

INSTRUCTIONS FOR USE

OXIDASE TEST STRIPS


 Oxidase Test Strips. From the top to bottom: *P.aeruginosa* (+) and *E.coli* (-)

1 - INTENDED USE

In vitro diagnostic device. For the detection of the cytochrome oxidase enzyme in bacteria isolated on solid culture media.

2 - COMPOSITION – PACK CONTENT

30 paper strips impregnated with N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride in a preservative solution.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The final stage of bacterial respiration may involve the use of the enzyme cytochrome oxidase, which catalyses the oxidation of cytochrome c while reducing oxygen to form water.¹ The cytochrome system is usually only present in aerobic organisms which are capable of utilising oxygen as the final hydrogen receptor.²

The test reagent, N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) acts as an artificial electron acceptor for the enzyme oxidase. When the reagent is oxidized by cytochrome c, it changes from colourless to a dark indophenol blue.

The oxidase test is used as an aid for the differentiation of *Neisseria*, *Moraxella*, *Campylobacter* and *Pasteurella* species (oxidase positive).² It is used to differentiate pseudomonads from related species; all *Pseudomonas* species are oxidase positive except a few *Pseudomonas* species that are oxidase negative; *P.luteola*, *P.oryzihabitans*, *P.syringae* and *P.viridiflava* are all oxidase negative.²

The performance of the oxidase test is essential for colonies suspected to belong to *Neisseria*; both *N.gonorrhoeae* and *N.meningitidis* give a positive reaction.³

Within *Bordetella* genus, *B.pertussis* and *B.bronchiseptica* are oxidase positive, *B.parapertussis* is oxidase negative.⁴

Within the *Burkholderia cepacia* complex all the species are oxidase positive with the exception of *B.contaminans* (variable) and *B.pyrrocinia* (variable)⁵

The oxidase test with the compound TMPD is recommended by ISO 16266,⁶ ISO 13720,⁷ ISO 11059⁸ for the confirmation test of *P.aeruginosa* or *Pseudomonas* spp. in water, meat and milk products and by ISO 9308⁹ for differentiating coliform bacteria (oxidase negative) from *Aeromonas* and other oxidase positive Gram negative bacteria.

Oxidase Test Strips are paper strips impregnated with Kovacs' oxidase reagent¹⁰. N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride in a preservative solution and dried.

4 - PHYSICAL CHARACTERISTICS

Paper strips appearance colourless

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Oxidase Test Strips	Paper strips for oxidase test	191040ST	30 paper strips 7.5 cm x 0.5 cm

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile platinum or plastic loop, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of colonies grown on plated or tubed media. Oxidase Test Strips cannot be used for the direct testing of clinical specimens.

8 - TEST PROCEDURE

Observe the culture for purity and sufficient growth on media such as blood agar, chocolate agar, Tryptic Soy Agar, Nutrient Agar.

Moist a portion of the paper strip with 2 drops of distilled water.

Use a platinum or plastic sterile loop and pick a well-isolated colony from a fresh (18 to 24-hour culture) bacterial plate and rub onto the moistened filter paper.

9 - READING AND INTERPRETATION

Examine the reaction area for the appearance of a colour ranging from blue to grey-blue.

Oxidase positive organisms produce a colour ranging from blue to grey-blue within 30 seconds.

Oxidase negative organisms produce no colour change within 30 seconds test period.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control: *P.aeruginosa* ATCC 27853; negative control: *E.coli* ATCC 25922

ATCC is a trademark of American Type Culture Collection





11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Oxidase Test Strips is tested with positive and negative strains.

Oxidase positive strains (blue to grey-blue colour appearance within 30 seconds): *N.gonorrhoeae* ATCC 19424, *P.aeruginosa* ATCC 27853, *P.aeruginosa* ATCC 10145, *P.fluorescens* ATCC 13525, *C.jejuni* ATCC 33291.

Oxidase negative strains (no colour appearance within 30 seconds): *E.coli* ATCC 25923, *C.sakasakii* ATCC 29544, *C.muytiensis* ATCC 51329.

12 - LIMITATIONS OF THE METHOD

- The oxidase test may be used in the presumptive identification of *Neisseria* spp. and in the differentiation and identification of Gram-negative bacilli. All oxidase-positive organisms should be examined by Gram stain to determine cellular morphology and Gram reaction
- Weak oxidase producers (e.g., *Pasteurella*) may appear negative within the time limits of the test.
- The test should not be performed on cultures from media containing tellurite and fermentable carbohydrates such as glucose, as these may prevent the reaction from occurring and give false negative results.²
- Bacteria grown on media containing dyes may give aberrant results.^{1,2}
- *Candida albicans* will occasionally give positive result with oxidase test when grown on chocolate agar but give negative reactions when grown on Sabouraud dextrose agar.²
- Use a young culture growing on an agar plate or agar slant, preferably less than 24 hr old. Older cultures are less metabolically active and results from these are unreliable.^{1,2}
- Using nickel, steel and other wire loops may give false-positive results and this may occur due to surface oxidation products formed during flame sterilisation. It is important to use only platinum or inert transfer loops, sterile wooden sticks, sterile plastic loops, sterile swabs, etc.^{1,2}
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the colonies. If relevant, perform antimicrobial susceptibility testing.
- This identification test is intended as an aid in the diagnostic procedures of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is classified as dangerous according to the current European legislation. Consult the Safety Data Sheet before use.
- Apply good laboratory practice guidelines when performing the test. The strip is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the strips, the culture media or the microbial strains.
- Sterilize all biohazard waste before disposal. Dispose the used and unused strips and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original pack at 2-8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. Do not use the strips with signs of deterioration (atypical colour).

15 - REFERENCES

1. Shields P, Cathcart L. Oxidase Test Protocol. ASM 11-11-2010. B, American Society for Microbiology © 2016.
2. Public Health England. (2019). Oxidase test. UK Standards for Microbiology Investigations. TP 26 Issue 4.
3. Elias J, Frosh M, Vogel U. *Neisseria*. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019
4. Kilgore PE, Coenye T. *Bordetella* and related genera. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019.
5. LiPuma JJ, Currie BJ, Peacock SJ, Vandamme PAR. Miscellaneous Gram-negative bacteria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019
6. ISO 16266:2006 Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane filtration
7. ISO 13720:2010 Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.
8. ISO/TS 11059:2009 [IDF/RM 225:2009] Milk and milk products — Method for the enumeration of *Pseudomonas* spp.
9. ISO 9308-1:2014 Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora.
10. Kovács, N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 1956; 178:703

101040ST OXIDASE TEST STRIPS

SDS rev 2

Regulation (EU) 2020/878

Contains: N,N,N',N', tetramethyl-p-phenylendiamine dihydrochloride

Classification

Eye irritation, category 2	H319	Causes serious eye irritation.
Skin irritation, category 2	H315	Causes skin irritation.





Labelling
Pictogram



Signal word

Warning

Hazard statements:

H319

Causes serious eye irritation.

H315

Causes skin irritation.

Precautionary statements:

P280

Wear protective gloves / eye protection / face protection.

P337+P313

If eye irritation persists: Get medical advice / attention.

P264

Wash . . . thoroughly after handling.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	Manufacturer	Store in a dry place	For single use only
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/04
Revision 3	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

