SULFIDE-INDOLE-MOTILITY (SIM) TEST

Principle and Purpose

Using Sulfide-Indole-Motility (SIM) media, various Gram-negative enteric bacilli can be distinguished based upon their ability to release sulfide gas (H_2S), produce indole from tryptophan, and move into the medium via the use of flagella. SIM medium contains ferrous ammonium sulfate and sodium thiosulfate. If a microbe hydrolyzes proteins rich in the amino acid cysteine, which contains a sulfur atom, the sulfur is released as H_2S. The sulfide gas then combines with ferrous ammonium sulfate to form ferrous sulfide. The latter is detected as a black precipitate in the medium (Fig. 1). Microbes may also reduce inorganic sulfur-containing compounds, like thiosulfate, and release sulfide gas. Again, the H_2S interacts with ferrous ammonium sulfate in the medium to form the black precipitate, ferrous sulfide.

Figure 1. Sulfide and Motility Test Using SIM media. The left image depicts a positive hydrogen sulfide reaction as well as motility of the test organism (*Salmonella enterica* serovar Typhimurium). The right image shows a negative hydrogen sulfide reaction, but the cloudy appearance of the medium indicates that the test organism (*Escherichia coli*) is motile.

With regard to motility, SIM is a semi-solid medium which allows motile organisms to migrate away from the inoculating stab line. If an H_2S-producing organism is motile, the resulting black precipitate becomes diffuse as the microbe spreads through the medium. In contrast, the motility of non-H_2S producing bacteria is visually detected by turbid or cloudy nature of the medium compared to an uninoculated control tube (Fig. 1). Non-motile organisms grow only along the stab line and shall not cause any turbidity or cloudiness of the surrounding medium.

Finally, indole production is detected in SIM medium due to the presence of casein peptone, an ingredient rich in tryptophan. Organisms that possess the enzyme tryptophanase degrade tryptophan to generate 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. Indole combines with the *p*-dimethylaminobenzaldehyde in the reagent to produce a red band at the top of the medium (Fig. 2). A negative indole test produces no color change upon the addition of Kovacs' Reagent.

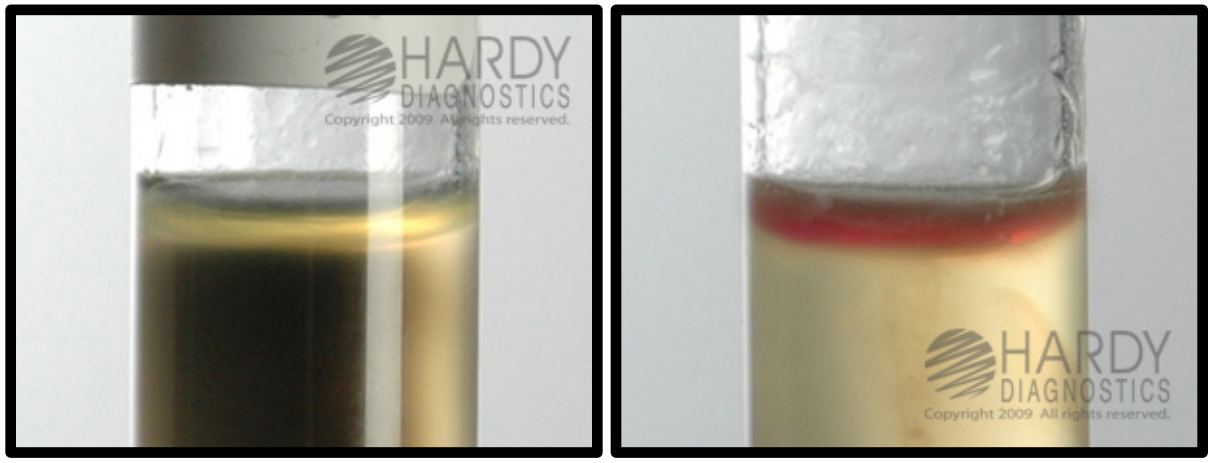


Figure 2. Indole Test Using SIM media. The left image depicts a negative indole reaction following the addition of Kovacs' Reagent organism (*Salmonella enterica* serovar Typhimurium). The right image shows a positive indole reaction (*Escherichia coli*).

In this exercise, students will examine the sulfide and indole production of selected bacterial species, as well as their motility. A commercially available medium will be used for this purpose (Hardy Diagnostics).

Learning Objectives

Upon completion of this exercise, a student will be able to demonstrate the ability to:

- Understand the various underlying mechanisms of each component of the sulfide-indole-motility test;
- Properly conduct the sulfide-indole-motility test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

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

Note: This exercise requires that the inoculum original from a solid medium. Use of an inoculum from a broth will delay growth, possibly leading to the misinterpretation of results.

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

Note: *Salmonella enterica* includes a number of 'serovars', i.e., serologically distinct groups. When used in this and other exercises, cultures of the specific serovars will be labeled according to their serovar, e.g., *S.* Cholerasuis, as opposed to the taxonomically specific name, e.g., *Salmonella enterica* serovar Choleraesuis.

Media and Reagents

- SIM medium (Cat. No. Q30; Hardy Diagnostics, Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/SIMMedium.htm)
- Kovacs' Reagent (Cat. No. Z67; Hardy Diagnostics Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/IndoleTestRgnts.htm)

Procedures

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

1) Obtain four (4) SIM agar deeps and allow them to warm to room temperature before use.
2) Label one of the tubes as '*E. coli*', a second as '*S.* Typhimurium', 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 obtain some cells on a microbiological needle from a TSA slant culture of *E. coli*, then stab the appropriately labeled SIM medium tube three-quarters deep. Be sure to withdraw the needle along the same line in which it was inserted.
   b) Aseptically obtain some cells on a microbiological needle from a TSA slant culture of *S.* Typhimurium, then stab the appropriately labeled SIM medium tube three-quarters deep. Be sure to withdraw the needle along the same line in which it was inserted.
   c) Aseptically obtain some cells on a microbiological needle from a TSA slant culture of *S. flexneri*, then stab the appropriately labeled SIM medium tube three-quarters deep. Be sure to withdraw the needle along the same line in which it was inserted.
   d) Aseptically obtain some cells on a microbiological needle from a TSA slant culture of *K. oxytoca*, then stab the appropriately labeled SIM medium tube three-quarters deep. Be sure to withdraw the needle along the same line in which it was inserted.

Note: Remove the inoculating needle along the same line used to inoculate the medium. Removal of the needle otherwise may result in a false-positive interpretation.

4) Incubate all the tubes at 37°C for 18-24 hours. Be sure that the screw-cap lid is loosened, but not to the degree at which it can fall off.
5) Remove the tubes from the incubator and examine the tube for hydrogen sulfide (H_2S) production and motility.

Interpretation of Results: A blackening of the medium along the line of inoculation indicates a positive H_2S test. The absence of any blackening indicates a negative H_2S test.

A diffuse zone of growth flaring from the line of inoculation indicates a positive motility test. The absence of such growth or its confinement to the stab line is indicative of a negative motility test. If H_2S-producing bacteria obscure the butt of the tube, assume this is a positive motility reaction.

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

6) To test for indole production, apply three drops of Kovacs' Reagent to the surface of the medium. Observe for the development of a pink to red color.

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 after the addition of Kovacs' Reagent. If a yellow color remains, this indicates a negative indole test.

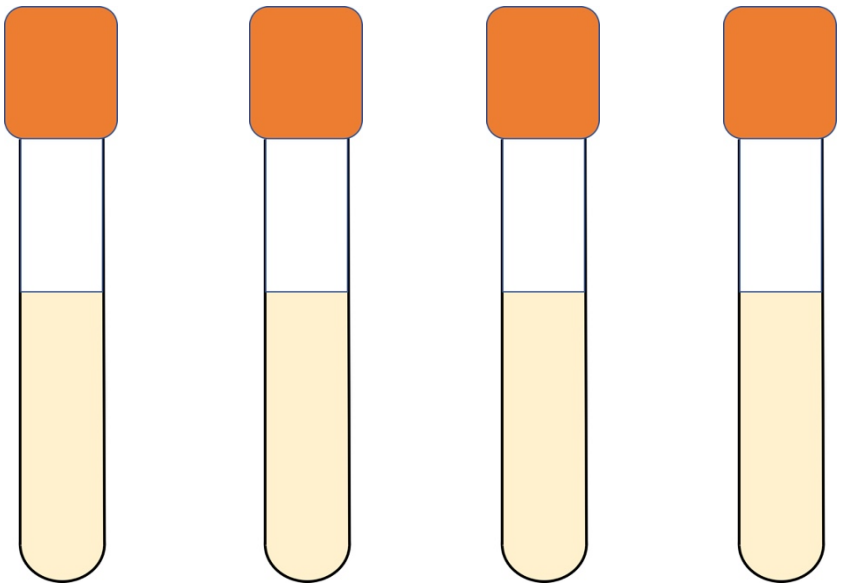
Record your observations on the report sheet attached to this exercise.

Note: H_2S production and the motility reaction must be read and recorded prior to testing for indole production.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS, THEN SKETCH THE RESULTS ON THE FIGURES BELOW EACH ORGANISM LISTED IN THE TABLE.

Observations of Inoculated SIM Media (+ or -)	**Bacteria Tested**			
	Escherichia coli	*Klebsiella oxytoca*	*Salmonella enterica* serovar Typhimurium	*Shigella flexneri*
H_2S Production				
Motility				
Indole Production				



E. coli *K. oxytoca* *S, enterica* *S. flexneri*

Discussion Question:

What are some common/known sources where bacterial hydrogen sulfide production occurs? Why do bacteria generate hydrogen sulfide from these sources?