ESCULIN-PYR TEST

Principle and Purpose

Esculin, a naturally occurring type of glucoside found in certain plants, is sometimes employed as a vasoprotective drug. Some bacterial species, like *Enterococcus*, possess an enzyme (esculinase) that degrades esculin into 6,7-dihydroxycoumarin (also known as esculetin) and glucose. The glucose is further metabolized as a carbon source. Esculetin can be detected in particular diagnostic microbiological media (e.g., bile esculin medium) by forming a brown pigment upon reacting with ferric citrate component.

Streptococcus and *Enterococcus* can be distinguished from each other by differences in their abilities to hydrolyze esculin. With the exception of *S. bovis*, all species of *Streptococcus* do not degrade esculin, whereas enterococci do hydrolyze this compound. In addition, among the Enterobacteriaceae, species of *Enterobacter*, *Klebsiella*, and *Serratia* are hydrolyze esculin.

Esculinase can also hydrolyze *p*-nitrophenol-ß-D-glucopyranoside (PNGP), a compound with structural similarity to esculin, to release *p*-nitrophenol. The latter is yellow in color. Students will take advantage of the latter property by using a commercially-available, two-hour test (ESC/PYR test; Key Scientific) to differentiate *Enterococcus* from *Streptococcus* as well as *Staphylococcus*.

Similarly, some bacterial species can hydrolyze L-pyroglutamic acid-ß-naphthylamide (PYR) to release ß-naphthylamine via the action of the enzyme pyrolidonyl arylamidase (also known as pyrrolidonyl aminopeptidase). Such microbes include *Enterococcus faecalis* and *E. faecium*, some uncommon staphylococcal species, *Streptococcus pyogenes* (but not other streptococci), and species of Gram-negative bacilli within the genera *Citrobacter, Klebsiella, Yersinia*, and *Serratia.* ß-naphthylamine can be detected as a color change upon the addition of PEP reagent. (PEP is a solution of *p*-dimethyl-amino-cinnamaldehyde in weak hydrochloric acid.) *Streptococcus* and *Staphylococcus* species cannot hydrolyze PYR, therefore no color change occurs upon exposure to PEP. Using the same commercial test to assess esculin hydrolysis note above, students will demonstrate differences in PYR hydrolysis among species of *Enterococcus*, *Streptococcus*, and *Staphylococcus.*

Learning Objectives

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

- Understand the underlying basis of both the esculin and PYR tests;
- Properly conduct the esculin and PYR tests; and
- Accurately interpret the results of these tests.

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 or Petri dish cultures:

- *Enterococcus faecalis* (ATCC 19433) [abbreviated as *E. faecalis*]
- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *Staph. aureus*]
- *Streptococcus pyogenes* (ATCC 19615) [abbreviated as *Strept. pyogenes*]

Reagents/Materials

- Sterile bulb pipette
- Sterile water
- ESC/PYR tubes (Cat. No. K1137; Key Scientific, Stamford, TX; http://www.keyscientific.com/files/New%20Website%20Files/ESC-PYR/K1137-0805.PDF)
- PEP reagent (Cat. No. K2375; Key Scientific, Stamford, TX; https://www.keyscientific.com/files/New Website Files/Reagents/K982375-0805.pdf)

Procedure

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

1) Obtain three vials containing the ESC/PYR tablet and allow these to reach room temperature.
2) Label one vial for inoculation with *E. faecalis*, one for inoculation with *Strept. pyogenes*, and one for inoculation with *Staph. aureus*. Be sure to label these vials with other pertinent identifying information.
3) Remove the cap of each vial, then use a sterile bulb pipette to add 3-5 drops of sterile water for each tablet.
4) Using a microbiological loop, aseptically transfer a heavy loopful of the appropriate bacterium to the respectively labeled vial. The inoculum should come from a 24-48-hour agar plate or slant culture. With the loop, thoroughly suspend the microbe.
5) *Loosely* replace the cap and incubate the vials at 37°C for 2 hours.
6) After incubation, remove the vials from the incubator and observe the color of the liquid medium.

Interpretation of Results: The presence of a yellow color any time during the incubation period indicates a positive esculin reaction. A negative esculin reaction is indicated if a yellow color does not appear after 2 hours of incubation.

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

7) After recording the results from Step 6, add 2 drops of PEP reagent to each vial. Incubate the vials for 15 minutes at 37°C. Remove the vials from the incubator and observe the color of the liquid medium.

Interpretation of Results: The appearance of a dark pink/red color indicates a positive PYR reaction. In contrast, a negative PYR test result will appear yellow to a very light peach in color. If a peach-like color appears, mix the tube vigorous (use a vortex, if available) to confirm the negative results, i.e., the color remains peach-like.

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

Note: If the inoculum was grown on indole-rich media (e.g., blood agar), the positive PYR reaction may appear dark blue or purple. However, on such media, development of a green or turquoise color indicates a negative PYR reaction.

Student Name: _____

ESC/PYR TEST

Observe and record the results of esculin (ESC) and L-pyroglutamic acid-ß-naphthylamide (PYR) hydrolysis by *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Be sure to record the color of each reaction and indicate if the test is positive or negative for the hydrolysis of ESC and PYR.

Results of ESC and PYR Hydrolysis Test

	Esculin Hydrolysis		PYR Hydrolysis	
Test Organism	**Color Reaction**	**Positive or Negative?**	**Color Reaction**	**Positive or Negative?**
Staphylococcus aureus				
Streptococcus pyogenes				
Enterococcus faecalis				

Discussion Questions

1) How does bile in bile esculin medium contribute to the isolation and presumptive identification of *Enterococcus*?

2) Other than the PYR test, what is another use of the PEP reagent? The answer to this question may require some background investigation.