CITRATE UTILIZATION TEST

Principle and Purpose

The primary use of Simmons Citrate Agar is to differentiate among various Gram-negative enteric bacilli based upon their ability to utilize citrate. The original medium was a broth formulation that was later modified to function as a solid medium, thereby helping to eliminate errors in determining if growth of the test organism did or did not occur.

Simmons Citrate Agar contains ammonium dihydrogen phosphate and sodium citrate. The former serves as the sole nitrogen source, whereas the latter is the sole carbon source. The medium also contains the pH indicator bromothymol blue. Microbes able to use the nitrogen and carbon sources grow well on this medium provided oxygen is present. Hence, the modification of the medium from a broth to an agar slant was made to facilitate this requirement. When citrate is metabolized, an alkaline carbonate is generated which causes the pH of the medium to increase, i.e., become more alkaline. In such a situation, the bromothymol blue in the medium changes from a green (neutral) to a blue (alkaline) color (Fig. 1). In contrast, the medium will remain green for organisms unable to metabolize citrate.

In this exercise, students will examine the citrate utilization patterns of selected bacterial species. A commercially-available Simmons Citrate Agar medium (Hardy Diagnostics) will be used for this purpose.

Figure 1. Simmons citrate test. The left image depicts a negative reaction (*Escherichia coli*), whereas the right image shows a positive citrate utilization reaction (*Salmonella enterica* serovar Typhimurium)

Learning Objectives

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

- Understand the underlying mechanism of the citrate utilization test;
- Properly conduct the citrate utilization 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 18-24 hour-old TSA slant cultures:

- *Enterobacter cloacae* (ATCC 23355) [abbreviated as *Ent. cloacae*]
- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Klebsiella pneumoniae* (ATCC 13883) [abbreviated as *K. pneumoniae*]
- *Proteus hauseri* (ATCC 13315) [abbreviated as *P. hauseri*]

Media and Reagents

- Simmons Citrate agar (Cat. No. L80; Hardy Diagnostics, Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/SimmonsCitrateAgar.htm)

Procedures

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

1) Obtain four (4) Simmons Citrate agar slants and allow them to warm to room temperature before use.
2) Label one of the tubes as '*E. coli*', a second as '*Ent. cloacae*', a third as '*K. pneumoniae*', and the remaining tube as '*P. hauseri*'. Be sure to add other identifying information as appropriate.
3) Using a microbiological loop and aseptic technique, lightly inoculate the appropriately labeled tube of medium with cells from a TSA slant culture by streaking the surface in a serpentine manner.
4) Incubate all the tubes at 37°C for 18-96 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 daily and examine for growth and a color change.

Interpretation of Results: A positive reaction is indicated by growth and concurrent development of a deep blue color reaction within the medium. Conversely, a negative reaction is evidenced by no growth and the medium remaining green in color.

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

If a positive reaction is observed, the tube can then be discarded. If no reaction has occurred, return the tube(s) to the 37°C incubator for another 24 hours up to a total incubation time of 96 hours and make periodic observations.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS

Observations	Bacteria Tested			
	Escherichia coli	*Enterobacter cloacae*	*Klebsiella pneumoniae*	*Proteus hauseri*
Growth visible?				
Color of tube?				
Positive or Negative Reaction				

Discussion Questions:

1. Why is a chemically defined medium necessary to detect the utilization of citrate by a test organism?

2. Is Simmons Citrate medium a selective medium? Why or why not?

3. Is Simmons Citrate medium a differential medium? Why or why not?