

Kovacs' Indole Reagent

Intended use

For Indole Test, particularly useful in the identification of *E. coli*.

Summary

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase. When tryptophan is broken down, the presence of indole can be detected through the use of Kovac's Reagent. Kovac's Reagent, which is yellow, reacts with indole and produces a red colour on the surface of the test tube. This test is particularly useful in the identification of *E. coli*.

Principle

Indole is tested for by a colorimetric reaction with Kovac's Reagent (p-dimethyl-amino-benzaldehyde). Indole reacts with the aldehyde present in Kovac's Reagent to give a reddish-pink colour in the alcohol layer, which indicates a positive result. The alcohol layer extracts and concentrates the red colour complex.

Reagents/contents

The Microxpress Kovac's Reagent is a reagent set for laboratory use only.

The Kovac's Reagent comprises of:

5% p-dimethyl-amino-benzaldehyde.

Storage and stability

1. Store the Kovac's Reagent at 15°C-25°C away from light.
2. Stability of the Kovac's Reagent is as per the expiry date mentioned on the label.

Procedure

Preparation of Inoculum

1. Isolate the organism to be identified on Nutrient Agar or Brain Heart Infusion Agar.
2. Pick up a single isolated colony and inoculate it in 4-5 mL Brain Heart Infusion Broth.
3. Incubate at 37°C for 6-8 hours until inoculum turbidity is between 0.1- 0.2 at 620 nm. Alternatively, a homogenous suspension made in 2-3 mL sterile saline adjusted to a turbidity of 0.1- 0.2 at 620 nm can also be used as inoculum.

Test procedure

1. Inoculate an aliquot (1 mL) of a suitable medium like Tryptone Water with the above-prepared inoculum (approx. 100 mL) and incubate for 6-8 hours at 35°C-37°C.
2. Observe for growth.
3. Add 2-3 drops of Kovac's Reagent directly to the tube and agitate.
4. Check for characteristics results.

Appearance: Greenish yellow coloured clear solution.

Interpretation of results

1. Formation of a red coloured ring in the alcohol layer is indicative of a positive test.
2. No change in colour is indicative of a negative test.

Quality Control

Organisms (ATCC)

Klebsiella aerogenes (13048)

Escherichia coli (25922)

Reaction in Indole test

-

+

Key:

+ = Red colour ring in alcohol layer

- = No Red colour ring

Precautions/limitations

1. The Kovac's Reagent is an in vitro diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Kovac's Reagent cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
4. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
5. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of gram reaction, morphology, and other biochemical and serological tests.
6. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited By J. G. Collee, J. P. Duguid.
2. Clarke P.H. And S.T. Cowan, 1952, Biochemical Methods For Bacteriology, J. Gen. Microbiol; 6:187-197.
3. Clinical Diagnosis & Management By Laboratory Methods, Todd, Sanford And Davidsohn, 17th Edition 1998, Edited By John Bernard Henry.
4. Spot Indole Test: Evaluation of Four Reagents, Journal of Clinical Microbiology, Apr. 1982, Vol. 15, No. 4, p: 589-592.
5. Quantitation of The Tetramethyl-P-Phenylenediamine Oxidase Reaction In Neisseria Species, By Peter Jurtshuk and Thomas W. Milligan, Applied Microbiology, Dec. 1974, Vol. 28, No.6, p: 1079-1081.
6. Evaluation of two spot-indole reagent, B.L.Lawrence, P.reich and W.H.Traub 1969, Appl. Microbiol; 17:p 923-924.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
204110090100	Kovacs' Indole Reagent	100 mL

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
