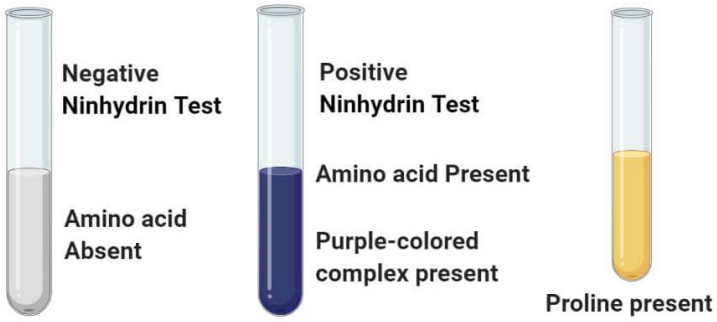
QUALITATIVE TESTS FOR AMINO ACIDS AND PROTEINS

1. Ninhydrin test

- The ninhydrin test is a chemical test which is used to check whether a given analyte contains amines or α-amino acids.
- In this test, ninhydrin is added to a test solution of the analyte. The development of a deep blue colour indicates the presence of ammonia, primary/secondary amines, or amino acids in the analyte

➢ Ninhydrin Test Principle

- The amino group belonging to a free amino acid undergoes a chemical reaction with ninhydrin, which behaves as an oxidizing agent. When exposed to ninhydrin, the amino acid undergoes oxidative deamination, resulting in the liberation of CO_2, NH_3, and an aldehyde along with hydrindantin (which is a reduced form of ninhydrin).
- Now, the ammonia goes on to react with another ninhydrin molecule to form diketohydrin (which is also known as Ruhemann's complex). This complex is responsible for the deep blue colour. When the analyte contains Imino-acids like proline, a yellow coloured complex is formed. When asparagine is used, the colour of the resulting complex is brown.



➢ Procedure

First, a 2% solution of ninhydrin must be prepared by dissolving 0.2 grams of ninhydrin in 10ml of either ethanol or acetone.

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Now a 1% solution of the amino acid (analyte) in distilled water must be prepared. A few drops of the 2% ninhydrin solution must be added to this solution.

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The test tube must be kept in a warm water bath for approximately 5 minutes.

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The development of a deep blue/violet colour indicates the presence of amino acids.

➢ Ninhydrin Test Result Interpretation

- For ammonia, primary/secondary amines, and amino acids, deep purple colour is obtained.
- For hydroxyproline and proline, a yellow colour is obtained.
- For asparagine, brown colour is obtained.
- If no colour change is observed, the analyte does not contain amino acids, amines, or ammonia.

2. Biuret test

- The biuret test is a chemical test that can be used to see if an analyte has peptide bonds or not. As a result, the biuret test may be used to figure out how much protein is in the analyte. In this test, the presence of peptides induces the copper (II) ion to form pale purple (or mauve) coordination complexes (when the solution is sufficiently alkaline).
- Biuret is a compound produced by heating urea at 180 ℃
- Biuret reagent is made of Copper sulphate (CuSO4), sodium hydroxide (NaOH) and sodium-potassium tartrate (also known as Rochelle salt). Despite the name, this reagent does not contain Biuret ((H2N-CO-)2NH).
- It is the Cu_2^+ in the Biuret reagent that forms a complex with the peptide bonds found in proteins. Hence, this test helps in determining peptide bonds in any substance.

➢ **Biuret Test Principle**

- In the presence of alkaline, when Biuret is reacted with dilute copper sulphate, a purple coloured substance is formed. The reason behind this colour is the formation of a chelate complex or the copper coordination complex.
- Cu (II) or cupric ions create a chelate complex of violet colour, using oxygen of water and the unshared electron pairs of peptide nitrogen. Since this complex absorbs light in 540 nm, it appears violet. In the presence of protein, it changes its colour from blue to violet.
- Naturally, the colour intensifies as the number of peptide bonds increases in protein.
- One cupric ion is generally attached to six nearby peptide linkages through coordinate bonds.

➢ **Procedure**

Take 3 clean and dry test tubes.
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Add 1-2 ml of the test solution, egg albumin, and deionized water in the respective test tubes.
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Add 1-2 ml of Biuret reagent to all the test tubes.
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Shake well and allow the mixtures to stand for 5 minutes.
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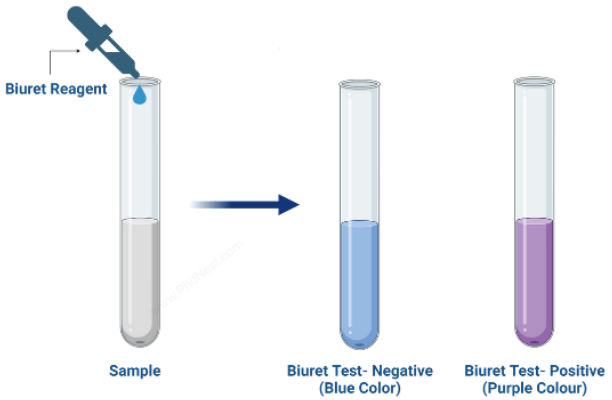
Observe for any color change.



➢ **Positive Result**

- The Colour turns purple.
- All proteins and peptides give positive results.
- Only amino acid, Histidine, gives a positive result

3. Xanthoproteic test

- Xanthoproteic 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 Xanthoproteic test due to the formation of a yellow precipitate of xanthoproteic 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.

➢ Principle-

- The Xanthoproteic test is based on the fact that aromatic groups in the amino acids are nitrated by heating with concentrated HNO_3 to yield intensely yellow-coloured nitro derivative. On the addition of alkali, however, the residue turns orange due to the formation of a salt of the tautomeric form of the nitro compound.
- Benzene ring-containing amino acids like phenylalanine don't give a positive test to this test because the phenyl group in phenylalanine is very stable, which doesn't react with nitric acid in the conditions of this test. However, phenylalanine might give a positive result after an extended period of heating.

➢ **Reagent required**

i) Concentrated Nitric acid
ii) 40% NaOH
iii) Test solution

➢ **Procedure**

About 1 ml of the sample solution is taken in a test tube. To this, the same amount of concentrated nitric acid is added.

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The test tube is allowed to cool down to room temperature. If the sample is a protein solution, a white precipitate might develop due to the denaturation of proteins.

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Then, 1 ml of 40% NaOH solution is added to the test tube and observed for colour change.

➢ **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.

➢ **Uses**

i) This is a biochemical test for the detection of proteins and amino acids.

ii) The test allows the differentiation of aromatic amino acids from non-aromatic amino acids.

4. Glyoxylic Acid Reaction(Hopkins-Cole test**)**

- 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.
- **Hopkin's Cole reagent(**Glyoxylic acid) It can be prepared by exposing glacial acetic acid to sunlight for a few days.
- **Objectives-**
  - → To detect the presence of indole ring containing amino acid in proteins.
  - → To detect the presence of tryptophan-containing proteins.
- **Principle**
  - → 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 glyoxylic acid added to the sample combines two tryptophan molecules by acting on the indole ring of the tryptophan molecules. The condensation product thus formed undergoes dehydration to form a violet colored pigment.

➢ **Procedure**

In a test tube, 2 ml of light-exposed glacial acetic acid and 2 ml of the sample liquid are taken.

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To this, concentrated H_2SO_4 is added along the sides of the test tube held at a slanting position. Two distinct layers of liquid are to be formed without mixing.

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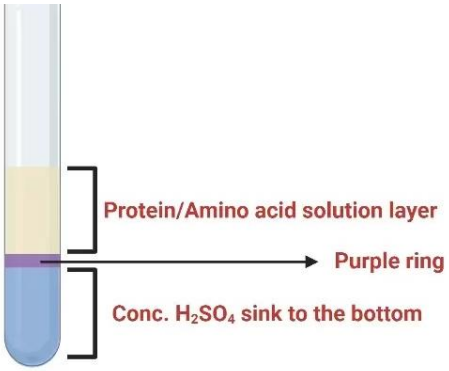
The test tube should be observed for the formation of a colored ring at the interface of two layers.

➢ **Result**

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.



➢ **Uses of Hopkin's Cole Test**

- The test is used for the detection of proteins and amino acids in a sample.
- The test is a simple and easy-to-perform test that helps to identify tryptophan from other amino acids.

5. **Millon's test**
    - 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.
    - Millon's test is a specific test for tyrosine, but it is not a specific test for **protein** as it also detects the phenolic group present in other compounds as well. Therefore, while performing Millon's test, it is essential that other tests like the **Biuret test** and **Ninhydrin test** also be performed.
    - The test was discovered by and named after the French Chemist Auguste Nicolas Eugene Millon.
    - **Millon's reagent:** Millon's reagent consists of mercuric nitrate and mercurous nitrate dissolved in nitric acid and distilled water

➢ **Objectives of Millon's Test**
- To detect the presence of tyrosine-containing proteins in a given sample.
- To detect the presence of phenol-containing compounds.
- To differentiate tyrosine from other amino acids.

➢ **Principle**
- Millon's test is based on the principle of nitrification 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.
- In some proteins containing tyrosine, the initial reaction between mercuric nitrate results in a white or yellow colored precipitate. After the addition of nitric acid and heating, however, the residue turns red in color.
- Both of these results are considered positive results and indicate the presence of tyrosine in the solution.

➢ **Procedure**

About 2 ml of the sample solution or the 1% tyrosine solution is taken in a test tube.

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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.

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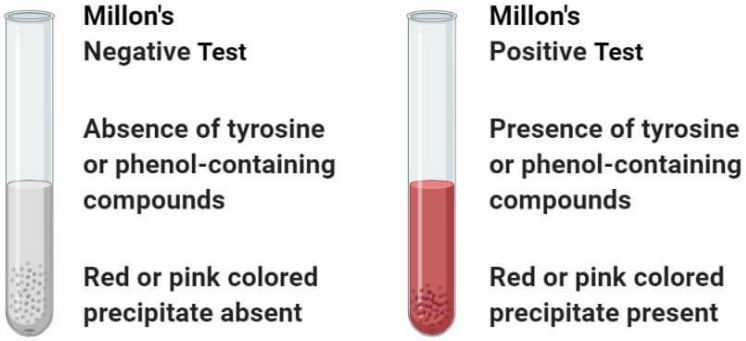
The tubes are then observed for the formation of the colored precipitate.



6. **Sakaguchi Test. Objective:**
    - Sakaguchi test used as a qualitative test for arginine that is either free or in protein.
    - The test was discovered by and named after the Japanese Food Scientist Shoyo Sakaguchi in 1925.
    - Sakaguchi test is an example of color reactions used for the detection of **amino acids** or **proteins**.
    - The test is a specific test for arginine where the guanidinium group of arginine reacts with 1-naphthol or α-naphthol to produce a colored product

- **Sakaguchi reagent:** 1% 1-naphthol in alcohol with a few drops of 10% sodium hypobromite solution of bromine water.
- **Objective of Sakaguchi Test**
  To detect the presence of arginine in either free form or in proteins
- **Principle of Sakaguchi Test**
  - Sakaguchi test is based on the principle of reaction between 1-naphthol and the guanidinium groups in arginine, in the presence of an oxidizing agent.
  - The reaction results in the formation of a red-colored complex due to the formation of an indole-like structure.
  - The Sakaguchi reagent consists of sodium hypobromite and 1-naphthol. The sodium hypobromite acts as an oxidizing agent that facilitates the hydrogen bonding between two arginine molecules.
- **Procedure of Sakaguchi Test**

About 3 ml of the test solution is added in a test tube, to which 1 ml of 40% NaOH is added and mixed correctly.

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Then, two drops of 1-naphthol are added to the same test tube and mixed thoroughly.

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Now, 4-5 drops of the 10% sodium hypobromite or bromine water is added.

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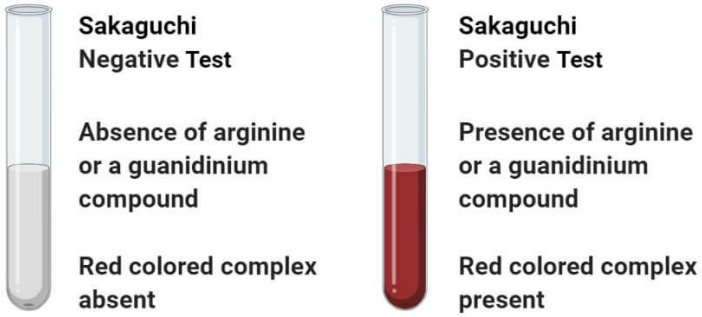
The test tube is observed for the development of color.



- **Positive result:** A positive result on the Sakaguchi's test is demonstrated by the formation of red color. This indicates the presence of an arginine or guanidinium compound.
- **Uses of Sakaguchi Test**
  Sakaguchi's Test is a biochemical test for the detection of arginine in the free or combined form in proteins.

7. Lead Sulfide Test Objective:

- Lead sulphide test (or Lead acetate test) is a biochemical test for the detection of **amino acids** like cysteine and cystine.
- The test is a specific test for the detection of amino acids containing sulphur, S-S group in cysteine, and S-H group in cystine.
- The test is also called a lead acetate test as the reagent for the test is lead acetate.
- Even though the test is specific for the detection of sulphur-containing amino acids, methionine doesn't give a positive result in this test.

- **Objectives of Lead Sulphide Test**
  - To detect the presence of sulphur-containing amino acids in a sample.
  - To detect **protein**-containing cysteine and cystine in a given sample.
  - To distinguish between sulphur-containing and non-sulphur containing amino acids.
- **Principle of Lead Sulphide Test**
  - The test is based on the principle of detection of sulphur in a solution by the degradation of the S-H or S-S group in amino acids under strongly alkaline conditions.
  - Amino acids like cysteine and cystine release sulphur in the presence of strong alkaline conditions at a high temperature. The sulphur then combines with the alkali (NaOH) to form Na_2S. The Na_2S thus formed reacts with lead acetate to form lead sulphide, which results in a black residue.
  - For the reaction to take place, free sulphur ions should be present in the medium.

➢ **Procedure of Lead Sulphide Test**

In a test tube, 2 ml of the amino acid solution is taken. To this, 2 ml of NaOH is added, and the solution is boiled for a minute.

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Once the test tube cools down, a few drops of lead acetate are added to the solution.

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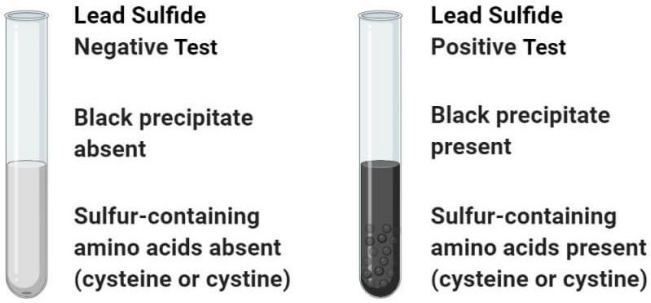
The test tube is then observed for the formation of a precipitate.



➢ **Positive test:**

A positive test in the Lead sulphide test is represented by the formation of black precipitate at the bottom of the test tube. This indicates the presence of cysteine or cystine in the solution.

➢ **Uses of Lead Sulphide Test**

- The test is used to detect sulphur-containing amino acids like cysteine and cystine.
- It helps to distinguish between different groups of amino acids.
- The detection of cystine in urine is a pathological symptom of diseases like cystine stones in the kidneys and bladder.