# **Luminol Chemiluminescence Test for Blood**

## **How to Use Luminol to Test for Blood**

By Anne Marie Helmenstine, Ph.D., About.com Guide

The luminol chemiluminescence reaction is responsible for the glow of lightsticks. The reaction is used by criminalists to detect traces of blood at crime scenes. In this test, luminol powder (C8H7O3N3) is mixed with hydrogen peroxide (H2O2) and a hydroxide (e.g., KOH) in a spray bottle. The luminol solution is sprayed where blood might be found. The iron from the hemoglobin in the blood serves as a catalyst for the chemiluminescence reaction that causes luminol to glow, so a blue glow is produced when the solution is sprayed where there is blood. Only a tiny amount of iron is required to catalyze the reaction. The blue glow lasts for about 30 seconds before it fades, which is enough time to take photographs of the areas so they can be investigated more thoroughly. Here's how you can detect blood yourself or demonstrate how to do it:

### **Luminol Materials**

- luminol stock solution (2 g luminol + 15 g potassium hydroxide + 250 mL water)
- 3% hydrogen peroxide in water (common over-the-counter concentration)
- potassium ferricyanide or a sterile blood lancet and sterile alcohol pad

# **Performing the Test or Demonstration**

- In a clear test tube or cup, mix 10 ml of the luminol solution and 10 ml of the peroxide solution.
- You can activate the glow either by adding ~0.1 g of potassium ferricyanide to the solution or with a drop of blood. The blood must be on the alcohol pad. The forensic test is for dried or latent blood, so the reaction between the alcohol and fresh blood is necessary.

### **Notes About the Luminol Test**

- In addition to iron and iron compounds, other substances can catalyze the luminol reaction. Copper and its compounds, horseradish, and bleach also cause the solution to glow. So, you could substitute any of these materials for the drop of blood or potassium ferricyanide in the demonstration. Similarly, the presence of these chemicals at a crime scene affects testing for blood. If a crime scene was washed in bleach, for example, the whole area would glow when sprayed with luminol, making it necessary to use a different test to find traces of blood.
- If you are doing the reaction as a chemiluminescence demonstration, you can kick the display up a notch by dissolving the potassium ferricyanide in the peroxide solution and using a fractionating column or glass spiral to react the solutions rather than a test tube. You could pour a small amount of fluorescein in the bottom of a flask, pour the potassium ferricyanide solution through the spiral into the flask, and (in a darkened room) finish by adding the luminol solution. The spiral will glow blue as it passes through the column, but the glow will change to bright green once the luminol touches the fluorescein in the flask.

- Don't drink the luminol solution. Don't get it on your skin or in your eyes. If you prepare
  a spray bottle of luminol solution to check for traces of blood, keep in mind the solution
  might be damaging to some surfaces. That's not a big factor at a crime scene, but it's
  something to keep in mind at home or in class. Don't spray upholstery or clothing or
  people.
- I am told you can use much less luminol (~50 mg) and still achieve enough luminescence for a demonstration or for crime work.

### **How the Test Works**

The iron in the hemoglobin found in blood catalyzes an oxidation reaction in which the luminol gains oxygen atoms while losing nitrogen and hydrogen. This produces a compound called 3-aminophthalate. The electrons in the 3-aminophthalate are in an excited state. Blue light is emitted as energy is released when the electrons return to the ground state.

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# **Luminol - Chemiluminescent Blood Detector**

Forensic Investigators' Essential Tool for Crime Scene Investigation Dec 19, 2007 Karen Lotter

Luminol glows in the dark. This chemiluminescent compound is an essential part of crime scene investigation as it detects blood, even invisible blood.

Cop shows make the heart beat faster with creepy music, car chases and evil looking baddies. But one of the more SFX-ish TV cop show forensic tools is a special chemical agent in a spray bottle that reveals invisible blood traces, by making it glow in the dark. Most of what one sees about this chemical is true.

### What is Luminol?

Commonly known as Luminol, this chemical exhibits a blue-green chemiluminescence when mixed with an appropriate oxidizing agent.

Luminol is a relatively simple chemical containing only carbon, nitrogen, oxygen and hydrogen and is a white to slightly yellow crystalline solid powder, soluble in water and most polar organic solvents.

Luminol is used by forensic investigators to detect trace amounts of blood left at crime scenes.

## The Crime Scene Investigator Sprays Luminol

On cop shows one always sees the CSI technicians take out a spray bottle, dim the lights spray and then like magic, luminous blue green splashes and shapes appear, glowing eerily in the darkened room. Everyone watching the show knows that a violent crime has been committed.

In reality, Luminol is used as a forensic tool by crime scene investigators to locate traces of blood, even if it the scene has been cleaned. Because luminol is a chemiluminescent compound the light is released because of a chemical reaction.

As Luminol doesn't have a long shelf life, the crime scene investigator prepares a solution of Luminol and the activator at the scene and sprays it throughout the area under investigation. The iron present in any blood in the area catalyzes the chemical reaction that leads to the luminescence revealing the location of the blood.

### What Does Luminol Actually Do?

Just like on the TV cop shows, the most well-known use of luminol is in the field of forensic science. Blood, which is slightly alkaline, contains cells, water, enzymes, proteins, and hemoglobin (iron) which is the part that reacts with the Luminol as the catalyst. Yes, it is true that Luminol can detect very small amounts of blood, many years old.

## **Blood and Violent Crime Scenes**

One of the principles of crime scene investigation is that everything leaves a trace. This is particularly true of violent crime scenes. A murderer can dispose of the victim's body and clean up the blood till nothing is visible to the naked eye, but tiny particles of blood will remain in cracks and on surfaces without anyone ever knowing they're there.

Forensic investigators apply Luminol to an area suspected to have blood, even if it has been cleaned. The truth is, Luminol has no special affinity for blood, it reacts with any sort of iron. This forensic tool also reacts with bleach, dyes, and other organic material.

## **Luminol, Blood Traces and Verification**

If Luminol reveals apparent blood traces, investigators will photograph or videotape the crime scene to record the pattern.

Remember, Luminol only shows investigators that there might be blood in an area, since other substances can also cause the luminol to glow, but experienced forensic investigators know the difference based on how quickly the reaction occurs. After the Luminol test, forensic investigators still need to run other tests to verify that it is really human blood.

## Forensic Investigators Have Problems with Luminol

Although Luminol has become one of the essential tools in forensic crime scene investigations, it is not perfect. What they don't show on TV Cop Shows is that Luminol's chemical reaction can destroy other evidence in the crime scene. For this reason, forensic investigators only use Luminol after exploring other options and often not with quite as much abandon as on TV cop shows.

If Luminol is used it can destroy important properties of the blood. While it can detect even small amounts of blood, the disadvantage is often that the small amount identified is diluted further by the Luminol solution. For these reasons, Luminol is encouraged to be used as a last resort in crime scene investigations to protect the physical evidence.

## Luminol a Useful Tool for Forensic Crime Scene Investigation

The use of Luminol alone won't solve a murder or rape case. It's only one step in the crime scene investigation process. But it can reveal essential information that may lead forensic investigators to more evidence. By assisting crime scene investigators, Luminol is contributing towards making the world a safer place.

Sources:

How Luminol works by Tom Harris Luminol, what is Luminol

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