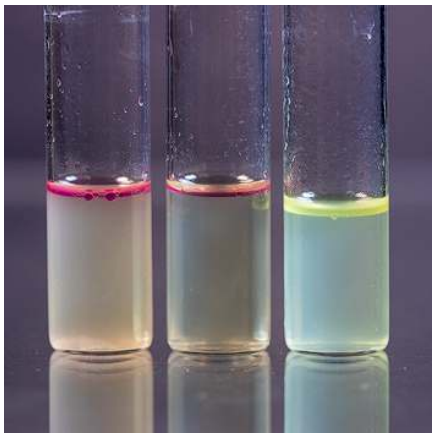
INSTRUCTIONS FOR USE

KOVACS' REAGENT



Kovacs'Reagent - from left: *E.coli* (+), *H.influenzae* (+), *S.*Typhimurium (-)

1 - INTENDED USE

In vitro diagnostic device. Reagent for indole test, used for the detection of tryptophanase production, as an aid in the differentiation of the *Enterobacteriaceae* and other genera.

2 - COMPOSITION – TYPICAL FORMULA (100 mL)

p-dimethylaminobenzaldehyde	5 g
N-amyl alcohol	75 mL
Hydrochloric acid (concentrated)	25 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bacteria that possess the enzyme triptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.[1] The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan. The indole test is based on the formation of a red colour complex when indole reacts with aldehyde group of p-dimethylaminobenzaldehyde, under acidic conditions. Typically indole test can be performed by tube method and by spot test.[1-3] Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E.coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative). It is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.[1] Kovács' Reagent for indole test is included in several ISO Standards[4-8] as an aid in the identification of *E.coli*, *E.coli* O157, *Salmonella*, *Vibrio*, *Yersinia*, isolated from the food chain.

4 - PHYSICAL CHARACTERISTICS

Appearance of the reagent limpid, yellow solution

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Kovacs' Reagent	Liquid reagent	19171000	50 mL, primary packaging: bottle with dropper; secondary packaging: cardboard box
Kovacs' Reagent	Liquid reagent	19171001	500 mL, primary packaging: crew capped bottle

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Screw capped tubes, filter paper, sterile loops, tryptophan rich culture media, ancillary reagents for the complete identification of the colonies.

7 - SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of colonies grown on plated media. Kovács' Reagent cannot be used for the direct testing of clinical specimens.

8 - TEST PROCEDURE

Indole tube test

Inoculate the Tryptone Tryptophan Broth (REF 402165) or Peptone-Tryptone Water (REF 401891) with the test organism and incubate at 37°C for 24 to 48 h.

- add several drops of the Kovács' Reagent and shake gently.
- examine the upper layer of liquid after about 1 min for the appearance of a red-pink colour.

Indole spot test

Inoculate the bacterium to be tested on an agar medium that contains tryptophan. Tryptic Soy Agar (REF 402150) or Blood Agar Sheep plates (REF 541136) can be used. Incubate for 18 to 24 hours at the appropriate temperature to allow the growth.
Place a piece of filter paper (Whatman no.1) into a sterile Petri dish and moisten with 1 -1.5 mL of Kovács' Reagent.
Smear an isolated pure colony onto the saturated surface of the filter paper using a sterile loop and examine for the appearance of a red-pink colour within 1-3 minutes.

9 - READING AND INTERPRETATION

Indole tube test

Positive result: formation of a pink to red colour within 1 minute (occurring normally within a few seconds)
Negative result: no colour change, the reagent layer remains yellow or slightly cloudy

Indole spot test

If indole is present, a red-pink colour will develop within 1 to 3 minutes.

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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: *Escherichia coli* ATCC 25922
Negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Kovacs' Reagent is tested with positive and negative strains.
Indole positive strains (appearance of a red-pink colour on the upper layer of Peptone-Tryptone Water within few seconds): *E.coli* ATCC 25922, *E.coli* O157 ATCC 43888, *P.vulgaris* ATCC 13315, *P.morgani* CB118, *P.rettgeri* ATCC 39944, *Y.enterocolitica* ATCC 23715.
Indole negative strains (no colour change of the upper layer of Peptone-Tryptone Water within 3 minutes): *P.mirabilis* ATCC 12453, *E.aerogenes ATCC 13048, S.*Typhimurium ATCC 14028.

12 - LIMITATIONS OF THE METHOD

- This test should be used only on colonies from media containing sufficient tryptophan and no glucose.[9]
- Colonies from mixed culture should not be used, because indole-positive colonies can cause indole negative colonies to appear weakly positive.[9]
- If Peptone Broth, other than the suggested reference, is used instead of Tryptophan broth, the batch should be checked with a positive control to ensure the peptone is adequate for indole production. This is because there are varieties of peptone broth media on the market, and some are unsuitable for indole production because they contain too little tryptophan.[2]
- Organisms to be tested by the spot indole method must be taken from a tryptophan-containing medium (for example blood agar) and never from MacConkey agar media as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult.[2]
- Indole is a diffusible product. To mitigate indole diffusion, select a well isolated colony for the spot indole test.[2]
- The tube test is a more sensitive method of detecting indole than the spot test.
- Kovacs Indole Reagent is not recommended for use with anaerobic bacteria.
- Change in colour of the reagent from yellow to brown indicates improper storage, which may cause weaker reactions.
- 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 culture medium 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 the use.
- Apply good laboratory practice guidelines when performing the test.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the reagent, the culture media or the microbial strains.
- Sterilize all biohazard waste before disposal. Dispose the used and unused tubes 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 with the cap tightly closed, at 10-30°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 reagent with atypical brown colour.

15 – REFERENCES

1. Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
2. Public Health England. UK Standards for Microbiology Investigation, Indole test. TP 19, Issue 4, 2018.
3. Cowan and Steel's Manual for identification of Medical Bacteria, 3rd ed. 1993.
4. ISO 7251:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive Escherichia coli. Most probable number technique.
5. ISO 16654:2001Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157.
6. ISO 6579-1:2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp.
7. ISO 21872-1:2017 Microbiology of the food chain. Horizontal method for the determination of Vibrio spp. — Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.
8. ISO 10273:2017 -Microbiology of the food chain. Horizontal method for the detection of pathogenic Yersinia enterocolitica.
9. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

19171000 KOVACS'REAGENT
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Classification

The product is classified as hazardous

Flammable liquid, category 3	H226	Flammable liquid and vapour.
Substance or mixture corrosive to metals, category 1	H290	May be corrosive to metals.
Acute toxicity, category 4	H332	Harmful if inhaled.
Eye irritation, category 2	H319	Causes serious eye irritation.
Skin irritation, category 2	H315	Causes skin irritation.
Specific target organ toxicity - single exposure, category 3	H335	May cause respiratory irritation.

Labelling
Pictograms

Signal words:	Warning
Hazard statements:	
H226	Flammable liquid and vapour.
H290	May be corrosive to metals.
H332	Harmful if inhaled.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H335	May cause respiratory irritation.
Precautionary statements:	
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P370+P378	In case of fire: use . . . to extinguish.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P312	Call a POISON CENTRE / doctor / . . . if you feel unwell.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.

Contains: HYDROCHLORIC ACID, PURE N-AMYL ALCOHOL

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2022/04
Revision 2	Removal of obsolete classification	2023/04

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

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