificity of the OXA-48 immunochromatographic K-SeT for the detection of OXA-48 like in Shewanella spp. 26th European Congress of Clinical Microbiology and Infectious Diseases, Infectious Amsterdam April 09 – 12, 2016


Last update: 27 NOVEMBER 2019

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

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This test is ready to use and is based on a membrane technology with colloidal gold nanoparticles. A nitrocellulose membrane is sensitized with a monoclonal antibody directed against one epitope of the OXA-23 carbapenemase. Another monoclonal antibody directed against a second epitope of the OXA-23 carbapenemase is conjugated to colloidal gold particles. This conjugate is dried on a membrane.
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## III. REAGENTS AND MATERIALS

**1. OXA-23 *K*-SeT (20)**
20 sealed pouches containing one device and one desiccant. Each device contains one sensitized 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)**
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We recommend the use of fresh bacterial colonies for optimal test performance.

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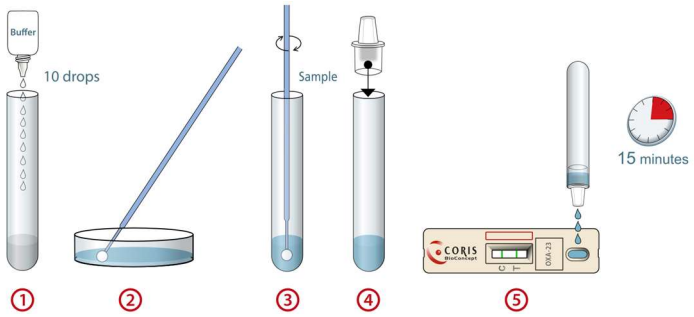


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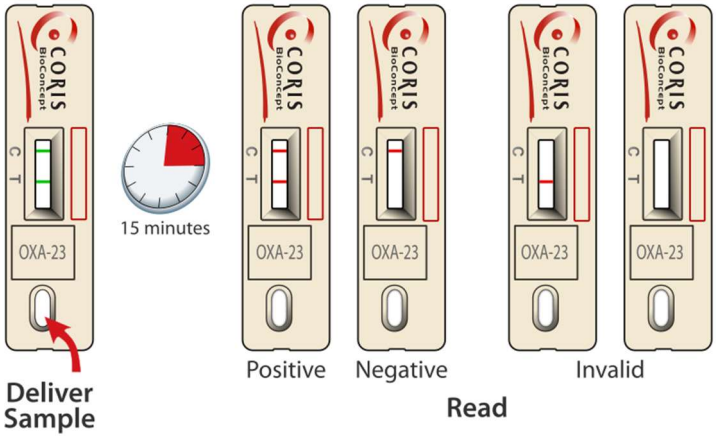

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| | | **5 strains** carrying class B carbapenemases | Including VIM-2, NDM-1, NDM-2 |

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| | Italy | Belgium | Total | Test OXA-23 K-SeT |
|---|---|---|---|---|
| $bla_{OXA\text{-}23\text{-}like}$ | 170 | 137 | 307 | 307 + |
| $bla_{OXA\text{-}24\text{-}like}$ | 5 | 25 | 30 | negative |
| $bla_{OXA\text{-}58\text{-}like}$ | 1 | 30 | 31 | negative |
| IS*Aba1* $bla_{OXA\text{-}51\text{-}like}$ | 11 | 0 | 11 | negative |
| $bla_{OXA\text{-}23\text{-}like}$ + $bla_{OXA\text{-}58\text{-}like}$ | 5 | 2 | 7 | 7 + |
| $bla_{OXA\text{-}23\text{-}like}$ + IS*Aba1* $bla_{OXA\text{-}51\text{-}like}$ | 4 | 0 | 4 | 4 + |
| $bla_{OXA\text{-}23\text{-}like}$ + $bla_{NDM}$ | 0 | 3 | 3 | 3 + |
| $bla_{OXA\text{-}58\text{-}like}$ + $bla_{VIM}$ | 0 | 1 | 1 | negative |
| $bla_{NDM}$ | 0 | 13 | 13 | negative |
| $bla_{OXA\text{-}143\text{-}like}$ | 0 | 1 | 1 | negative |
| $bla_{IMP}$ | 0 | 3 | 3 | negative |
| $bla_{VIM}$ | 0 | 1 | 1 | negative |
| $bla_{GES}$ | 0 | 1 | 1 | negative |
| $bla_{OXA\text{-}48\text{-}like}$ | 0 | 2 | 2 | negative |
| $bla_{OXA\text{-}198\text{-}like}$ | 0 | 1 | 1 | negative |
| *non-carbapenemase producer* | 0 | 46 | 46 | negative |
| **Total** | 196 | 266 | 462 | 321 + |

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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.
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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.
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U. **Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, Bogaerts P.** Evaluation of two new commercial immunochromatographic assays for the rapid detection of OXA-48 and KPC carbapenemases from cultured bacteria. J Antimicrob Chemother. 2016 May;71(5):1217-22

V. **B.M. Willey, X. Trimi, R. Ioboni, D.A. Boyd, G. Ricci, D.N. Grohn, D. Terenzi, A. Mazzulli, L. Mataseje, M. Mulvey, P. Lo, T. Mazzulli, S.M. Poutanen**. The Coris BioConcept OXA48 K-SeT Immuno-Chromatographic Assay Detects OXA48-type Carbapenemases with High Sensitivity and Specificity. 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam April 9-12, 2016

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Last update: 27 NOVEMBER 2019

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

# OXA-23 *K*-SeT

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
**IFU-58R7/EN/02**

***In vitro* rapid diagnostic test for the detection of OXA-23 carbapenemase in bacterial culture**

***FOR IN VITRO DIAGNOSTIC USE*** **EN**
**FOR PROFESSIONAL USE ONLY**
**References: K-15R7, 20 cassettes, buffer, 20 tubes and droppers**

## I. INTRODUCTION

*Acinetobacter baumannii* is an important opportunistic and multidrug-resistant Gram-negative bacterium 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*. Among the OXAs, OXA-23 is the most prevalent carbapenem-resistance determinant in *A. baumannii* isolates.
OXA-23 has been detected in other bacterial species as chromosomal (*P. mirabilis,* Bonnet et al 2002 and Osterblad et al 2016; *A. radioresistans*) or plasmidic gene (*E. coli*, La et al, 2014), which can constitute reservoirs for horizontal transmission of this resistance factor (Poirel et al 2016). The detection of this resistance factor OXA-23, 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 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 action by international experts and health authorities.
The OXA-23 K-SeT test aimed at a rapid identification of the OXA-23 carbapenemase (and variants of the OXA-23 group) ensures effective treatment of patients and prevention of spread of OXA-23 *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. A nitrocellulose membrane is sensitized with a monoclonal antibody directed against one epitope of the OXA-23 carbapenemase. Another monoclonal antibody directed against a second epitope of the OXA-23 carbapenemase is conjugated to colloidal gold particles. This conjugate is dried on a membrane.
This test is aimed at the detection of OXA-23 like carbapenemases in a single bacterial colony growing on agar plate. The sample must be diluted in the dilution buffer supplied with the test. When the provided buffer containing the resuspended bacteria comes into contact with the strip, the solubilized conjugate migrates with the sample by passive diffusion and both the conjugate and sample material come into contact with the anti-OXA-23 antibody that it is adsorbed onto the nitrocellulose strip. If the sample contains the OXA-23 carbapenemase, the conjugate–OXA-23 complex will remain bound to the anti-OXA-23 antibody adsorbed onto the nitrocellulose and a red line will develop. Solution continues to migrate to reach a second reagent (control reagent) that binds the migration control conjugate, thereby producing a red control line that confirms that the test is valid. Result is visible within 15 minutes.

## III. REAGENTS AND MATERIALS

**1. OXA-23 *K*-SeT (20)**
20 sealed pouches containing one device and one desiccant. Each device contains one sensitized 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)**
**5.**

## 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.
- 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 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.

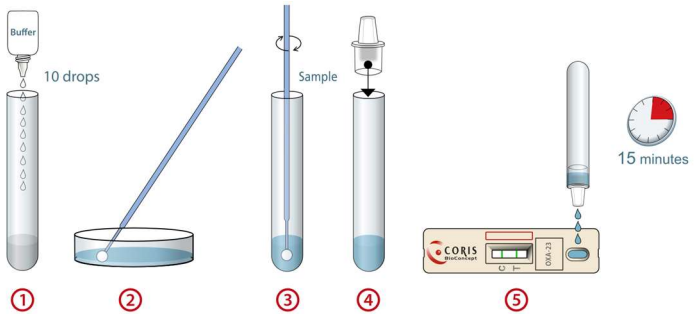
## VII. SPECIMEN HANDLING AND COLLECTION

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/OXA-23/FAQ.php

## 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, run the test immediately. Indicate the patient's name or specimen number on the device (one device per sample).
SPECIMEN PREPARATION PROCEDURE:
We recommend the use of fresh bacterial colonies for optimal test performance.

1. Prepare one semi-rigid tube provided in the kit and add **10** 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 the cassette. Alternatively, add 100µl with a micropipette into the sample well of the cassette.
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 is passed.**
**The result must be read on still wet strip.**

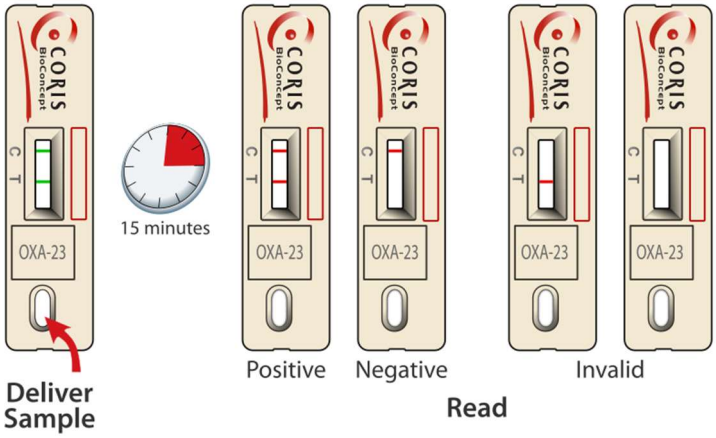
## IX. INTERPRETING RESULTS

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 band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the test line may vary according to the quantity of antigens present in the sample. Any reddish-purple line (T), even weak, should be considered as a positive 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 at the Test line position. It should not be regarded as a positive result.



## X. PERFORMANCE

### A. Detection Limit

The detection limit was determined with a purified recombinant OXA-23 protein and has been evaluated at 0,156 ng/mL.

### B. Validation on collection of reference strains

The OXA-23 *K*-SeT was evaluated on a collection of 108 clinical isolates of carbapenem-resistant *Acinetobacter* spp. fully characterized resistance mechanisms to beta-lactams by phenotypic and molecular tests (Germany).

| | | | |
|---|---|---|---|
| **108 strains** | **35 strains** tested positive with the OXA-23 *K*-SeT | **35 strains** carrying OXA-23 carbapenemase | *Acinetobacter baumannii, Acinetobacter pittii, Acinetobacter nosocomialis, Acinetobacter radioresistens* |
| | **73 strains** tested negative with the OXA-23 *K*-SeT | **68 strains** carrying a non-OXA-23 carbapenemase | OXA-40, OXA-51, OXA-58, OXA-143, OXA-235 |
| | | **5 strains** carrying class B carbapenemases | Including VIM-2, NDM-1, NDM-2 |

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R. **Wareham DW, Shah R, Betts JW, Phee LM, Abdul Momin MH.** Evaluation of an Immunochromatographic Lateral Flow Assay (OXA-48 K-SeT) for the Rapid Detection of OXA-48-like Carbapenemases in Enterobacteriaceae. . J Clin Microbiol. 2016 Feb;54 (2):471-3

S. **Fernández J, Fleites A, Rodcio MR, Vazquez F.** Evaluation of OXA-48 K-Se T: an immunochromatographic assay for rapid detection of OXA-48-producing Enterobacteriaceae. Diagn Microbiol Infect Dis. 2016 May;85 (1):12-5

T. **Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T.** Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. J Antimicrob Chemother. 2016 Jul;71 (7):1834-40

U. **Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, Bogaerts P.** Evaluation of two new commercial immunochromatographic assays for the rapid detection of OXA-48 and KPC carbapenemases from cultured bacteria. J Antimicrob Chemother. 2016 May;71(5):1217-22

V. **B.M. Willey, X. Trimi, R. Ioboni, D.A. Boyd, G. Ricci, D.N. Grohn, D. Terenzi, A. Mazzulli, L. Mataseje, M. Mulvey, P. Lo, T. Mazzulli, S.M. Poutanen**. The Coris BioConcept OXA48 K-SeT Immuno-Chromatographic Assay Detects OXA48-type Carbapenemases with High Sensitivity and Specificity. 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam April 9-12, 2016

W. **P. Bogaerts, S. Evrard, G. Cuzon, TD. Huang, T. Naas and Y. Glupczynski** Specificity of the OXA-48 immunochromatographic K-SeT for the detection of OXA-48 like in Shewanella spp. 26th European Congress of Clinical Microbiology and Infectious Diseases, Infectious Amsterdam April 09 – 12, 2016


Last update: 27 NOVEMBER 2019

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

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

Manufacturer:

Coris BioConcept
CREALYS Science Park
Rue Guillaume Fouquet, 11
5032 GEMBLOUX
BELGIUM
Tel.: +32(0)81.719.917
Fax: +32(0)81.719.919
info@corisbio.com
Produced in BELGIUM

www.corisbio.com
**IFU-58R11/EN/06**

***In vitro* rapid diagnostic test for the detection of OXA-48, KPC, NDM, VIM and IMP carbapenemases in bacterial culture**

***FOR IN VITRO DIAGNOSTIC USE*** **EN**
**FOR PROFESSIONAL USE ONLY**
**References: K-15R11, 2x20 cassettes, buffer, 20 tubes and transfer pipets**

## 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 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. The rapid spread of CPOs and genes encoding these resistances has led to nosocomial outbreaks and endemic situations 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. VIM is not only present in Enterobacteriaceae but is also highly prevalent in non-fermenting bacteria. IMP should be regarded as a potential problem since they degrade not only C3G but also carbapenem antimicrobial drug like Imipenem. IMP prevalence is the lowest, apart from Japan where it is more prevalent.
Inhibitor-based phenotypic confirmatory tests exist for the confirmation of class A (KPC) and class B (VIM, IMP, NDM) carbapenemases, Nowadays, definitive confirmation of CPO resistance mechanism relies on molecular assays. These tests are expensive and can only be performed in dedicated environment and by skilled personnel, hence limiting their more generalized usage.
O.K.N.V.I. RESIST-5 test is part of Coris BioConcept RESIST range of antimicrobial resistance diagnostic tests.

## 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 detect and identify the carbapenemases from a bacterial colony isolate of Enterobacteriaceae or NFGNB growing on agar plate. Each pouch contains: 2 lateral-flow cassettes for the identification of (i) OXA-48, KPC, NDM and (ii) VIM and IMP.
**Identification of OXA-48, KPC and NDM.** A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against OXA-48 carbapenemase and variants (except OXA-163-like enzymes) (“O” line)
(2) a monoclonal antibody directed against KPC carbapenemase (“K” line)
(3) a monoclonal antibody directed against NDM carbapenemase (“N” line)
(4) a control capture reagent (upper “C” line).
Four different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against a second epitope of the OXA-48 carbapenemase, a conjugate directed against a second epitope of the KPC carbapenemase, a third conjugate specific to NDM carbapenemase and a control conjugate to validate the test conditions.
**Identification of VIM and IMP**. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against VIM carbapenemase (“V” line),
(2) a monoclonal antibody directed against IMP carbapenemase (“I” line)
(3) a control capture reagent (upper “C” line).
Three different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against VIM carbapenemase, a conjugate directed against IMP carbapenemase and a control conjugate.
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 OXA-48, KPC, NDM, VIM or IMP carbapenemase, the respective complexes made of the conjugates and either OXA-48, or KPC, or NDM or VIM or IMP will remain bound to their respective specific lines (OXA-48 : “O” line; KPC : “K” line; NDM : “N” line, VIM : “V” line, IMP : “I” 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. O.K.N.V.I. RESIST-5 (2x20 cassettes)**
20 sealed pouches containing two lateral-flow cassettes and one desiccant.
Each cassette contains one sensitised strip.
**2. LY-D buffer vial (7 mL)**
Tris-EDTA solution containing $NaN_3$ (<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 with blood culture
- ReSCape (S-1002): reagents kits for use with 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 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

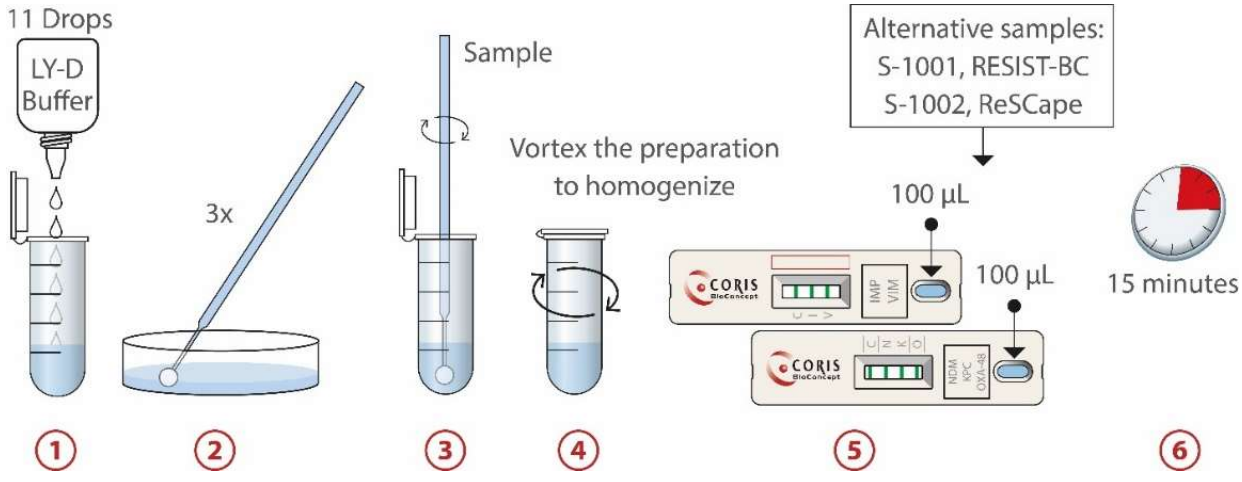
Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIST kits are listed on the website: https://www.corisbio.com/products/oknvi-resist-5/faq

## VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens (in 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 been established for rectal swabs and blood cultures.
With rectal swabs and blood cultures, the preparation procedure has to be followed as described in the respective kits (S-1002, ReSCape and S-1001, RESIST-BC)
With bacterial colonies, we recommend the use of fresh agar cultures for optimal test performance and as followed:

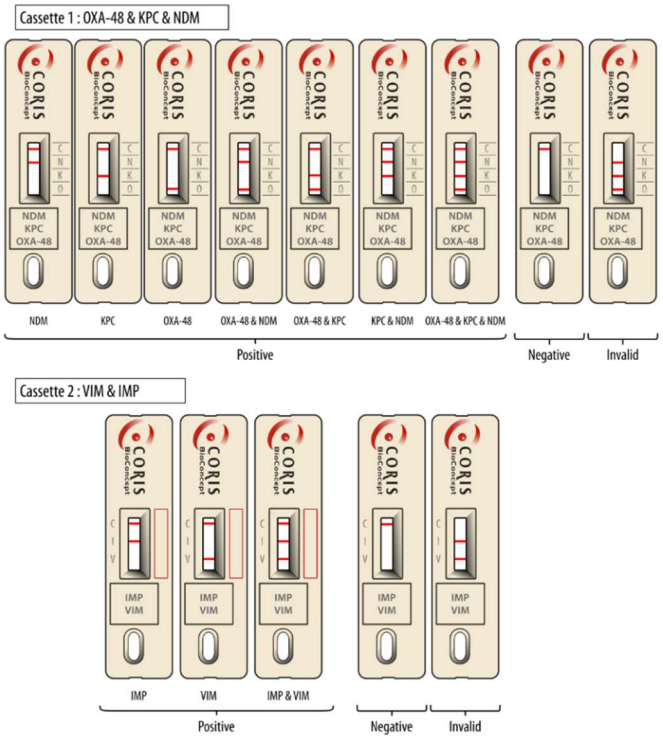
1. Prepare one collection tube and add **11 drops** of LY-D buffer in the tube
2. Harvest bacteria by taking **3 colonies** with a disposable bacteriological loop and dip the loop in the bottom of the tube containing the buffer. The same bacteriological loop can be used to collect the 3 colonies.
3. Stir thoroughly before removing the loop.
4. Close de tube and vortex the preparation to homogenize.
5. Use the transfer pipette provided in the kit and add 100 µL of diluted sample into the sample well of each of the two cassettes labelled (i) NDM, KPC and OXA-48 and (ii) IMP and VIM (**diluted sample must reach the black line indicated on the transfer pipette to accurately aspirate 100 µL**).
6. Allow to react for 15 minutes 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 ("N" or "K" or "O") on cassette labelled (i) NDM, KPC, OXA-48 or at one of the Test lines position ("I" or "V") on cassette labelled (ii) IMP 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 (OXA-48, KPC, NDM, VIM and IMP), even weak, should be considered as a positive result.
If a positive test line appears beside of the "O" mark, the sample contains OXA-48 or OXA-48-like variants. If it appears beside the "K" mark, the sample contains KPC variants; beside the "N" mark, the sample contains NDM; the "V" mark, the sample contains VIM; and beside of the "I" mark, IMP is present in the sample. 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.




## X. PERFORMANCE

### A. Detection Limit

The detection limit determined with purified recombinant proteins of OXA-48, KPC, NDM, VIM and IMP have been evaluated at 0.25 ng/mL, 0.5 ng/mL, 0.0625 ng/mL, 0.23 ng/mL and 0.781 ng/mL, respectively.

### B. Retrospective study

The test cassettes were validated by comparison with reference molecular method (validated in house multiplex PCR including sequencing) in a retrospective study performed on 180 non duplicated, consecutive suspected CPE clinical isolates collected between 2012 and 2021 from Belgian hospitals.

| Molecular method / OXA-48 test | Positive | Negative | Total |
|---|---|---|---|
| **Positive** | 41 | 0 | 41 |
| **Negative** | 0 | 139 | 139 |
| **Total** | 41 | 139 | 180 |

95 % Confidence Interval [1]

| | | |
|---|---|---|
| **Sensitivity**: | 100 % | (89.3 to 100 %) |
| **Specificity**: | 100 % | (96.6 to 100 %) |
| Positive Predictive value: | 100 % | (89.3 to 100 %) |
| Negative predictive value: | 100 % | (96.7 to 100 %) |
| Agreement: | 100 % | (180/180) |

| Molecular method / KPC test | Positive | Negative | Total |
|---|---|---|---|
| **Positive** | 24 | 0 | 24 |
| **Negative** | 0 | 156 | 156 |
| **Total** | 24 | 156 | 180 |

95 % Confidence Interval [1]

| | | |
|---|---|---|
| **Sensitivity**: | 100 % | (82.8 to 100 %) |
| **Specificity**: | 100 % | (97.0 to 100 %) |
| Positive Predictive value: | 100 % | (82.8 to 100 %) |
| Negative predictive value: | 100 % | (97.0 to 100 %) |
| Agreement: | 100 % | (180/180) |

| Molecular method / NDM test | Positive | Negative | Total |
|---|---|---|---|
| **Positive** | 40 | 0 | 40 |
| **Negative** | 0 | 140 | 140 |
| **Total** | 40 | 140 | 180 |

95 % Confidence Interval [1]

| | | |
|---|---|---|
| **Sensitivity:** | 100 % | (89.1 to 100 %) |
| **Specificity:** | 100 % | (96.7 to 100 %) |
| Positive Predictive value: | 100 % | (89.1 to 100 %) |
| Negative predictive value: | 100 % | (96.7 to 100 %) |
| Agreement: | 100 % | (180/180) |

| Molecular method / VIM test | Positive | Negative | Total |
|---|---|---|---|
| **Positive** | 43 | 0 | 43 |
| **Negative** | 3 | 134 | 137 |
| **Total** | 46 | 134 | 180 |

95 % Confidence Interval [1]

| | | |
|---|---|---|
| **Sensitivity:** | 93.5 % | (81.1 to 98.3 %) |
| **Specificity:** | 100 % | (96.5 to 100 %) |
| Positive Predictive value: | 100 % | (89.8 to 100 %) |
| Negative predictive value: | 97.8 % | (93.2 to 99.4 %) |
| Agreement: | 98.3 % | (177/180) |

| Molecular method / IMP test | Positive | Negative | Total |
|---|---|---|---|
| **Positive** | 19 | 0 | 19 |
| **Negative** | 0 | 161 | 161 |
| **Total** | 19 | 161 | 180 |

95 % Confidence Interval [1]

| | | |
|---|---|---|
| **Sensitivity:** | 100 % | (79.1 to 100 %) |
| **Specificity:** | 100 % | (97.1 to 100 %) |
| Positive Predictive value: | 100 % | (79.1 to 100 %) |
| Negative predictive value: | 100 % | (97.1 to 100 %) |
| Agreement: | 100 % | (180/180) |

The O.K.N.V.I. RESIST-5 kit 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.

## 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


A. **J. Wesley MacDonald and V. Chibabhai Evaluation of the RESIST-4 O.K.N.V immunochromatographic lateral flow assay for the rapid detection of OXA-48, KPC, NDM and VIM carbapenemases from cultured isolates** Access Microbiology 2019;1
B. T. Pilate, S. Desmet Detection of carbapenemase production in pseudomonas aeruginosa in a tertiary care centre Annual Meeting of the Royal Belgian Society of Laboratory Medicine November 15th, 2019 Belgium
C. **Oueslati S, Iorga BI, Tlili L, Exilie C, Zavala A, Dortet L, Jousset AB, Bernabeu S, Bonnin RA, Naas T.** Unravelling ceftazidime/avibactam resistance of KPC-28, a KPC-2 variant lacking carbapenemase activity. **J Antimicrob Chemother. 2019 Aug 1;74(8):2239-2246**
D. **Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, Kohlenberg A.** Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, **assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019 Feb; 24 (9) 1560-7917**
E. **Oliveira J, Reygaert WC.** Gram Negative Bacteria. **StatPearls Publishing; 2019 Jan-2019**
F. **Baeza LL, Pfennigwerth N, Greissl C, Göttig S, Saleh A, Stelzer Y, Gatermann SG, Hamprecht A.** Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm. **Clin Microbiol Infect. 2019 Mar 18. pii: S1198-743X(19)30104-1**
G. **Rösner S, Kamalanabhaiah S, Küsters U, Kolbert M, Pfennigwerth N, Mack D.** Evaluation of a novel immunochromatographic lateral flow assay for rapid detection of OXA-48, NDM, KPC and VIM carbapenemases in multidrug-resistant Enterobacteriaceae. **J Med Microbiol. 2019 Mar;68(3):379-381.**
H. **Glupczynski Y, Evrard S, Huang TD, Bogaerts P.** Evaluation of the RESIST-4 K-SeT assay, a multiplex immunochromatographic assay for the rapid detection of OXA-48-like, KPC, VIM and NDM carbapenemases. **J Antimicrob Chemother. 2019 Feb 6. doi: 10.1093**


**Last update : 20 FEBRUARY 2023**

| | | | |
|---|---|---|---|
| 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 |
| UDI | Unique device identifier | | |

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

# Oxidase Discs

**DD018**

Oxidase Discs are used for detection of oxidase production by microorganisms like Neisseria, Alcaligenes, Aeromonas, Vibrio's, Campylobacter and Pseudomonas, which give positive reactions and for excluding Enterobacteriaceae, which give negative reactions.

## Directions

Oxidase reaction is carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction is observed within 5-10 seconds at 25-30°C. A change later than 10 seconds or no change at all is considered negative reaction.

Precautions

1. ,,Do not use stainless steel or nichrome inoculating wires, as false positive reaction may result from surface oxidation products formed during flame sterilization.

2. ,,Growth from media containing dyes is not suitable for testing.

3. ,,Timing is critical (5-10 sec) for interpretation of results.

4. ,,Perform oxidase test on all gram-negative bacilli.

5. ,,Cytochrome oxidase production may be inhibited byacid production. False negative reactions may be exhibited by Vibrio, Aeromonas and Plesiomonas species when grown on a medium containing fermentable carbohydrate e.g. MacConkey Agar (M081). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto-oxidation which may be avoided by adding 0.1% ascorbic acid (3).

## Principle And Interpretation

Certain bacteria posses either cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein), which catalyzes the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). In the oxidase test, a colourless dye such as N, N-dimethy-p-phenylenediamine serves as an artificial electron acceptor for the enzyme oxidase. The dye is oxidized to form indophenol blue, a coloured compound. The test is useful in the initial characterization of aerobic gram-negative bacteria of the genera Aeromonas, Plesiomonas, Pseudomonas, Campylobacter and Pasteurella.

Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. These discs overcome the neccessity of daily preparation of fresh reagent. Gordon and McLeod (1) introduced oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and a-naphthol. Gaby and Hadley (2) introduced a more sensitive method by using N, N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative. In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

## Quality Control

**Appearance**

Filter paper discs of 10 mm diameter

**Cultural response**

Typical oxidase reaction given by 18-48 hour culture observed within 5-10 seconds at 25-30°C.

| Organism | Reaction Observed |
|---|---|
| *Pseudomonas aeruginosa* ATCC 27853 | positive : deep purplish blue colouration of disc |

| | |
|---|---|
| *Neisseria gonorrhoeae ATCC 19424* | positive : deep purplish blue colouration of disc |
| *Escherichia coli ATCC 25922* | negative : purplish blue colouration after 10 sec/ no colour change |
| *Staphylococcus aureus ATCC 25923* | negative : no colour change |

## Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

## Reference

1.Gordon J. and Mcleod J.W., 1928, J. Path. Bact., 31:185
2.Gaby W.L and Hadley C., 1957. J. Bact., 74:356
3.Steel. K.J. 1962. J. Appl. Bact. 25:445

Revision : 1 / 2011

# SS Agar (Salmonella Shigella Agar) M108

## Intended Use:

Recommended for the isolation of *Salmonella* and some *Shigella* species from pathological specimens, suspected foodstuffs etc.

## Composition**

| Ingredients | g / L |
|---|---|
| Peptone | 5.000 |
| HM peptone B # | 5.000 |
| Lactose | 10.000 |
| Bile salts mixture | 8.500 |
| Sodium citrate | 10.000 |
| Sodium thiosulphate | 8.500 |
| Ferric citrate | 1.000 |
| Brilliant green | 0.00033 |
| Neutral red | 0.025 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.0±0.2 |

**Formula adjusted, standardized to suit performance parameters
# - Equivalent to Beef extract

## Directions

Suspend 63.02 grams in 1000 ml purified /distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

## Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (1) and suspected foodstuffs (2,3,4,5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Peptone, HM peptone B provides nitrogen and carbon source, long chain amino acids, vitamins and essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and $H_2S$ gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of $H_2S$ gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of $H_2S$ with ferric ions or ferric citrate, indicated in the center of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centers. Growth of *Salmonella* species appears as colourless colonies with black centers resulting from $H_2S$ production. *Shigella* species also grow as colourless colonies which do not produce $H_2S$.

## Type of specimen

Clinical: faeces, rectal swabs; Suspected food stuffs.

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3,4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1.The medium is highly selective and may be toxic to certain *Salmonella* or *Shigella* species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of *Shigella* species (9).

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

**Appearance**

Light yellow to pink coloured homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Reddish orange coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| # *Klebsiella aerogenes* ATCC 13048 (00175*) | 50-100 | fair | 20-30% | cream pink |
| *Escherichia coli* ATCC 25922 (00013*) | 50-100 | fair | 20-30% | pink with bile precipitate |
| *Salmonella Choleraesuis* ATCC 12011 | 50-100 | good-luxuriant | >=50% | colourless with black centre |
| *Salmonella* Typhi ATCC 6539 | 50-100 | good-luxuriant | >=50% | colourless with black centre |
| *Enterococcus faecalis* ATCC 29212 (00087*) | 50-100 | none-poor | <=10% | colourless |
| *Proteus mirabilis* ATCC 25933 | 50-100 | fair-good | 30-40% | colourless, may have black centre |
| *Shigella flexneri* ATCC 12022 (00126*) | 50-100 | good | 40-50% | colourless |
| *Salmonella* Typhimurium ATCC 14028 (00031*) | 50-100 | good-luxuriant | >=50% | colourless with black centre |
| *Salmonella* Enteritidis ATCC 13076 (00030*) | 50-100 | good-luxuriant | >=50% | colourless with black centre |

Key : *Corresponding WDCM numbers. # Formerly known as *Enterobacter aerogenes*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

## Reference

1. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
5. Williams S., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed.,AOAC,Washington, D.C.
6. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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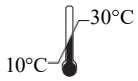
HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device




Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE CE Marking

Do not use if package is damaged

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