

UREASE TEST

Principle and Purpose

The urease test detects those microbes that are capable of breaking down urea into ammonia and carbon dioxide. The enzyme urease carries out this reaction, but it is uncommon among enteric bacteria. Those genera that typically do not exhibit urease activity include *Salmonella*, *Shigella* and *Escherichia*. Notable species that do express urease activity include *Proteus mirabilis* and *Helicobacter pylori*. The former is a significant cause of urinary tract infections, where urea would naturally occur, and the latter is a cause of peptic ulcers in which the urease likely produces a localized alkaline environment in the stomach, thereby protecting this bacterium from digestive acids.

In this exercise, two different urease tests will be conducted, but both operate in similar ways. One test is combined with the possibility to also detect indole production. Urease is detected in both tests by a color change of the incorporated pH indicator. When urea is hydrolyzed by urease, ammonia is released and accumulates in the medium. This causes the medium to become alkaline and increases the pH. The indicator dye changes to a deep pink or purple red (cerise) color, which indicates a positive test for urease action (Fig. 1). If no such color appears, then the test is negative for urease activity.

Students will conduct urease tests using the commercially available Urease Test Tablets and the Urease/Indole tablets (Key Scientific). Using the latter test tablets in conjunction with Kovac's reagent, the production of indole by particular organisms can also be detected (Fig. 2).

Learning Objectives

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

- Understand the biochemical basis of the urease test;
- Properly perform the urea test and distinguish between positive and negative reactions; and
- Discern how this information can be used to differentiate and identify microbial species.

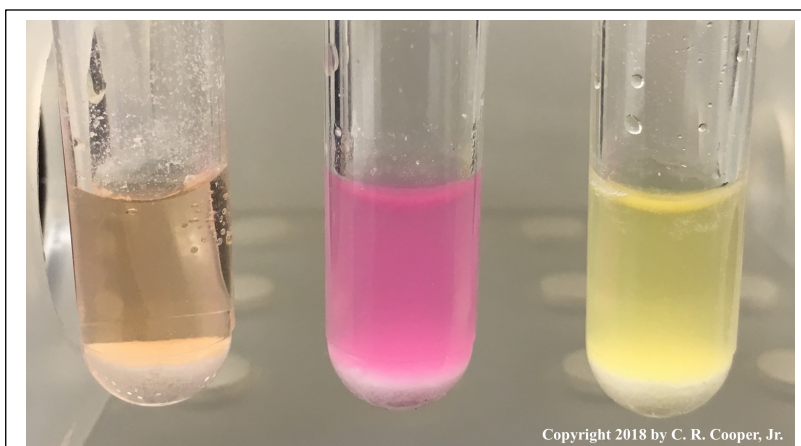


Figure 1. Urease Test Results. The three tubes contain rehydrated Urease Test tablets. The left tube was not inoculated and served as a control. The middle tube was inoculated with *Proteus hauseri*, whereas the right tube was inoculated with *Escherichia coli*. All tubes were incubated at 37°C for 24 hours. The red-pink color of the middle tube indicates that *P. hauseri* possesses urease activity. The yellow color of the right tube depicts a negative urease test.

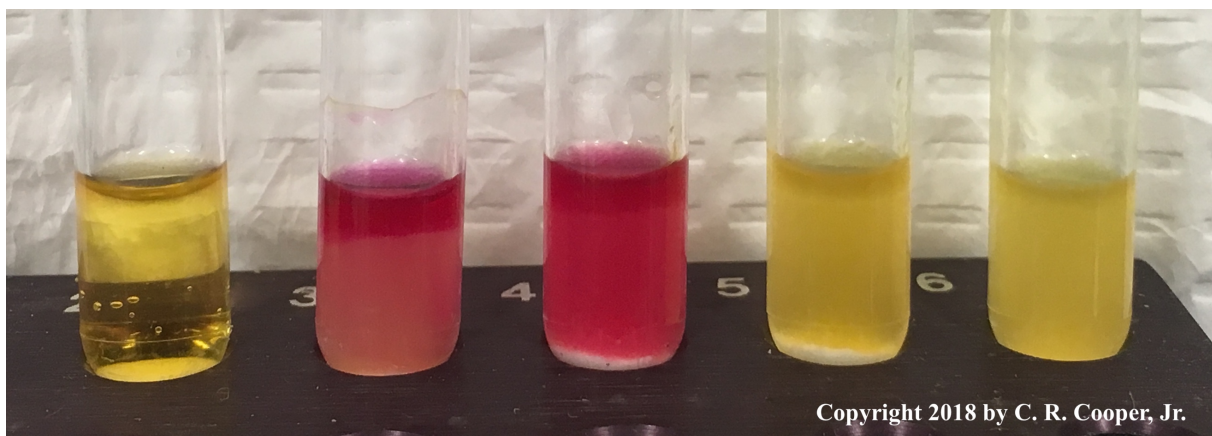
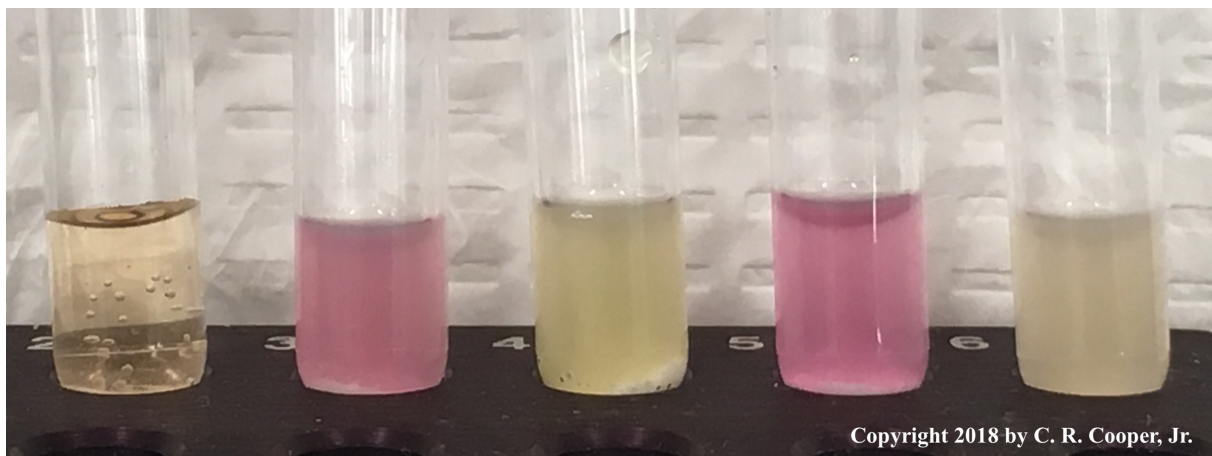


Figure 1. Urease/Indole Test Results. All five tubes in each image contain rehydrated Urease/Indole tablets. The first tube on the left was not inoculated, whereas the remaining tubes were inoculated with *Proteus hauseri*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella enterica* serovar Cholerasuis, respectively. The top image depicts the results of the urease test which indicates that *P. hauseri* and *E. coli* possess this activity. The bottom image is shown following the addition of Kovac's reagent. Both *P. hauseri* and *K. pneumoniae* show positive results.

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:

- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Klebsiella pneumoniae* (ATCC 13883) [abbreviated as *K. pneumoniae*]
- *Proteus hauseri* (ATCC 13315) [abbreviated as *P. hauseri*]
- *Salmonella enterica* serovar Cholerasuis (ATCC 10708) [abbreviated as *S. Cholerasuis*]

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.

Materials

- Urease Test Tablets (Cat. No. K650; Key Scientific, Stamford, TX; https://www.keysscientific.com/files/New_Website_Files/Urease/K650-0805.PDF)
- Urea/Indole Wee-Tabs (Cat. No. K1651; Key Scientific, Stamford, TX; https://www.keysscientific.com/files/New_Website_Files/Urea-Indol/K1651-0805.PDF)
- Sterile water
- Sterile plastic bulb pipettes
- Alcohol wipes
- Sterile test tubes with caps (12 x 75 mm; glass or polystyrene)
- 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.

Students shall perform two different urease tests simultaneously and compare the results.

Urease Test Tablets

- 1) Obtain four (4) sterile 12 x 75 mm test tubes. Label each tube with the microbe to be tested and other relevant information.
- 2) Use an alcohol wipe to sterilize a set of forceps by thoroughly wiping the outside and inside tip area of the instrument.
- 3) Using the sterile forceps, add a single Urease Test Tablet to each labeled tube.

Note: Be sure to retrieve tablets from the small white bottle marked "Urease Test Tablet K650". Several other products come in similar containers.

- 4) Using a sterile plastic bulb pipet, aseptically add 1 ml of sterile water to each labeled tube. Discard the pipet in the appropriate waste bin.
- 5) Using a microbiological loop, *heavily* inoculate each tube with the appropriate bacterial species (preferably from a culture grown on a slant or plate for 18-24 hours).
- 6) Incubate all the tubes at 37°C for 1-24 hours.

Note: *Proteus* often gives a positive test result in less than 8 hours and sometimes within one hour. Some *Klebsiella* strains may require up to 24 hours to show a positive reaction.

- 7) Over the next 24 hours, remove the tubes periodically from the incubator and observe them for any color change.

Record any observations on the data report sheet attached to this document.

Interpretation of Results: If any pink or red color is observed, this is a positive result. Reactions yielding straw, peach, or yellow colors, or if the reaction tube remains colorless, the urease test is negative.

Urea/Indole Wee-Tab Test

- 1) Obtain four (4) sterile Urea/Indole tubes. Label each tube with the microbe to be tested and other relevant information.

Note: Be sure to retrieve tablets from the small white bottle marked "Urea/Indole Wee-Tab K1651". Several other products come in similar containers.

- 2) Using a sterile plastic bulb pipet, aseptically add 0.5 ml of sterile water to each labeled tube.
- 3) Using a microbiological loop, *heavily* inoculate each tube with the appropriate bacterial species (preferably from a culture grown on a slant or plate for 18-24 hours).
- 4) Incubate all the tubes at 37°C for 1-6 hours.
- 5) After 1 hour, remove the tubes and observe if any are urease is positive.

Record any observations on the data report sheet attached to this document.

Interpretation of Results: If any pink or red color is observed, this is a positive result. Reactions yielding straw, peach, or yellow colors, or if the reaction tube remains colorless, the urease test is negative.

If this initial observation shows any tubes not to be urease positive, return the tubes to the incubator for up to six (6) hours of total incubation time before considering any negative test result valid.

- 6) For any positive urease reaction observed at or after 1 hour of incubation, perform the indole test by adding two (2) drops of Kovac's reagent to the tube. Let the tube stand for 3-4 minutes without shaking.

Interpretation of Results: A positive indole test is indicated by the development of a cherry red color in the top layer. The absence of a cherry red color is indicative of a negative indole test.

Record any observations on the data report sheet attached to this document.

- 7) If tubes have incubated for 6 hours, regardless of the urease reaction, perform the indole test as described in step 6 above.

Record any observations on the data report sheet attached to this document.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR VISUAL OBSERVATIONS

Organism	Urease Test Tablet	Urea/Indole Wee-Tab	
	Urease Activity	Urease Activity	Indole Production
<i>Salmonella enterica</i> serovar Cholerasuis	Color?	Color?	Color?
	Urease Pos./Neg.?	Urease Pos./Neg.?	Indole Pos./Neg.?
<i>Klebsiella pneumoniae</i>	Color?	Color?	Color?
	Urease Pos./Neg.?	Urease Pos./Neg.?	Indole Pos./Neg.?
<i>Escherichia coli</i>	Color?	Color?	Color?
	Urease Pos./Neg.?	Urease Pos./Neg.?	Indole Pos./Neg.?
<i>Proteus hauseri</i>	Color?	Color?	Color?
	Urease Pos./Neg.?	Urease Pos./Neg.?	Indole Pos./Neg.?

Discussion Question:

What is the advantage of possessing the enzyme urease for each particular urease positive microbe that you identified above?