

# AL-RASHEED PHARMACY

Dpt.

3rd year 1st semester  
2021

Biochemistry

Lab 6

Xanthoproteic Test

Millon's Test

Hopkin-Cole's Test

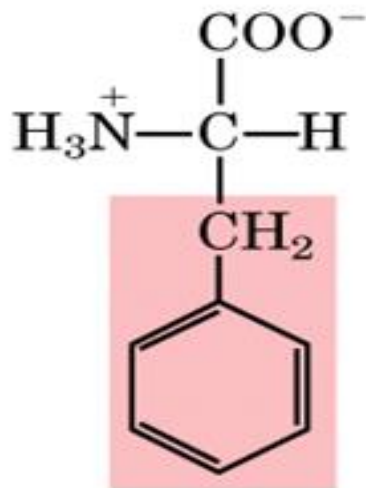
---

# Xanthoprotic test Definition

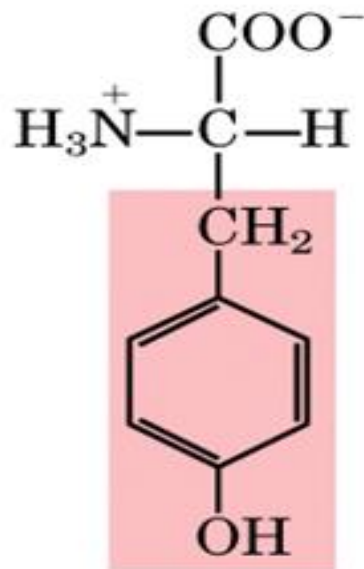
Xanthoprotic test is a **biochemical test** for the detection of **amino acids** containing phenolic or indolic groups like phenylalanine, tyrosine, and tryptophan (aromatic amino acids). The test is named Xanthoprotic test due to the formation of a yellow precipitate of xanthoprotic acid. The term 'Xantho' refers to 'yellow', so the test is often termed as the Yellow Protein Test. The test gives a positive result for amino acids containing benzene rings or other aromatic groups. The test is a qualitative test that provides information only on the presence or absence of the amino acids.

# Aromatic amino acid structures

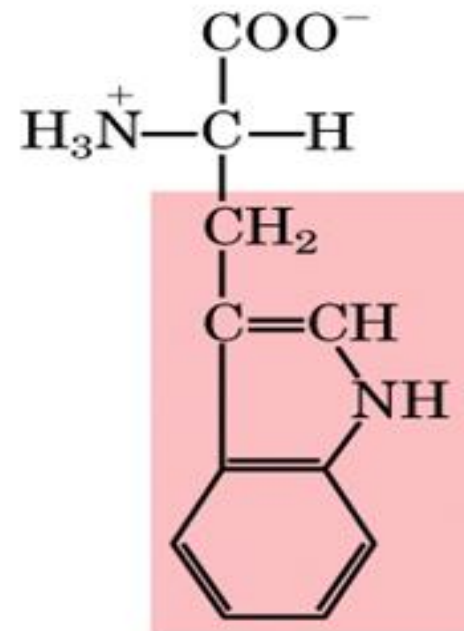
## Aromatic R groups



Phenylalanine



Tyrosine



Tryptophan

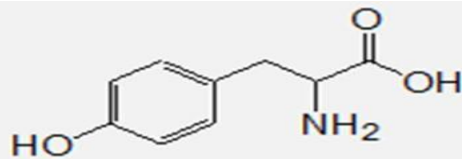
# Objectives of Xanthoproteic Test

- To detect the presence of aromatic groups-containing amino acids like tyrosine and tryptophan.
- To differentiate tyrosine and tryptophan from other amino acids.

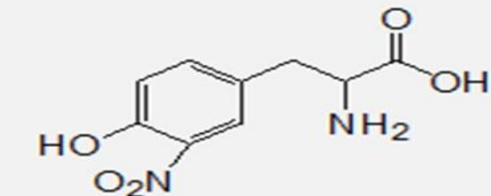
# Principle of Xanthoproteic Test

Treating aromatic amino acids with the concentrated nitric acid leads to the nitration of the aromatic ring and formation of yellow nitro-products (nitro derivatives). When the strong basic solution is added the colour of obtained products turns darker (from yellow to orange). The positive result of xanthoproteic reaction gives: tyrosine, tryptophan and phenylalanine (only after extended heating time). It is difficult to nitrate phenylalanine under normal conditions so it does not respond to this test without extended heating.

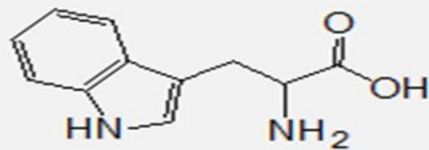
# Reaction



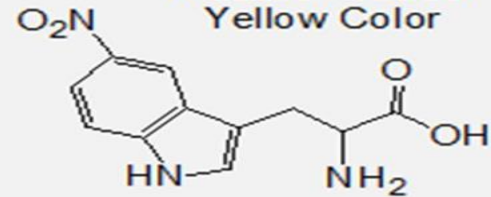
Tyrosine



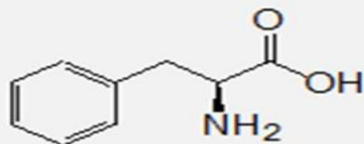
Nitrated Product  
Yellow Color



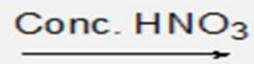
Tryptophan



Nitrated Product  
Yellow Color



Phenylalanine



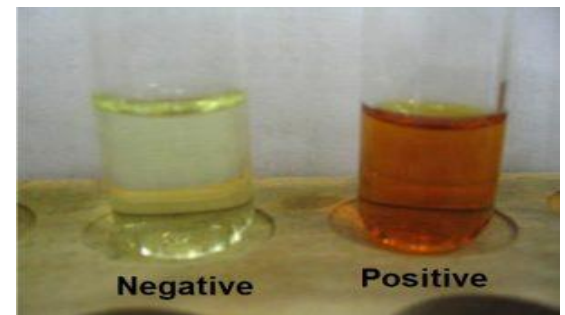
No Reaction

# Procedure of Xanthoproteic Test

- To 1 ml of test solution, add 1 ml of concentrated  $\text{HNO}_3$
- Mix and heat (In case of protein solution, initially white precipitate appears due to denaturation of protein, which turns yellow on heating).
- Cool under tap water
- Add 1 ml of 40% NaOH to make the solution alkaline
- Observe whether the mixture turns orange in color.

# Result and Interpretation

- **Positive result:** The appearance of a dark yellow or orange-colored solution represents a positive test. This indicates the presence of aromatic groups in the proteins and amino acids.
- **Negative result:** The absence of a dark yellow or orange-colored solution represents a negative test. This indicates the absence of aromatic groups in proteins and amino acids.





# Millon's Test

## Definition

Millon's test is an analytical test used for the detection of the amino acid tyrosine, which is the only amino acid containing the phenol group.

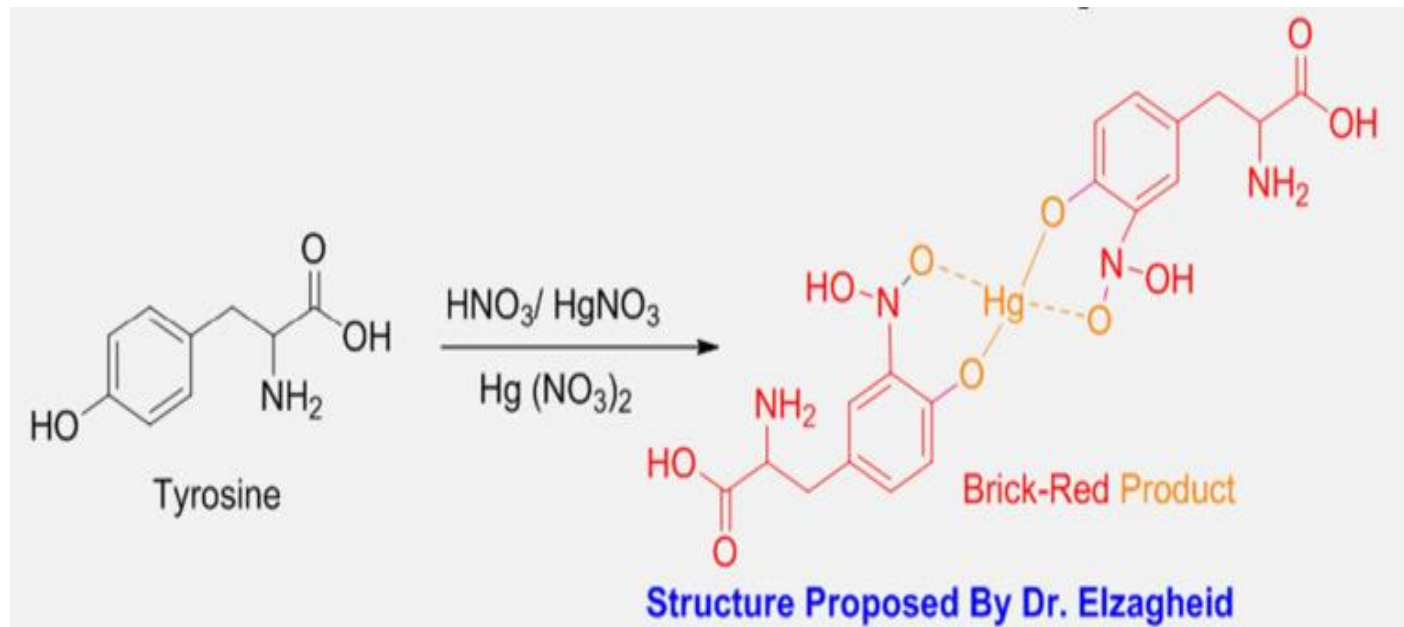
# Objectives of Millon's Test

- To detect the presence of tyrosine-containing proteins in a given sample.
- To differentiate tyrosine from other amino acids.

# Principle of Millon's Test

Millon's test is based on the principle of nitration of the phenol group in tyrosine, which then forms complexes with heavy metals like mercury. The reagent used for the test is called Millon's reagent, and it consists of mercuric nitrate and mercurous nitrate that is dissolved in concentrated nitric acid. In the test, the phenol group on the tyrosine molecule is nitrated by the nitric acid present in the reagent. The nitrated tyrosine then combines with the mercury ions in the solution to form a red-colored precipitate or solution.

# Reaction



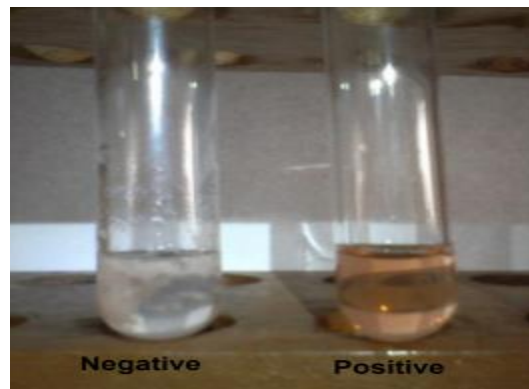
# Procedure of Millon's Test

- About 2 ml of the sample solution or the 1% tyrosine solution is taken in a test tube.
- To this, about 2 ml of Millon's reagent is added. The test tubes are then kept in the water bath for about 2 minutes if red colored precipitate is not observed immediately.

## Result and Interpretation of Millon's Test

Positive result: A positive result in the Millon's test is demonstrated by the formation of a red or pink colored precipitate. This indicates the presence of tyrosine or tyrosine containing protein.

Negative result: A negative result in the Millon's test is demonstrated by the absence of colored precipitate in the test tube. This indicates the absence of tyrosine or tyrosine-containing protein.



# Hopkin's Cole Test

Definition:

Hopkin's Cole test is a specific test used for the detection of indole ring and thus, tryptophan in proteins. The test is also termed as 'glyoxylic acid test' as the reagent contains glyoxylic acid.

## Objectives of Hopkin's Cole Test

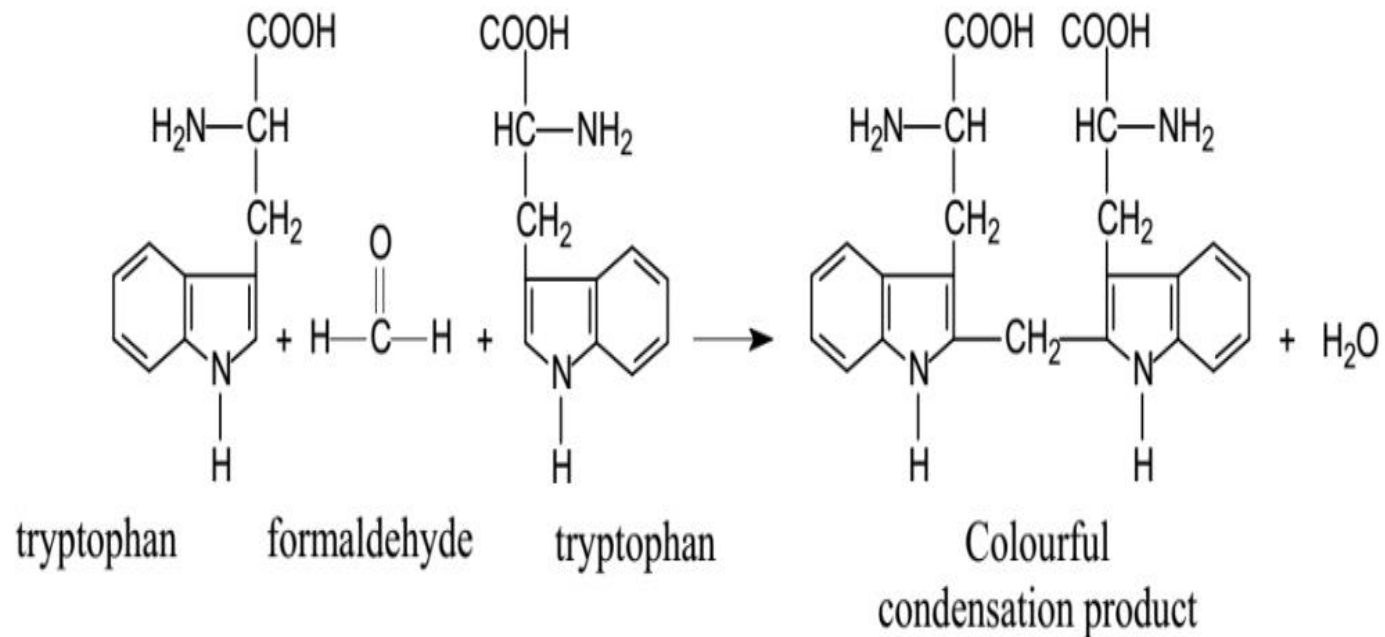
- To detect the presence of indole ring containing amino acid in proteins.
- To detect the presence of tryptophan-containing proteins.



# Principle of Hopkin's Cole Test

The test is based on the principle that the layering of concentrated sulfuric acid over a mixture of tryptophan-containing proteins with the Hopkin's Cole reagent results in the formation of a violet ring at the interface. The formalin (40% solution of formic aldehyde in water) added to the sample combines two tryptophan molecules by acting on the indole ring of the tryptophan molecules.

# Reaction:



# Procedure

- Add some drops of formalin (40% solution of formic aldehyde in water) to 1 ml of tryptophan solution.
- Mix the test tube contents and cautiously introduce down the side of the tube 1 ml of concentrated sulphuric acid.
- A purple ring appears between the two layers if the test is positive for tryptophan.

# Result and Interpretation

- **Positive result:** A positive result is represented by the formation of a purple-colored ring at the junction of two layers. This indicates the presence of tryptophan-containing proteins.
- **Negative result:** A negative result is represented by the absence of a purple-colored ring in the test tube. This indicates the absence of tryptophan-containing proteins.





THANK YOU FOR LISTINING

Prepared by:

Assis.Lect.Nabigh Al-SHARIFI