What Do You Think?

Every good episode of a crime show has some dealings with blood—what would a good murder be without it? In many episodes of murder mysteries, you can watch the investigators “spray for blood.”

- What do detectives spray when they are spraying for blood?
- How do they know blood is present at the scene after spraying?

Record your ideas about these questions in your Active Chemistry log. Be prepared to discuss your responses with your small group and the class.

Investigate

Although it may sound like science fiction, the spraying of a chemical on blood can create light and make the invisible blood “glow.” In this activity, you will investigate how the light is produced and whether other materials besides blood can produce the light.

Part A: Experiencing Chemiluminescence

1. The luminol reaction you’ll use in the lab today has quite a bit in common with the typical crack-n-glow lightstick you may have played with. Take a look at an uncracked lightstick and complete the following questions.
Activity 3 Presumptive Blood Testing: The Luminol Reaction

Part A: Observations

a) Make some initial observations of the uncracked lightstick. Write or draw these in your Active Chemistry log.

b) Crack and shake the lightstick. Write what you observe happening. These are observations on the macroscopic level.

c) Describe in words what you think is happening at the nanoscopic level (to the atoms and molecules).

Part B: Using Luminol to Test for Blood

1. Obtain a spray bottle containing some luminol reagent. The luminol reagent contains both luminol and hydrogen peroxide. Add 100 mL of distilled water to the spray bottle, then cap and mix. Allow it to sit for five minutes while occasionally stirring it. This solution will be good for one day.

2. While someone in your group is making the solution, have another group member obtain a small pipette of bovine hemoglobin solution. Bovine hemoglobin solution is made from the blood of cows. The red blood cells, which contain hemoglobin, are separated from the rest of the cow’s blood, and then they are sterilized and crystallized. Make sure you wash your hands and clean your pipettes and beakers with a dilute bleach solution when you are done.

a) Record your observations of the hemoglobin solution in your Active Chemistry log.

3. Obtain a piece of wax paper and two medium circles of filter paper. Label one circle “blood solution: experimental group” and one circle “distilled water: control group.” Place the filter paper circles on the piece of wax paper at your lab bench.

4. Place 10 drops of bovine hemoglobin solution into the circle labeled “blood solution.” Place 10 drops of distilled water in the second circle.

a) Once the circles are dry, record your observations.

b) Why does this experiment need a control? Record your answer in your Active Chemistry log.

5. Turn off the lights in the room and allow your eyes one minute to adjust to the dark. Alternatively, you can place the sample in a box.

6. In a dark room, or inside the box spray the luminol solution onto the test paper over each circle and observe. It will take 5-10 minutes to develop. While you are waiting, discuss your crime-scene story and how blood can be a creative element in your plot.

a) Record your observations in your Active Chemistry log.

b) What evidence is there that the hemoglobin causes the luminol to glow and not the paper or the distilled water? Record your answer after the lights are back on.

c) Compare the light produced by the lightstick to the light produced by the luminol-blood mixture. How are they alike? How are they different? Record your answers in your Active Chemistry log.

If any solution comes in contact with your skin, wash the area with soap and water immediately. Report all accidents to your teacher.

Avoid breathing dust from the bovine hemoglobin.
7. Rinse the paper with the bovine solution in a small amount of dilute bleach solution before throwing it out. Dispose of the materials as directed by your teacher. Clean up your workstation.

Part C: The Luminol Reaction

As you have seen, in the presence of blood, luminol reacts to produce light. Can something other than blood produce a similar reaction?

1. Obtain a piece of wax paper, and a sheet of white paper with circles on it, similar to the one shown. Label each circle with one of the following labels: 0.1 M Fe$^{2+}$/H$_2$O, 0.1 M Fe$^{3+}$/H$_2$O, bleach, ketchup, horseradish, a rusty iron nail, hemoglobin mixed with food coloring, and plain food coloring.

2. In this activity you will be observing how the luminol solution reacts with these different substances. You may want to record your observations in a table like the one shown.

3. Place three drops of each solution on the appropriately labeled circle. To save time, you may want to share pipettes with another group. Excess solutions should be disposed of as directed by your instructor. The hemoglobin pipette needs to be rinsed with a dilute solution of bleach before it is thrown away. All other pipettes may be rinsed with water and thrown out. The horseradish and iron nail can be placed directly on top of their circles.

4. In a dark room or inside a box, spray each circle with the luminol solution.

   a) Record your observations in your data table in your *Active Chemistry* log.

   b) Compare the results of tests on the different substances. Is the luminol test specific to blood? Explain.

   c) Based on your results, describe what is meant by the statement that the luminol test is a presumptive test for blood.

5. Rinse the hemoglobin circle with a small amount of bleach solution before you throw the circle away.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Color of paper before treatment</th>
<th>Does it glow?</th>
<th>Brightness of glow</th>
<th>Duration of glow</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M Fe$^{2+}$/H$_2$O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 M Fe$^{3+}$/H$_2$O</td>
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<tr>
<td>Bleach</td>
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<tr>
<td>Ketchup</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Horseradish</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Rusty iron nail</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin mixed with food coloring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain food coloring</td>
<td></td>
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</tr>
</tbody>
</table>
HOW ATOMS PRODUCE LIGHT

There are many techniques investigators use to find evidence at a crime scene. One of the methods popularized by many of today’s crime shows and movies uses a light-producing chemical reaction to visualize hidden bloodstains. As you observed in this activity, when the light-producing molecules of luminol and hydrogen peroxide are sprayed on areas contaminated with blood, a blue glow is produced. Due to the sensitivity of this technique, even invisible blood splatters that have been supposedly “cleaned” off the surface will appear. To learn how the luminol test works, you need to understand the structure of an atom.

Structure of the Atom

The atom is composed of protons, neutrons, and electrons. The protons and neutrons are located in the center of the atom, known as the nucleus. Neutrons, $n^0$, are neutral particles located in the nucleus of the atom. Protons, $p^+$, are positively charged particles also located in the nucleus. Atoms of the same element contain the same number of protons. For example, every atom of iron has 26 protons. However, every atom of the element copper has 29 protons. The number of protons in a single atom of an element is equal to the element’s atomic number. The atomic number is found above an element’s symbol on the periodic table.

Electrons, $e^-$, are negatively charged particles located outside the nucleus. The electrons exist outside the nucleus in certain allowable states called energy levels. If an atom has an equal number of electrons and protons, then the atom is neutral. For example, a neutral iron atom has 26 protons and 26 electrons. The positive charge of the protons is equal to the negative charge of the electrons. A neutral carbon atom contains 6 protons and 6 electrons.

Atoms Lose or Gain Electrons to Form Ions

Electrons, $e^-$, can be lost or gained in chemical reactions. If an atom loses an electron, the number of protons is larger than the number of electrons and the atom becomes positively charged. The atom has...
one positive charge for every electron it loses. If an iron atom loses 2 electrons it will have a (+2) charge. If an iron atom loses 3 electrons it will have a (+3) charge. The charges are written in the upper right corner above the element’s symbol.

\[
\begin{align*}
\text{Fe} & \rightarrow \text{Fe}^{3+} + 3e^- \\
26 \text{ protons(+) & 26 \text{ protons(+) & 26 \text{ protons(+) & 26 \text{ protons(+)} \\
26 \text{ electrons(–) & 23 \text{ electrons(–) & 26 \text{ electrons(–) & 24 \text{ electrons(–) & 3 \text{ charge & no charge & +2 \text{ charge} \\
\text{no charge & +3 charge & no charge & +2 charge}
\end{align*}
\]

In both examples given, only the number of electrons changes. The number of protons in each atom always remains the same. Atoms that have lost or gained electrons are called ions. Atoms of most elements can form ions. Metal elements, like iron, lose electrons when they form ions, so they become positively charged. Nonmetal atoms form ions, too, but when they do, they gain electrons. Thus, they become negatively charged because they have more electrons than they do protons. If a chlorine atom gains an electron it will have a (–1) charge (shown as Cl⁻). If sulfur gains 2 electrons it will have a (–2) charge.

\[
\begin{align*}
\text{Cl} + 1e^- & \rightarrow \text{Cl}^- \\
17 \text{ protons(+) & 17 \text{ protons(+)} & 16 \text{ protons(+) & 16 \text{ protons(+)} \\
17 \text{ electrons(–) & 18 \text{ electrons(–) & 16 \text{ electrons(–) & 18 \text{ electrons(–) & –1 \text{ charge & no charge & –2 \text{ charge} \\
\text{no charge & –1 charge & no charge & –2 charge}
\end{align*}
\]

Ions have very different properties than the neutral atoms they are formed from. For example, Fe atoms are very different from Fe²⁺ ions. Atoms of some elements, like iron, can form more than one type of ion, because atoms of those elements can lose varying numbers of electrons. Those different ions of the same element, such as Fe²⁺ and Fe³⁺, not only have different properties from their neutral atoms, but they also are very distinct from each other. You observed one significant difference between Fe²⁺ and Fe³⁺, that they have different effects on some chemical reactions. In this activity, Fe²⁺ ions speed up the reaction between luminol and hydrogen peroxide, but Fe³⁺ ions do not.

**How Substances Produce Light: The Nanoscopic Perspective**

Electrons exist outside the nucleus in certain allowable states called energy levels. When an electron gains energy, it can move from a lower energy level to a higher level. However, the energy that the electron gains must be equal to the energy difference between the two levels. Think of the energy levels as the rungs of a ladder. If you only give your
foot enough energy to make it halfway between one rung and the next, it is not going to reach the next rung. Instead, it will remain on the lower rung. If an electron does not gain enough energy, it will not be promoted to a higher energy level. When an electron gains enough energy to move to a higher state it is in an excited state. This excited state has a higher energy level than the ground state where the electron began. The energy the electron gains may come from heat, collisions, chemical reactions, light, or other forms of electromagnetic radiation.

The electron does not remain in the excited state for very long. It quickly falls to a lower energy state and in the process loses the energy it gained. The energy lost from the electron’s return to the ground state is emitted as light energy and so the atom gives off light. With this basic understanding of how some atoms are able to produce light, you can now make sense of the luminol reaction.

**Production of Light with the Luminol Mixture**

The glowstick reaction and the luminol reaction are examples of chemiluminescence. As you observed in this activity with the lightstick, chemiluminescence is the production of light without heat through a chemical reaction. In chemiluminescence, the starting substances (reactants) react and their atoms rearrange themselves to form new substance (products) and light. In the luminol blood test, luminol reacts with hydrogen peroxide to form a new compound (called 3-aminophthalate). The energy released in this reaction excites some of the electrons in the new compound. When the electrons in the new compound return to the ground state, blue light is released.

However, at room temperature, luminol and hydrogen peroxide molecules do not react at a significant rate. Some light may be emitted but not enough to easily observe. A catalyst is needed in order to speed up the reaction so that more light will be produced in a given time and the light can be more easily observed. A catalyst is a substance that speeds up a chemical reaction without being changed itself.
In every chemical reaction, an energy barrier must be overcome for the reactants to be converted into products. You can picture that energy barrier like a hill, as shown in the diagram. Point A represents the energy level of the reactants and Point B represents the products at the end of the reaction. For the luminol reaction, this energy barrier is high enough that the reaction does not take place at any visible extent at room temperature. This explains why the luminol solution that you made does not glow in the spray bottle, before it comes in contact with a catalyst. A catalyst works by lowering the energy barrier. The iron in the blood, $\text{Fe}^{2+}$, is the catalyst for the luminol and water reaction.

When the luminol-peroxide mixture comes into contact with blood, the $\text{Fe}^{2+}$ ions that are in the center of the hemoglobin molecules catalyze the reaction. A visible blue glow is produced. You can picture this as lowering the hill in the diagram, making it a faster process to get the reaction from Point A to Point B.

**Why Is the Test Presumptive?**

You found other substances also catalyze the reaction and cause the solution to glow. The luminol test is not specific for blood. Examples of other ions that catalyze this reaction include $\text{Cu}^{2+}$ and $\text{Co}^{2+}$ ions. Different plant materials such as horseradish can catalyze the luminol reaction as well. These materials contain enzymes. Enzymes are molecules that catalyze specific reactions in living things. Some enzymes also happen to catalyze the luminol reaction. Because the luminol test is not specific for blood, it is called a presumptive test. A positive result, glowing, indicates that blood may be present. A negative result, though, does indicate that no blood or blood residue is present.

Other tests need to be performed on the sample to confirm that the substance is blood. You may have also noticed, however, that each substance speeds up the reaction to a different degree. For example, bleach causes the reaction to glow intensely for a few seconds whereas hemoglobin causes the reaction to glow more uniformly, more dimly, and for a longer period of time. An experienced forensic chemist will have a good idea whether or not the glow is caused by blood. However, no matter how experienced the investigator, a sample will have to be taken back to the lab to determine if the substance is actually blood.
What Do You Think Now?
At the beginning of this activity you were asked:

- What do detectives spray when they are spraying for blood?
- How do they know blood is present at the scene after spraying?

You investigated the answers to these questions during the activity. Now, think about the lightstick you looked at in Part A. The diagram shows how a lightstick works. Assume luminol is causing the glow of this lightstick. (There are other possibilities besides luminol.) What substances could be in the glass and plastic tubes?

Chem Essential Questions

What does it mean?
Chemistry explains a macroscopic phenomenon (what you observe) with a description of what happens at the nanoscopic level (atoms and molecules) using symbolic structures as a way to communicate. Complete the chart below in your Active Chemistry log.

<table>
<thead>
<tr>
<th>MACRO</th>
<th>NANO</th>
<th>SYMBOLIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>What are the observable changes when the luminol is sprayed onto blood?</td>
<td>Describe how light can be produced by an atom.</td>
<td>Graphically and symbolically, how can you represent the reaction you observed both before you sprayed (what is happening in the bottle) and after you sprayed the reaction mixture on your sample?</td>
</tr>
</tbody>
</table>

How do you know?
Making specific reference to your data and using terms from the Chem Talk section, explain why the luminol test is called a presumptive test for the presence of blood.

Why do you believe?
A neon sign is nothing more than electrodes, powered through the electrical socket of your house, in atoms of a gas, like neon. When you turn a neon sign on, electrical energy is gained and lost by the gas atoms, and the sign glows. When you stop the electricity, it doesn’t glow. How does this relate to the luminol reaction studied in this activity? How is it different?

Why should you care?
How could you use the idea that luminol is a presumptive test in your story? Give a specific example.
Reflecting on the Activity and the Challenge

The powdered luminol reagent you used contains both luminol and hydrogen peroxide. The luminol reacts with the hydrogen peroxide once the reagent is dissolved in water, producing light. However, this reaction does not occur fast enough to produce enough light for your eyes to detect it until the hemoglobin comes in contact with the luminol and hydrogen peroxide. The hemoglobin in the blood contains a particular form of iron, and that iron speeds up the reaction. When that happens, the reacting luminol produces enough light for you to see a blue glow. The more blood present, the faster the reaction takes place and the brighter the glow is.

Part of the problem you are facing in creating a crime scene is how to observe something that is not initially visible. In this activity you focused on how the chemiluminescence of luminol could be used to detect the presence of blood. You also examined other substances that caused the luminol to glow. A positive luminol test does not mean the substance is definitely blood. Think how this may mislead you when you are examining a crime scene. Finally, you can now use the concepts that you have learned to describe the chemistry behind this technique, another important element of the Chapter Challenge.

1. Why are the two chemicals in a lightstick separated by glass?
2. Why do lightsticks eventually stop producing light?
3. What prevents the hydrogen peroxide and the luminol from glowing in the spray bottle?
4. What is the symbol for the element that has 16 protons?
5. Identify each as an atom or ion.
   a) 15 protons and 15 electrons      b) 7 protons and 10 electrons
   c) 3 protons and 2 electrons
6. Write the symbol for the element that has 16 protons and 18 electrons.
7. Examine Group 1 and Group 2 below.
   Group 1: Ti$^{2+}$  Na$^+$  Mg$^{2+}$  Al$^{3+}$
   Group 2: P$^{3-}$  F$^-$  As$^{3-}$  O$^{2-}$
   Which group was formed when electrons were lost?
   Which group was formed when electrons were gained?
8. **Preparing for the Chapter Challenge**

Using density to identify glass and using luminol to indicate blood, are two ways that can lead to a false identification of a suspect. In your *Active Chemistry* log, write two scenes; one that uses the properties in the last two activities to correctly identify a suspect as the criminal and one that uses both properties to falsely identify a suspect as the criminal. In the false statement, give an explanation as to how it was a false identification. Use this process and your teacher’s feedback to start thinking about how you’ll make your crime scene have possible twists for the future investigators.

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**Inquiring Further**

1. **Other luminol reaction catalysts**

   Research the luminol blood test. Design an experiment to determine if other household chemicals will catalyze the luminol reaction. Under the supervision of your teacher perform your investigation.

2. **Sensitivity of the luminol test**

   Design an experiment to determine the sensitivity of the luminol test. Before designing your experiment research the concepts of solution, dilution and concentration. Under the supervision of your teacher perform your experiment and present your results to the class.

3. **Other presumptive blood tests**

   There are many other types of presumptive blood tests. One of the more popular alternatives to the luminol test is the Kastle-Meyer test. In the Kastle-Meyer test, the reagent turns pink in the presence of blood. Research the Kastle-Meyer test. Under the supervision of your teacher, investigate the sensitivity and specificity of this test. Compare your results to the luminol test.