

## INDOLE TEST

### Principle and Purpose

The amino acid tryptophan is incorporated into nearly all proteins. Proteins are readily degraded by microorganisms using a slew of enzymes collectively termed proteases. Some bacteria possess an additional enzyme, tryptophanase, that metabolizes any released tryptophan to indole, pyruvic acid, and ammonia. The latter two are used for a cell's nutritional needs, whereas indole is not and accumulates in the medium.

Indole can be detected upon the addition of Kovacs' Reagent. When indole combines with *p*-dimethylamino-benzaldehyde, a red band forms at the top of the medium (Fig. 1). A negative indole test produces no color change upon the addition of Kovacs' Reagent. This test is part of those employed with SIM media, also. Yet, in this particular exercise, students will instead employ tryptic soy broth which is rich in tryptophan.

### Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying mechanism of the indole test;
- Properly conduct the indole test; and
- Accurately interpret the test results.

### Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 24-48 hour TSA slant cultures:

- *Klebsiella oxytoca* (ATCC 49131) [abbreviated as *K. oxytoca*]
- *Salmonella enterica* serovar Typhimurium (ATCC 14028) [abbreviated as *Sal. enterica*]
- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Shigella flexneri* (ATCC 12022) [abbreviated as *S. flexneri*]

Media and Reagents

- Tryptic Soy Broth (TSB) tubes

**Note:** Tryptone Broth or Peptone Broth can be used in place of TSB.

- Kovacs' Reagent (Cat. No. Z67; Hardy Diagnostics;  
[https://catalog.hardydiagnostics.com/cp\\_prod/Content/hugo/IndoleTestRgnts.htm](https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/IndoleTestRgnts.htm))



**Figure 1. Indole Test Results.** Four tubes of TSB were inoculated, from left to right, with *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Shigella sonnei*. Kovacs' reagent was added to each tube after incubation at 37°C for 24 hours. Both the *K. oxytoca* and *E. coli* cultures reveal the formation of a red layer indicating a positive indole test. The other two cultures did not form a red layer which indicates that indole was not produced by these bacteria.

## Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

- 1) Obtain four (4) TSB tubes and allow them to warm to room temperature before use.
- 2) Label one of the tubes as '*E. coli*', a second as '*Sal. enterica*', a third as '*S. flexneri*', and the remaining tube as '*K. oxytoca*'. Be sure to add other identifying information as appropriate.
- 3) Inoculate the media as indicated below:
  - a) Aseptically transfer some cells on a microbiological loop from a TSA slant culture of *E. coli* to the appropriately labeled TSB tube.
  - b) Aseptically transfer some cells on a microbiological loop from a TSA slant culture of *Sal. enterica* to the appropriately labeled TSB tube.
  - c) Aseptically transfer some cells on a microbiological loop from a TSA slant culture of *S. flexneri* to the appropriately labeled TSB tube.
  - d) Aseptically transfer some cells on a microbiological loop from a TSA slant culture of *K. oxytoca* to the appropriately labeled TSB tube.
- 4) Incubate all the tubes at 37°C for 18/24-48 hours.
- 5) Remove the tubes from the incubator. To test for indole production, apply 3-5 drops of Kovacs' Reagent to the surface of the broth and mix by gentle shaking. Observe if development of a pink to red color occurs.

**Interpretation of Results:** A positive test for indole is indicated by the formation of a pink to red color band at the top of the medium (in an alcohol layer) after the addition of Kovacs' Reagent (see Fig. 1). If the layer remains colorless or yellow color, a negative indole test reaction is indicated.

*Record your observations on the report sheet attached to this exercise.*

Student Name: \_\_\_\_\_

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS

Observations	Bacteria Tested			
	<i>Escherichia coli</i>	<i>Klebsiella oxytoca</i>	<i>Salmonella enterica</i> serovar Typhimurium	<i>Shigella flexneri</i>
Color After Adding Kovacs' Reagent				
Indole Production?				

**Discussion Question:**

Is the bacterial production of indole a curious side effect of tryptophan degradation or does its production serve purpose? Speculate why and support your argument. (This answer should be supported with some background literature research. Be sure to cite your sources.)