

INDOLE SPOT REAGENT

1 - INTENDED USE

Liquid reagent for indole spot test, as an aid in the differentiation of the *Enterobacteriaceae* and other genera.

2 - COMPOSITION – BOTTLE CONTENTS

p-dimethylaminocinnamaldehyde 0.1 g
HCl 10% 10 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. Typically, indole test can be performed by tube method and by spot test.¹⁻³ Indole Spot Reagent has been devised for spot test. The indole spot test is based on the formation of a green-blue colour complex when indole reacts with aldehyde group of p-dimethylaminocinnamaldehyde. 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*.

4 - PHYSICAL CHARACTERISTICS

Appearance of the reagent limpid, yellow solution

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Indole Spot Reagent	Liquid reagent	19171003	10 mL, primary packaging: bottle with dropper

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

Inoculate the strain 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.

Touch the colonies to be tested with the tip of a swab or place a piece of filter paper into a sterile Petri dish and smear an isolated pure colony using a sterile loop. Squeeze 2 drops of Indole Spot Reagent onto the swab or the filter paper.

9 - READING AND INTERPRETATION

Examine the reaction area and record the colour.

Production of a distinct green-blue colour within 30 seconds: indole positive strain.

No colour production or orange or violet colour production within 30 seconds: indole negative strain.

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 Indole Spot Reagent is tested with positive and negative strains.

Indole positive strains: *E. coli* ATCC 25922, *P. vulgaris* ATCC 13315, *P. morgani* CB118*, *P. rettgeri* ATCC 39944, *P. incostans* ATCC 33672.

Indole negative strains: *P. mirabilis* ATCC 12453, *E. aerogenes* ATCC 13048.

* CB: Collezione Microbica Biolife

12 - LIMITATIONS OF THE METHOD

- This test should be used only on colonies from media containing sufficient tryptophan and no glucose.⁴
- Colonies from mixed culture should not be used, because indole-positive colonies can cause indole negative colonies to appear weakly positive.⁴





- 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
- 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.

13 - PRECAUTIONS AND WARNINGS

- This product is for laboratory use and 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.
- 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 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 reagent with atypical brown colour.

15 – REFERENCES

- Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
- Public Health England. UK Standards for Microbiology Investigation, Indole test. TP 19, Issue 4, 2018.
- Cowan and Steel's Manual for identification of Medical Bacteria, 3rd ed. 1993.
- 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.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Fragile, handle with care	Manufacturer	This side up
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 3	Updated layout and content	2023/03

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

