

# AG- KOVAC'S REAGENT FOR INDOLE TEST (IFU)

# **PRINCIPLE:**

AG-Kovac's reagent is a chemical reagent used to detect the presence of indole in a bacterial sample. Indole is a nitrogen-containing aromatic organic compound that is produced by some bacteria as a byproduct of tryptophan metabolism, along with two other major end products: indole, pyruvic acid, and ammonia. Detection of indole indicates tryptophan degradation and can be accomplished by the addition of certain aldehydes to form colored end products.

Kovac's reagent consists of p-dimethylaminobenzaldehyde. When Kovacs reagent is added to a bacterial sample that has produced indole through metabolic reaction, the reagent reacts with the indole to produce a red-pink color. This reaction is known as the Kovac's test. The Kovacs test is commonly used in the identification of bacteria belonging to the Enterobacteriaceae family, such as *Escherichia coli* and *Klebsiella pneumoniae*, which are indole-positive. Other bacterial species, such as *Salmonella* and *Shigella*, are indole-negative and do not produce a color change when treated with Kovac's reagent.

#### **PACKAGE CONTENTS:**

Description	Catalogue Number	Quantity
Kovac's Reagent	AG/Reagent/KR/22/01	200 mL

## **STORAGE & STABILITY:**

- Store at 2-8°C in the dark.
- Seal the container tightly after use.
- Under recommended conditions, the stain is stable until the mentioned expiry date

## **TYPES OF SPECIMENS:**

#### Bacterial cultures.

## **DIRECTIONS/PROCEDURES:**

- 1. Using a sterile inoculating loop, lightly inoculate 4 ml of Casein or Tryptone Broth using growth from an overnight, pure culture plate.
- 2. Incubate at 35°C for 24 or 48 hours. If testing is performed after 24 hours it is recommended that a 2ml portion be removed aseptically for the test. If negative, the remaining broth should be re-incubated an additional 24 hours and retested.
- 3. Add five drops (0.5mL) of Kovac's Reagent and shake the tube gently.
- 4. Check for a color change immediately.

## **INTERPRETATION RESULTS:**

- Positive: Development of a pink to cherry red color at the interface of the reagent and the broth within 30 seconds.
- Variable: Orange color at the surface of the medium (usually occurs after 24 hrs., re-incubate for additional 24 hrs. and re-test).
- Negative: No color change (yellow).

## LIMITATIONS:

- Only experienced personnel should carry out the interpretation of results.
- A variable result may occur due to the formation of skatole, a methylated compound that can be a precursor to indole formation.
- Some organisms form indole but break it down as rapidly as it is produced and therefore false negative reactions may occur. This
- occurs mainly among some *Clostridium* species.
  Madia used for growth must be tested using a known positive and negative control as some type
- Media used for growth must be tested using a known positive and negative control as some types of media may affect the result.

# WARNINGS & PRECAUTIONS:

- For In vitro diagnostic and professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiry dates.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Samples should be processed in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.

#### SYMBOLS:





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