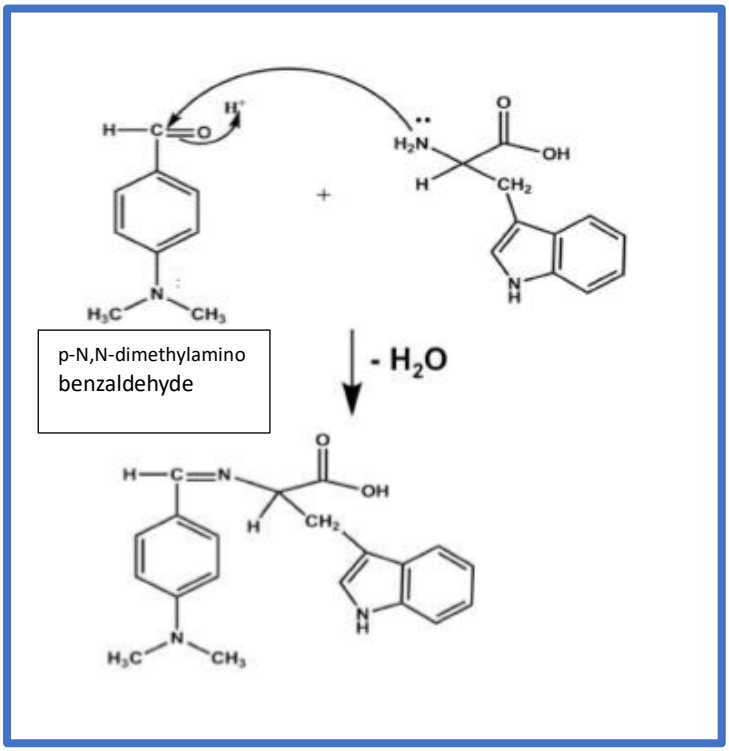
Qualitative Test of Amino Acids

Principle:

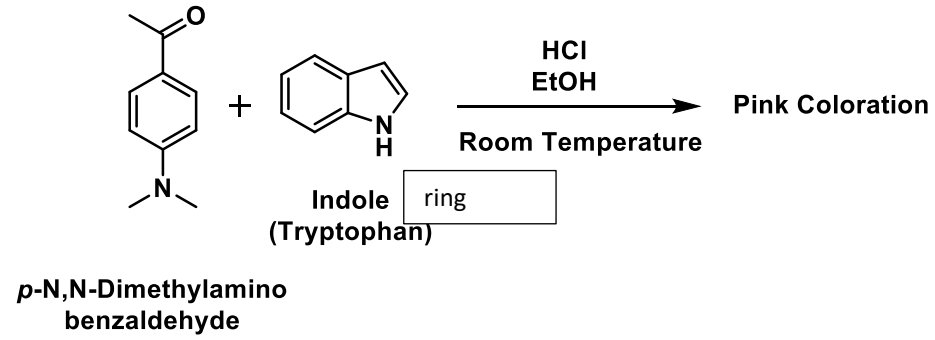
Cost-effective colorimetry tests were developed to identify qualitatively certain amino acids having functional sidechains.

Erlisch test for Tryptophan:

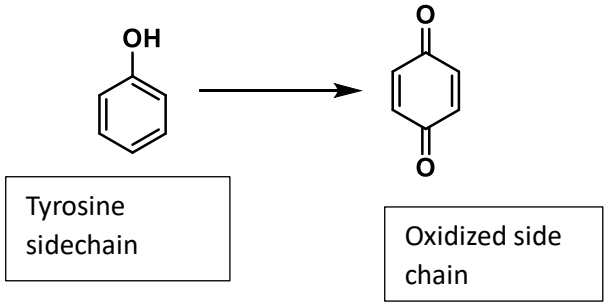
The p-N,N-dimethylamino benzaldehyde reacts with indole ring of tryptophan to form a pink/red colored product. Initially, it is **pink/red** and gradually it becomes **light purple-blue**.



p-N,N-dimethylamino benzaldehyde reaction with main chain amine group. This reaction can happen with 19 natural amino acids, except proline and is not responsible for colour formation in Erlisch's test



1. **Folins-Ciocalteu test for Tyrosine:**
   The phenolic group of tyrosine residue (amino acid) in a protein will produce a **blue-purple** color complex, with λ_{max} in the region of 660 nm wavelength, with Folin-Ciocalteu reagent which consists of sodium tungstate molybdate and phosphate. **Cysteine and Tryptophan also respond to this test and Cystine also shows mild blue-purple color.**



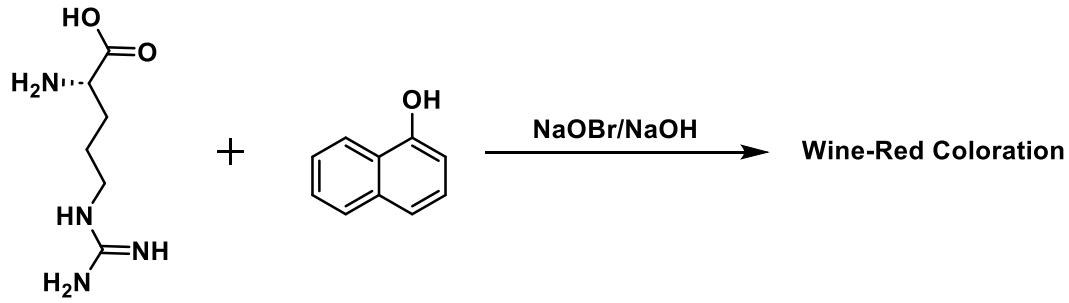
2. **Lead Acetate test for Cysteine:**
   Sulfur containing amino acids, such as cysteine, upon boiling with sodium hydroxide (hot alkali) yield sodium sulfide. Then, lead acetate solution is added and the solution is boiled for some time. The conversion of the organic sulfur to inorganic sulfide, which can be detected by the **black precipitation of lead sulfide**, confirms sulphur-containing amino acid. **Methionine does not respond to this test but Cystine responds to this test.**

$$S.(Protein) + 2\ NaOH \rightarrow Na_2S$$

$$Na_2S + (CH_3COO)_2Pb \rightarrow PbS\downarrow\ (\textbf{Black}) + 2\ CH_3COONa$$

Scheme 1. Black PbS formation in $Pb(OAc)_2$ test

3. **Sakaguchi test for Arginine:**
   The only amino acid which contains a guanidine group is arginine. Arginine gives a red color with α-naphthol in the presence of an oxidizing agent like Bromine solution. This test is specific for the presence of guanidine. The NaOH helps to bring the arginine into Zwitterionic form that undergoes a condensation reaction with α–naphthol and develops a **red/wine color**.



Arginine Naphthalen-1-ol

Experimental Observation

Unknown Sample No.	Erlisch's test	Folins-Ciocalteu's test	Lead Acetate Test	Sakagauchi Test
1	Pink Colour gradually tuned blueish pink Trp present	Purple Blue colour Trp/Tyr/Cys present	No black ppt in suspension observed. Cys absent	Brown colour. Arg absent. Trp may be present.
2	Yellowish green colour. Trp absent	Purple Blue colour Trp/Tyr/Cys present	No black ppt in suspension observed. Cys absent	Initially no colour formation. wine red colour formed after 10 minutes. Arg absent. Tyr may be present
3	Yellowish green colour. Trp absent	Purple Blue colour Trp/Tyr/Cys present	Black ppt in suspension. Cys present	No colour formation. Arg absent. Cys may be present
4	Yellowish green colour. Trp absent	Faint blue color formed. Trp/Tyr/Cys absent. Arg may be present	No black ppt in suspension observed. Cys absent	Wine red color appeared immediately. Arg present

Conclusion:

Based on observations for all the tests,

Unknown sample 1 is Tryptophan.

Unknown sample 2 is Tyrosine

Unknown sample 3 is Cysteine

Unknown sample 4 is Arginine