# Kovac's Indole Reagent

**R008**

## Intended use

For detection of presence of indole produced by microorganisms due to tryptophan deamination.

## Composition**

| Ingredients | - |
|---|---|
| p-dimethylamino benzaldehyde | 5.000 |
| Amyl alcohol | 75.000 |
| Hydrochloric acid, concentrated | 25.000 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Add 0.2 - 0.3 ml of Kovac's reagent to 5 ml of a 24 - 48 hours old culture of the organism under investigation. Formation of a red coloured ring indicates positive indole test.

## Principle And Interpretation

Peptone Water is particularly suitable as a substrate in the study of indole production. Peptone used in Peptone Water, is rich in tryptophan content (1). Other peptones which contain tryptophan can be used to study indole production. Tryptone Water is also recommended by APHA (2) for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. It is used as part of the IMViC procedures. Most strains of *E. coli*, *P. vulgaris*, *P. rettgeri*, *M. morgani* and *Providencia* species break down the amino acid tryptophan with the release of indole. The presence of indole can be detected by the addition of Ehrlich's or Kovac's reagent (p-dimethylaminobenzaldehyde).

Kovacs reagent is a biochemical reagent consisting of isoamyl alcohol, para-dimethylaminobenzaldehyde (DMAB), and concentrated hydrochloric acid. It is used for the diagnostic test, to determine the ability of the organism to split tryptophan into indole and alpha-aminopropionic acid by hydrolytic activity of bacteria that express tryptophanase enzyme (3). The indole produced is indicated by formation of a red coloured ring, soluble in ether, chloroform and alcohol. This was invented by the Hungarian-Swiss Chemist, Ervin Kovats. Indole production is used as, a tests designed to distinguish among members of the family Enterobacteria.

## Type of specimen

Clinical samples ; Water samples

## Specimen Collection and Handling

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

## Warning and Precautions :

In Vitro diagnostic 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 guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets

## Limitations :

1. Growth media must contain an adequate amount of tryptophan. Do not use Mueller- Hinton Agar for test, because tryptophan is destroyed during the acid hydrolysis of casein.
2. Do not used media that contain dyes (e.g., EMB, MAC).
3. Do not use medium with a nitrate disc/strip to perform the indole test, as nitrate can interfere with indole test by including false positive results.

## Performance and Evaluation

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

## Quality Control

**Appearance**
Greenish yellow coloured solution

**Solubility**
Immiscible with water

**Clarity**
Clear with no insoluble particles.

**Cultural Response**
Characteristic reactions observed when Kovac's Indole Reagent is added to growth in Tryptone Broth (M463).

| Organism | Indole production |
|---|---|
| *Enterobacter aerogenes* ATCC 13048 | negative reaction ,no red ring |
| *Escherichia coli* ATCC 25922 | positive reaction, red ringreaction, red ring at the interface of the medium |

## Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

## 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 (4,5).

## Reference

1.MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.
2.Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
3.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
4.Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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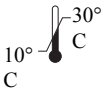
IVD — In vitro diagnostic medical device

CE Marking




Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited, B /4-6 , MIDC, Dindori, Nashik MH
www.himedialabs.com

EC REP CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner 4u.eu