# OXA-23 K-SeT

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Produced in BELGIUM

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**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.

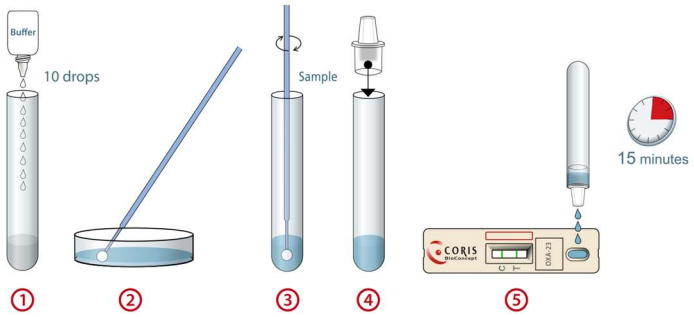
## VII. SPECIMEN HANDLING AND COLLECTION

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.**

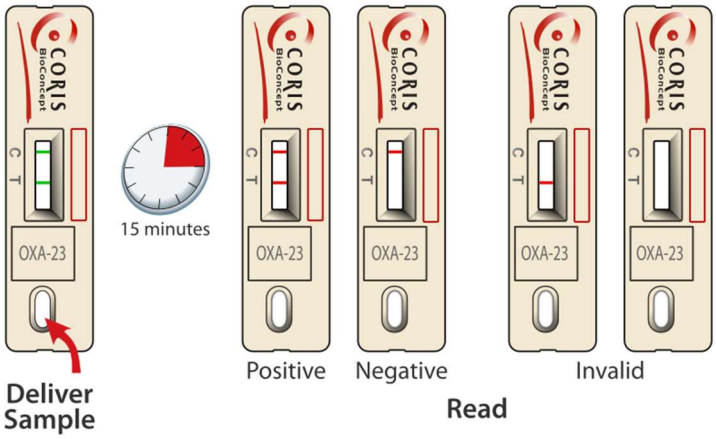
## IX. INTERPRETING RESULTS

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 |

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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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 |

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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.
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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.

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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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- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
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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
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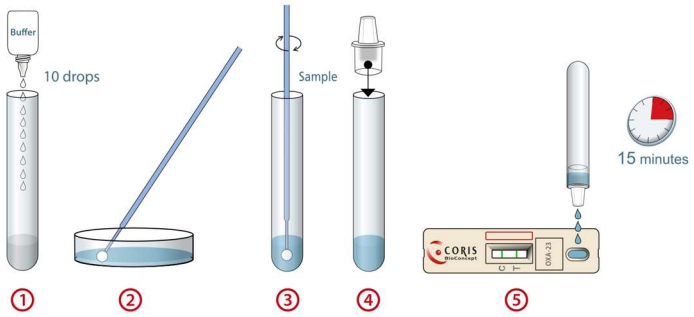


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The results are to be interpreted as follows:

**Negative test result**: a reddish-purple line appears across the central reading window at the Control line (C) position. No other band is present.

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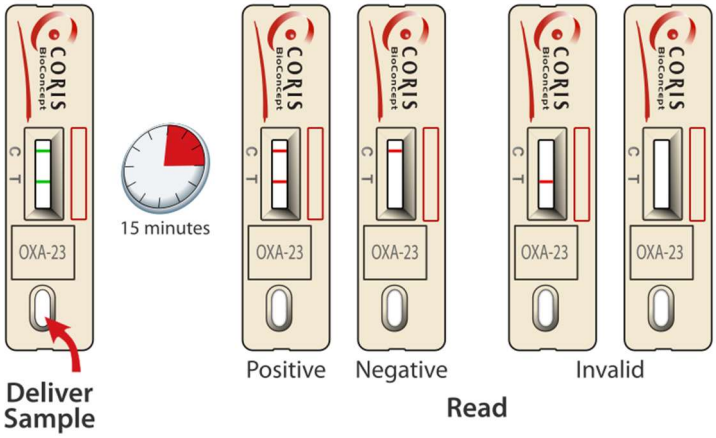

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| | Italy | Belgium | Total | Test OXA-23 K-SeT |
|---|---|---|---|---|
| $bla_{OXA\text{-}23\text{-}like}$ | 170 | 137 | 307 | 307 + |
| $bla_{OXA\text{-}24\text{-}like}$ | 5 | 25 | 30 | negative |
| $bla_{OXA\text{-}58\text{-}like}$ | 1 | 30 | 31 | 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 + |

### 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
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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.

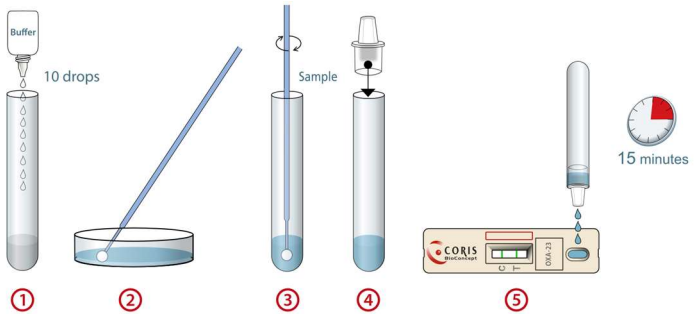
## VII. SPECIMEN HANDLING AND COLLECTION

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.**

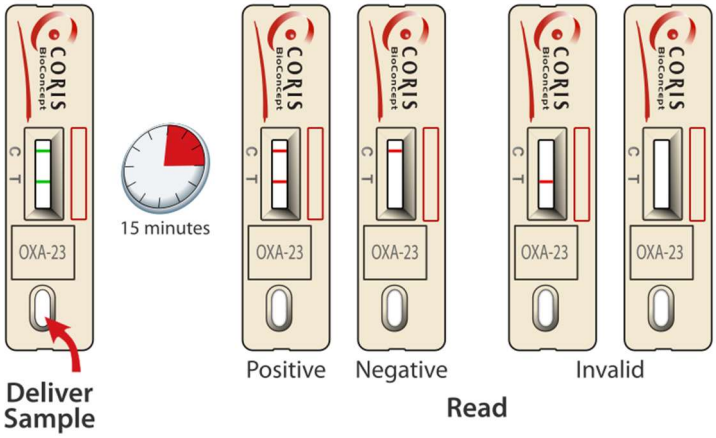
## IX. INTERPRETING RESULTS

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 |

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

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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 |

# 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
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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

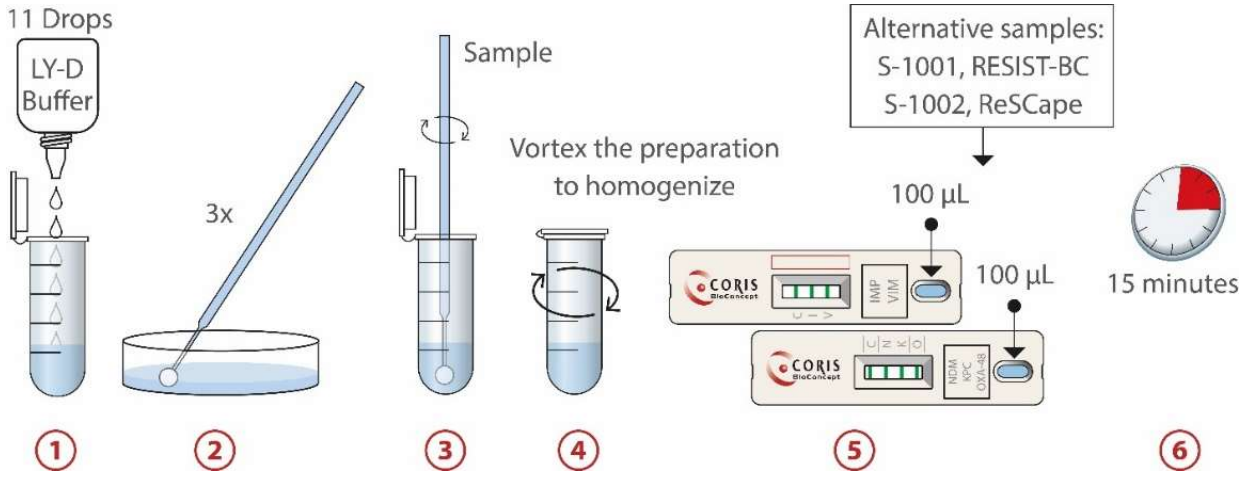
Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIST kits are listed on the website: https://www.corisbio.com/products/oknvi-resist-5/faq

## 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:

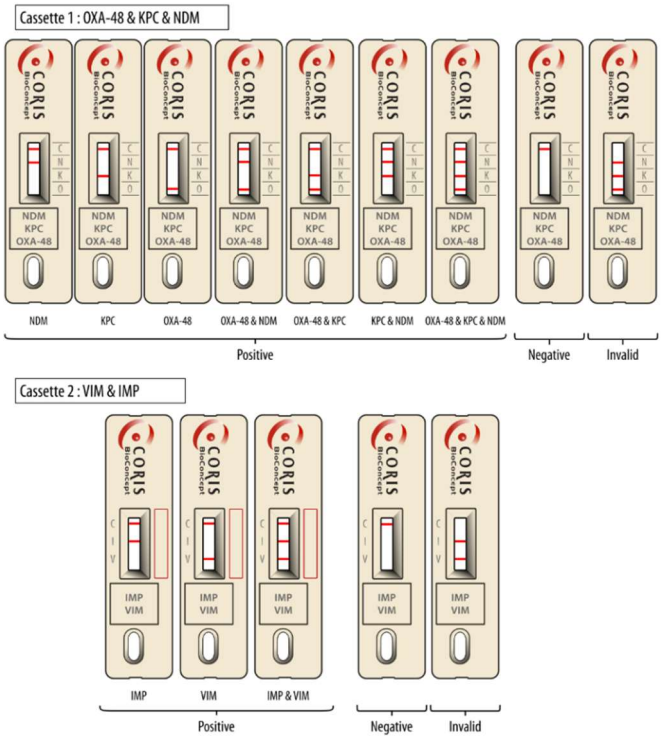
1. Prepare one collection tube and add **11 drops** of LY-D buffer in the tube
2. Harvest bacteria by taking **3 colonies** with a disposable bacteriological loop and dip the loop in the bottom of the tube containing the buffer. The same bacteriological loop can be used to collect the 3 colonies.
3. 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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E. **Oliveira J, Reygaert WC.** Gram Negative Bacteria. **StatPearls Publishing; 2019 Jan-2019**
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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

# Kligler Iron Agar

**M078**

## Intended Use:

Recommended for differential identification of gram-negative enteric bacilli from clinical and non-clinical samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptone | 15.000 |
| HM Peptone B # | 3.000 |
| Yeast extract | 3.000 |
| Proteose peptone | 5.000 |
| Lactose | 10.000 |
| Dextrose | 1.000 |
| Ferrous sulphate | 0.200 |
| Sodium chloride | 5.000 |
| Sodium thiosulphate | 0.300 |
| Phenol red | 0.024 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.4±0.2 |

**Formula adjusted, standardized to suit performance parameters

# - Equivalent to Beef extract

## Directions

Suspend 57.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

***Note:*** *Avoid overheating otherwise it may produce precipitate in the medium.*

## Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1,2) and Russels Double Sugar Agar (3) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (4). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other Salmonellae and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (5). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow. Kligler Iron Agar, in addition to Peptone, HM peptone B and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

## Type of specimen

Isolated microorganism from clinical, food, dairy and water samples.

## Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). 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. Results should be noted after 18-24 hours to avoid erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.
4. Other biochemical and serological tests must be performed for complete identification

## 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 homogeneous free flowing powder
**Gelling**
Firm, comparable with 1.5% Agar gel
**Colour and Clarity of prepared medium**
Red coloured, clear to slightly opalescent gel forms in tubes as slants
**Reaction**
Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2
**pH**
7.20-7.60
**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 18 - 48 hours.

| Organism | Inoculum (CFU) | Growth | Gas | H2S | Slant | Butt |
|---|---|---|---|---|---|---|
| *Escherichia coli* ATCC 25922 (00013*) | 50-100 | luxuriant | positive reaction | negative reaction, no blackening of medium | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium |
| #*Klebsiella aerogenes* ATCC 13048 (00175*) | 50-100 | luxuriant | positive reaction | negative reaction, no blackening of medium | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium |
| *Citrobacter freundii* ATCC 8090 | 50-100 | luxuriant | positive reaction | positive reaction, blackening of medium | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium |
| *Proteus vulgaris* ATCC 6380 | 50-100 | luxuriant | negative reaction | positive reaction, blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |
| *Klebsiella pneumoniae* ATCC 13883 (00087*) | 50-100 | luxuriant | positive reaction | negative reaction,no blackening of medium | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium |
| *Salmonella* Paratyphi A ATCC 9150 | 50-100 | luxuriant | positive reaction | negative reaction,no blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Salmonella* Schottmuelleri ATCC 10719 | 50-100 | luxuriant | positive reaction | positive reaction, blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |
| *Salmonella* Typhi ATCC 6539 | 50-100 | luxuriant | negative reaction | positive reaction, blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |
| *Salmonella* Enteritidis ATCC 13076 (00030*) | 50-100 | luxuriant | positive reaction | positive reaction, blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |
| *Shigella flexneri* ATCC 12022 (00126*) | 50-100 | luxuriant | negative reaction | negative reaction,no blackening of medium | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50-100 | luxuriant | negative reaction | negative reaction, blackening of medium | alkaline reaction, red colour of the medium | alkaline reaction,red colour of the medium |
| *Yersinia enterocolitica* ATCC 27729 | 50-100 | luxuriant | variable reaction | negative reaction,no blackening of medium | alkaline reaction,red colour of the medium | acidic reaction, yellowing of the medium |
| *Enterobacter cloacae* ATCC 13047 (00083*) | 50-100 | luxuriant | positive reaction | negative reaction,no blackening of medium | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium |

Key :* Corresponding WDCM numbers

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (10,11).

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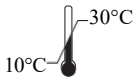


**Storage temperature**

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# 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

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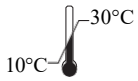


Storage temperature

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# Columbia Blood Agar Base

**M144**

## Intended Use:

For preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

## Composition**

| Ingredients | g / L |
|---|---|
| Peptone, special | 23.000 |
| Corn starch | 1.000 |
| Sodium chloride | 5.000 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.3±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 44.0 grams of in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.
For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.
The medium can be made selective by adding different antimicrobials to sterile base.
For *Brucella* species: Add rehydrated contents of 1 vial of NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.
For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.
For *Gardnerella* species: Add rehydrated contents of 1 vial of GNA Selective Supplement (FD056) to 500 ml sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

## Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.
Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.
Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae,* lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% $CO_2$.
*Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% $CO_2$. Campylobacter species are best grown at 42°C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% $CO_2$ (2).*

## Type of specimen

Clinical samples : throat swabs, pus.

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use only. 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. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-hemolytic *Streptococci* may exhibit a greenish hemolytic reaction which may be mistaken for the alpha haemolysis.
3. Biochemical characterization is required on colonies of pure culture for complete identification.

## 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**

Cream to yellow homogeneous free flowing powder.

**Gelling**

Firm, comparable with 1.5% Agar gel.

**Colour and Clarity of prepared medium**

Basal medium: Light amber coloured clear to slightly opalescent gel.
After addition of 5%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

**Reaction**

Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

**pH**

7.10-7.50

**Cultural Response**

Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Haemolysis |
|---|---|---|---|---|
| *Neisseria meningitidis* ATCC 13090 | 50-100 | luxuriant | >=70% | none |
| *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*) | 50-100 | luxuriant | >=70% | beta / gamma |
| *Staphylococcus epidermidis* ATCC 12228 (00036*) | 50-100 | luxuriant | >=70% | gamma |
| *Staphylococcus aureus* subsp. *aureus* ATCC 6538 (00032*) | 50-100 | luxuriant | >=70% | beta / gamma |
| *Streptococcus pneumoniae* ATCC 6303 | 50-100 | luxuriant | >=70% | alpha |
| *Streptococcus pyogenes* ATCC 19615 | 50-100 | luxuriant | >=70% | beta |
| *Clostridium sporogenes* ATCC 19404 (00008*) | 50-100 | luxuriant | >=50 % | |
| *Clostridium sporogenes* ATCC 11437 | 50-100 | luxuriant | >=50 % | |
| *Clostridium perfringens* ATCC 13124 (00007*) | 50-100 | luxuriant | >=50 % | |
| *Clostridium perfringens* ATCC 12934 | 50-100 | luxuriant | >=50 % | |

Key : (*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (5,6).

## Reference

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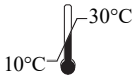


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# Mueller Hinton Agar

M173

## Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

## Composition**

| Ingredients | g / L |
|---|---|
| HM infusion solids B # (from 300g) | 2.000 |
| Acicase ## | 17.500 |
| Starch | 1.500 |
| Agar | 17.000 |
| Final pH ( at 25°C) | 7.3±0.1 |

**Formula adjusted, standardized to suit performance parameters
# - Equivalent to Beef heart infusion
## - Equivalent to Casein acid hydrolysate

## Directions

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

## Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (1). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.
ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
iii. It supports the growth of most non-fastidious bacterial pathogens and
iv. Many data and much experience regarding its performance have been recorded (4).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae.*

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (7,1,2). A standardized suspension of the organism is swabbed over the entire surface of the medium.

Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (4). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (4,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and

capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (10).

## Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13).
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use only. 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. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

## 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**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.7% agar gel.

**Colour and Clarity of prepared medium**

Light amber coloured clear to slightly opalescent gel froms in Petri plates

**Reaction**

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

**pH**

7.20-7.40

**Cultural Response**

Antibiotic susceptibility tests are performed in accordance with, and meet the acceptance limits of the current ISO/TS 16782 (15). Performance of the medium is checked in accordance with the CLSI/ EUCAST guidelines.

**For testing *S. pneumoniae* :** The medium was supplemented with 5% Horse blood and 20 mg/l NAD , incubated at 34-36°C for 18-20 hours in 5% $CO_2$ .

**For testing *H. influenzae* :** The medium was supplemented with 5% Horse blood and 20 mg/l -NAD, incubated at 34-36°C for 18-20 hours in 5% CO2 .

**Antibiotic Sensitivity test**

Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100).

**Thymine/Thymidine Content**

# The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

**Divalent Cation Content**

$ The zones for these discs are indicative of the Divalent Cation content of the medium

| Organism | Growth | Standard Zone | Incubation temperature | Incubation period |
|---|---|---|---|---|
| ***Escherichia coli* ATCC 25922 (00013*)** | luxuriant | | 34-36°C | 16-20 hours |
| Cephalothin CEP 30mcg | | 15-21 mm | | |
| Ampicillin AMP 10mcg | | 15-22 mm | | |
| Chloramphenicol C 30 mcg | | 21-27 mm | | |
| Gentamicin GEN 10mcg | | 19-26 mm | | |
| Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg | | 23-29 mm | | |
| Sulphafurazole SF 300 mcg | | 15-23 mm | | |
| Cefotaxime CTX 5 mcg | | 25-31 mm | | |
| Tigecycline TGC 15mcg | | 20-27 mm | | |
| Tetracycline TE 30 mcg | | 18-25 mm | | |
| Amoxicillin- clavulanate AMC 30 mcg | | 18-24 mm | | |
| Ciprofloxacin CIP 5mcg | | 29-38 mm | | |
| ***Escherichia coli* ATCC 35218** | luxuriant | | 34-36°C | 16-20 hours |
| Amoxicillin- clavulanate AMC 30 mcg | | 17-22 mm | | |
| Piperacillin/Tazobactam PIT 100/10 mcg | | 24-30 mm | | |
| Ticarcillin TI 75 mcg | | 6 mm | | |
| Ticarcillin/Clavulanic acid TCC 75/10mcg | | 21-25mm | | |
| Ampicillin AMP 10 mcg | | 6 mm | | |
| Ampicillin/Sulbactam A/S 10/10 mcg | | 13-19 mm | | |
| ***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)** | luxuriant | | 34-36°C | 16-20 hours |
| Erythromycin E 15 mcg | | 22-30 mm | | |
| Linezolid LZ 30 mcg | | 24-30 mm | | |
| Tetracycline TE 30 mcg | | 24-30 mm | | |
| Ciprofloxacin CIP 5mcg | | 22-30 mm | | |
| Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg | | 28-36 mm | | |
| Co-Trimoxazole COT 25 mcg | | 24-32 mm | | |
| Cefoxitin CX 30 mcg | | 23-29 mm | | |
| Oxacillin OX 1mcg | | 18-24 mm | | |
| Pristinomycin RP 15 mcg | | 21-28 mm | | |
| Gentamicin GEN 10 mcg | | 19-27 mm | | |
| Penicillin-G 10 units | | 26-37 mm | | |
| Ampicillin/Sulbactam A/S 10/10 mcg | | 29-37 mm | | |
| ***Staphylococcus aureus* subsp. *aureus* ATCC 29213 (00131*)** | luxuriant | | 34-36°C | 16-20 hours |
| Penicillin-G P 1 unit | | 12-18 mm | | |
| Cefoxitin CX 30 mcg | | 24-30 mm | | |
| Erythromycin E 15 mcg | | 23-29 mm | | |
| Linezolid LZ 10 mcg | | 21-27 mm | | |
| Gentamicin GEN 10 mcg | | 19-25 mm | | |
| Tetracycline TE 30 mcg $ | | 23-31 mm | | |
| Ciprofloxacin CIP 5mcg | | 21-27 mm | | |

| | | | | |
|---|---|---|---|---|
| ***Staphylococcus aureus* subsp. *aureus*ATCC 43300 (MRSA) (00211*)** | luxuriant | | 34-36°C | 24 hours |
| Oxacillin OX 1 mcg | | Very Hazy to No Zone | | |
| Cefoxitin CX 30 mcg | | <=21 mm | | |
| ***Pseudomonas aeruginosa* ATCC 27853 (00025*)** | luxuriant | | 34-36°C | 16-20 hours |
| Ceftazidime CAZ 30 mcg | | 22-29 mm | | |
| Ciprofloxacin CIP 5mcg | | 25-33 mm | | |
| Tobramycin TOB 10 mcg $ | | 20-26 mm | | |
| Amikacin AK 30 mcg $ | | 20-26 mm | | |
| Aztreonam AT 3mcg | | 23-29 mm | | |
| Cephotaxime CTX 30 mcg | | 18-22 mm | | |
| Gentamicin GEN 10 mcg $ | | 17-23 mm | | |
| Imipenem IPM 10 mcg | | 20-28 mm | | |
| Piperacillin PI 100 mcg | | 25-33 mm | | |
| Piperacillin Tazobactum PIT 30/6 mcg | | 23-29 mm | | |
| ***Enterococcus faecalis* ATCC 29212 (00087*)** | luxuriant | | 34-36°C | 16-20 hours |
| Trimethoprim TR 5 mcg # | | 24-32 mm | | |
| Ampicillin AMP 2 mcg | | 15-21 mm | | |
| Imipenem IPM 10 mcg | | 24-30 mm | | |
| Linezolid LZ 10 mcg | | 19-25 mm | | |
| Nitrofurantoin NIT 100 mcg | | 18-24 mm | | |
| Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg | | 26-34 mm | | |
| Vancomycin VA 5 mcg | | 10-16 mm | | |
| ***Enterococcus faecalis* ATCC33186 (00210*)** | luxuriant | | 34-36°C | 16-20 hours |
| Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg | | <=20 mm | | |
| ***Streptococcus pneumoniae* ATCC 49619** | luxuriant | | 34-36°C | 18-20 hours |
| Vancomycin VA 5 mcg | | 17-23 mm | | |
| ***Haemophilus influenzae* ATCC 49247** | luxuriant | | 34-36°C | 18-20 hours |
| Ampicillin AMP 2 mcg | | 6-12 mm | | |
| ***Haemophilus influenzae* ATCC 49766** | luxuriant | | 34-36°C | 18-20 hours |
| Cefixime CFM 5 mcg | | 29-35 mm | | |

Key : *Corresponding WDCM numbers.

## 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 (3,5).

## References

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11.ISO/TS 16782:2016, Confirmed in 2021 Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing
12.European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 14.0, valid from 2024-01-01.
13.European Committee on Antimicrobial Susceptibility Testing Routine and extended internal quality control as recommended by EUCAST Version 14.0, valid from 2024-01-01.

Revision : 06/2024

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**

10°C–30°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

**CE Marking**

**Do not use if package is damaged**

Disclaimer :

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# HiCrome™ UTI Agar

**M1353**

## Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

## Composition**

| Ingredients | g / L |
|---|---|
| Peptone, special (containing phenylalanine and tryptophan) | 15.000 |
| Chromogenic mixture | 2.450 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 6.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 32.45 gram in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

## Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli,* but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

## Type of specimen

Clinical samples : urine, faeces, Food samples, Water samples.

## 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 (10,11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

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.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.
4.Further biochemical and serological test are necessary for confirmation.

## 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**
Cream to yellow homogeneous free flowing powder
**Gelling**
Firm, comparable with 1.5% Agar gel
**Colour and Clarity of prepared medium**
Light amber coloured, clear to slightly opalescent gel forms in Petri plates
**Reaction**
Reaction of 3.24% w/v aqueous solution at 25°C. pH : 6.8±0.2
**pH**
6.60-7.20
**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of Colony |
|---|---|---|---|---|
| *Enterococcus faecalis* ATCC 29212 (00087*) | 50-100 | luxuriant | >=70% | blue (0.5 to 1.5 mm) |
| *Escherichia coli* ATCC 25922 (00013*) | 50-100 | luxuriant | >=70% | pink-purple |
| *Klebsiella pneumoniae* ATCC 13883 (00097*) | 50-100 | luxuriant | >=70% | blue to purple, mucoid |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50-100 | luxuriant | >=70% | colourless (greenish pigment may be observed) |
| *Proteus mirabilis* ATCC 12453 | 50-100 | luxuriant | >=70% | light brown |
| *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*) | 50-100 | luxuriant | >=70% | cream to golden yellow |
| # *Klebsiella aerogenes* ATCC 13048 (00175*) | 50-100 | luxuriant | >=70% | blue to purple |
| *Salmonella* Typhi ATCC 6539 | 50-100 | luxuriant | >=70% | colourless to amber |
| *Salmonella* Typhimurium ATCC 14028 (00031*) | 50-100 | luxuriant | >=70% | colourless to amber |

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

## Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°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

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Revision : 07/2024

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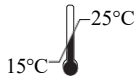
IVD *In vitro* diagnostic medical device




Storage temperature

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CE CE Marking

Do not use if package is damaged

Disclaimer :

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## Ceftazidime CAZ 30 mcg SD062

Ceftazidime CAZ 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

## Composition

| *Ingredients | Concentration |
|---|---|
| Ceftazidime | 30 mcg/disc |

## Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

## Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## Interpretation:

Use following interpretive criteria for susceptibility categorization*

| Antimicrobial agent | Interpretative criteria for | Sensitive mm or more | Intermediate mm | Resistant mm or less |
|---|---|---|---|---|
| Ceftazidime 30 mcg | *Enterobacteriaceae, B.cepacia* | 21 | 18-20 | 17 |
| | *P.aeruginosa, Acientobacter & Staphylococcus* | 18 | 15-17 | 14 |
| | *Haemophilus influenzae & Haemophilus parainfluenzae* | 26 | - | - |
| | *Neisseria gonorhoeae* | 31 | - | - |

## Quality Control:

**Appearance:** Filter paper discs of 6mm diameter with printed "CAZ 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

| Organisms (ATCC) | Std. zone of diameter (mm)* |
|---|---|
| *E.coli* (25922) | 25-32 |
| *S.aureus* (25923) | 16-20 |
| *P.aeruginosa* (27853) | 22-29 |
| *K.pneumonaie* (700603) | 10-18 |

* = Interpretive criteria & QC ranges as per CLSI standards.

## Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

## References:

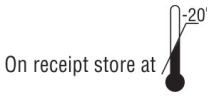
1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE Marking




Storage temperature

Do not use if package is damaged

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-400604, MS, India

EC REP

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Revision : 03 / 2022

## Amoxyclav AMC 30 mcg (Amoxycillin/Clavulanic acid) (20/10mcg) SD063

Amoxyclav (Amoxycillin/Clavulanic acid) AMC 30mcg (20/10mcg) discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

## Composition

| *Ingredients | Concentration |
|---|---|
| Amoxyclav (Amoxycillin/Clavulanic acid) | 30mcg/disc |

## Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

## Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## Interpretation:

Use following interpretive criteria for susceptibility categorization*

| Antimicrobial agent | Interpretative criteria for | Sensitive mm or more | Intermediate mm | Resistant mm or less |
|---|---|---|---|---|
| Amoxyclav (Amoxycillin/Clavulanic acid) 30mcg (20/10mcg) | *Enterobacteriaceae* | 18 | 14-17 | 13 |
| | *Haemophilus influenzae* & *Haemophilus parainfluenzae* | 20 | - | 19 |

## Quality Control:

**Appearance:** Filter paper discs of 6mm diameter with printed "AMC 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

| Organisms (ATCC) | Std. zone of diameter (mm)* |
|---|---|
| *E. coli* (25922) | 18-24 |
| *S.aureus* (25923) | 28-36 |
| *E.coli* (35218) | 17-22 |

* = Interpretive criteria & QC ranges as per CLSI standards.

## Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

## References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE Marking


Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

## Ceftriaxone CTR 30 mcg SD065

Ceftriaxone CTR 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

## Composition

| *Ingredients | Concentration |
|---|---|
| Ceftriaxone | 30 mcg/disc |

## Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

## Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## Interpretation:

Use following interpretive criteria for susceptibility categorization*

| Antimicrobial agent | Interpretative criteria for | Sensitive mm or more | Intermediate mm | Resistant mm or less |
|---|---|---|---|---|
| Ceftriaxone 30 mcg | *Enterobacteriaceae* | 23 | 20-22 | 19 |
| | *P.aeruginosa, Acientobacter & Staphylococcus* | 21 | 14-20 | 13 |
| | *Haemophilus influenzae & Haemophilus parainfluenzae* | 26 | - | - |
| | *Neisseria meningitidis* | 34 | - | - |
| | *Neisseria gonorhoeae* | 35 | - | - |
| | *Streptococcus spp. Viridians group* | 27 | 25-26 | 24 |
| | *Streptococcus spp. beta haemolytic gruop* | 24 | - | - |

## Quality Control:

**Appearance:** Filter paper discs of 6mm diameter with printed "CTR 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

| Organisms (ATCC) | Std. zone of diameter (mm)* |
|---|---|
| *E.coli* (25922) | 29-35 |
| *S.aureus* (25923) | 22-28 |
| *P.aeruginosa* (27853) | 17-23 |
| *K.pneumoniae* (700603) | 16-24 |

* = Interpretive criteria & QC ranges as per CLSI standards.

## Storage and Shelf-life:

On receipt discs should always be stored at -20°Cunder dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

## References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
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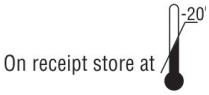
*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking




Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

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