**Kastle-Meyer Test**

The Kastle-Meyer solution is a phenolphthalein indication solution which has been reduced, usually by reacting it with powdered zinc. The basis of the test is that the peroxidase-like activity of the hemoglobin in blood catalyzes the oxidation of the colorless reduced phenolphthalein into bright pink.

**PROCEDURE OR ANALYSIS**

 1. A small cutting, swabbing, or extract of the suspected bloodstain is placed on filter paper or spot test paper.

 2. Two or three drops of ethanol are placed on the stain.

3. Two drops of the working solution of phenolphthalin are added to the stain.

4. After waiting to insure that no color develops at this stage, two or three drops of 3% H2O2 are added. 5. An intense pink color is a positive test for peroxidase activity, indicative of hemoglobin.

This is not a confirmatory test for blood.

**Principle:**

This test relies on the peroxidase-like activity of the heme groups associated with the hemoglobin contained in red blood cells. In the presence of hydrogen peroxide, this peroxidase-like activity will catalyze the oxidation of phenolphthalin, which is colorless in solution, into phenolphthalein resulting in a pink colored solution. The presence of a pink color is a positive test result indicating the presumptive presence of blood.

 **AH2 + H 2O 2 –[heme]- → A + 2H 2O**

**phenolphthalin phenolphthalein**

**(colorless) (pink)**

**TMB Test (Tetra Methyl Benzidine)**

The tetramethylbenzidine (TMB) presumptive test for blood is a catalytic test which is based on the peroxidase-like activity of hemoglobin. Hemoglobin has the ability to cleave oxygen molecules from H2O2 and catalyze the reaction from the reduced form of 3,3’,5,5’-tetramethylbenzidine to the oxidized blue-green colored product.

**PREPARATIONS**

* **TMB Reagent**

1. Weigh out 0.2 grams of TMB

 2. Add to a 50 ml beaker

3. Measure out 10 ml glacial acetic acid and add to the TMB

 4. Mix thoroughly until the TMB is dissolved

5. Store refrigerated – expiration date 1 week

* **Hydrogen Peroxide 3%**

1. Measure out 10 ml of 30% hydrogen peroxide

2. Add 90 ml of deionized water.

**PROCEDURE OR ANALYSIS**

1. Swab suspected blood stain with clean filter paper or a swab, which may be moistened if necessary with deionized water, ethanol or saline.
2. Apply 1-2 drops of the TMB reagent.
3. Note any blue-green color change. A blue-green color change at this step indicates a chemical oxidant and the test should be considered inconclusive. If there is no color change, proceed to the next step.
4. Add 1-2 drops of 3% hydrogen peroxide.
5. Note any immediate blue-green color change 6. An immediate blue-green color change indicates a positive result. No color change indicates a negative result. A negative result indicates that either no blood is present or is below the limit of detection of the test.

**Luminol Test**

**Luminol** (C8H7N3O2) is a [chemical](https://en.wikipedia.org/wiki/Chemical) that exhibits [chemiluminescence](https://en.wikipedia.org/wiki/Chemiluminescence%22%20%5Co%20%22Chemiluminescence), with a blue glow, when mixed with an appropriate [oxidizing agent](https://en.wikipedia.org/wiki/Oxidizing_agent). [Forensic](https://en.wikipedia.org/wiki/Forensic) investigators use luminol to detect trace amounts of [blood](https://en.wikipedia.org/wiki/Blood) at [crime scenes](https://en.wikipedia.org/wiki/Crime_scene), as it reacts with the iron in [hemoglobin](https://en.wikipedia.org/wiki/Hemoglobin). In case of the detection of blood, the Luminol solution, which contains hydrogen peroxide, is applied to blood. The iron of the hemoglobin of the blood works as catalyst which strongly accelerates the local oxidation reaction – the blood becomes visible through intense blue illumination in the dark. The term “Luminol” refers to this reaction: the creation of “luminescence”, the light emission.