The Effect of the Presence of Iron in Common Household Substances on the Luminol Blood Detection Test

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Abstract: Forensic science encompasses a multitude of different sub-disciplines. One of these is crime scene investigation, in which investigators collect and analyze evidence found at crime scenes. Blood is a critical piece of evidence found at crime scenes, as it can provide investigators with a wealth of information about the case. Luminol is one of the most frequently used presumptive blood tests and determines if a substance may be blood or not. When luminol comes into contact with the hemoglobin in blood, it produces chemiluminescence because of an oxidation reaction. There are some substances that are known to produce false positives for the luminol test, such as strong oxidants and true peroxidases. This study focused on the presence of iron in the substances tested and whether the iron would produce a false-positive. Half of the substances tested contained iron and the other half didn't. The substances containing iron didn't produce a higher level of fluorescence than the substances not containing iron. The results don't support the assumption that the presence of iron in a substance would affect its fluorescence when sprayed with luminol.

Keywords: luminol, chemiluminescence, presumptive test, iron, crime scene investigation

Blood evidence is a crucial part of crime scene investigation. Both the determination of blood type and extraction of DNA can be accomplished when blood is found at the crime scene. Bloodstain patterns can also give key information for crime scene reconstruction. Before analysis can be done, the substance found at the crime scene needs to be determined to be blood (Vandewoestyne et al., 2015). There are several presumptive and confirmatory tests that can do this. Presumptive tests are faster and can be done at the scene but are not as accurate. One presumptive test that is frequently used by crime scene investigators is luminol. Luminol has the ability to recover traces of blood after the blood has already been cleaned. Luminol ($C_8H_7N_3O_2$) is a chemical that produces light when it is oxidized, through a process called chemiluminescence (Huang et al., 2020). Hydrogen peroxide oxidizes luminol which forms 3-aminophthalate anions with their electrons in an excited state. The electrons can't stay in the excited state and eventually return to their ground state. When the electrons return to their ground state, they release the energy they used to get to the excited state in the form of light (Huang et al., 2020). Crime scene investigators take advantage of this chemical reaction when searching for latent blood. They use a

solution with several reagents, which include luminol and hydrogen peroxide. When the solution is sprayed on blood, the iron in hemoglobin catalyzes the oxidation of the luminol by the hydrogen peroxide, resulting in chemiluminescence (Quickenden and Creamer, 2001). The catalyzation of the oxidation reaction leads to the production of light, so substances that also catalyze the reaction would be able to produce light as well. This is possibly problematic for investigators, as they could mistakenly believe that a substance is blood if luminol produces light when sprayed on the substance (Creamer et al., 2003). The iron in hemoglobin is the catalyst in the reaction, so this study assesses whether certain substances that contain iron will produce light when sprayed with luminol. The experiment tests the assumption that if a substance contains iron, then it will react with luminol and produce light. The results of this study indicate whether luminol is reliable in visualizing blood for crime scene investigation or is unreliable and should be used less frequently by crime scene investigators.

Materials and Methods

To conduct this experiment, six different substances were tested with the luminol solution. Three of the substances contained iron and the other three didn't. The first substance tested was beet juice (Knudsen & Sons, Inc., Chico, California), which is high in iron content (Liang et al., 2013). The next two substances tested were egg yolks (The Country Hen, Luberski Inc., Hubbardston, Massachusetts) and spinach leaves (H-E-B Grocery Company, San Antonio, Texas), which are also high in iron content. Hill Country Fare bleach (H-E-B Grocery Company, San Antonio, Texas) was tested next and was one of the substances not containing iron. Then, Mrs. Meyer's lavender disinfectant (The Caldrea Company, Racine, Wisconsin) was tested and was another substance that didn't contain iron. The last substance not containing iron that was tested was Lysol lemon breeze disinfectant (Reckitt Benckiser, Parsippany, New Jersey). Synthetic blood (MEDTECH Forensics, Tallahassee, Florida) was also used as a control to compare fluorescence to the other substances. The luminol solution was prepared by mixing 14 grams of sodium perborate tetrahydrate, 3-aminophthalhydrazide, and sodium carbonate (Sirchie, Youngsville, North Carolina) with 236 mL of distilled water. The experiment consisted of five trials for each substance in addition to one control trial. Two sprays of luminol solution with a plastic spray bottle (manufacturer, city, state) were used for each trial. 1 mL of synthetic blood was used for the control trial, it was poured on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. Pictures were taken and observations were recorded about the intensity of the fluorescence. 1 mL of beet juice was used for each trial, it was poured out on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. Pictures were taken and observations were recorded. These observations were about the intensity of the fluorescence and about how it

compared to the fluorescence of the synthetic blood when sprayed with the luminol solution. 1 mL of egg yolk was used for each trial, the yolks of the eggs were separated from the whites and were whisked together. The egg yolk was poured on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. The same method for observation was used. One spinach leaf was used for each trial, the leaves were each cut up into smaller pieces. The pieces of the spinach leaf were placed on hardwood flooring and sprayed with luminol. The lights were turned off and any fluorescence that occurred was timed. The same method for observation was used. 1 mL of bleach was used for each trial, it was poured out on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. The same method for observation was used. 1 ml of Mrs. Meyer's lavender disinfectant was used for each trial, it was poured out on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. The same method for observation was used. 1 mL of Lysol lemon breeze disinfectant was used for each trial, it was poured out on hardwood flooring and sprayed with the luminol solution. The lights were turned off and any fluorescence that occurred was timed. The same method for observation was used.

Results

Several substances, some containing iron, were tested with luminol in order to compare their fluorescence to that of hemoglobin. The luminol test for blood is presumptive, so there are two possible results, positive or negative. Positive meaning the substance may be blood and negative meaning the substance isn't blood. Consequently, the results of this experiment were mostly qualitative rather than quantitative. The results of the presumptive tests are found in Table 1.

Table 1. Positive and negative results of luminol blood detection test.

Trial	Substance	Presence of Fluorescence
Control trial	Synthetic blood	+
1	Beet juice	+
	Egg yolk	-
	Spinach	-
	Bleach	+
	Mrs. Meyer's disinfectant	+
	Lysol disinfectant	+

2	Beet juice Egg yolk Spinach Bleach Mrs. Meyer's disinfectant Lysol disinfectant	+ - + + - +
3	Beet juice Egg yolk Spinach Bleach Mrs. Meyer's disinfectant Lysol disinfectant	- - - + + -
4	Beet juice Egg yolk Spinach Bleach Mrs. Meyer's disinfectant Lysol disinfectant	+ - - + - -
5	Beet juice Egg yolk Spinach Bleach Mrs. Meyer's disinfectant Lysol disinfectant	- - + + + -

The beet juice produced fluorescence in three out of five trials but was barely visible. Compared to the synthetic blood, the fluorescence of the beet juice was very weak. The egg yolk didn't fluoresce for any of the five trials. The spinach produced fluorescence in two out of the five trials, but the fluorescence was also very weak when compared to that of the synthetic blood. The bleach produced fluorescence in every trial. The intensity of the fluorescence matched the intensity of the fluorescence of the synthetic blood for the first second, then became less vibrant for the rest of the time. The Mrs. Meyer's disinfectant produced fluorescence for three out of the five trials and was visible but much less vibrant than the fluorescence of the synthetic blood. The Lysol disinfectant produced fluorescence for two out of the five trials and was barely visible and very weak when compared to the fluorescence of the synthetic blood. Picture were taken of all of the substances, but the fluorescence for some of the substances didn't last long enough or wasn't visible enough to show up in the pictures. The fluorescence of the substances that were visible in the pictures are shown in Figures 1-3. The different substances

produced fluorescence that lasted different amounts of time. The average time in seconds for each substance's fluorescence is found in Figure 4.

Figure 1. Fluorescence of synthetic blood.

Figure 2. Fluorescence of bleach.

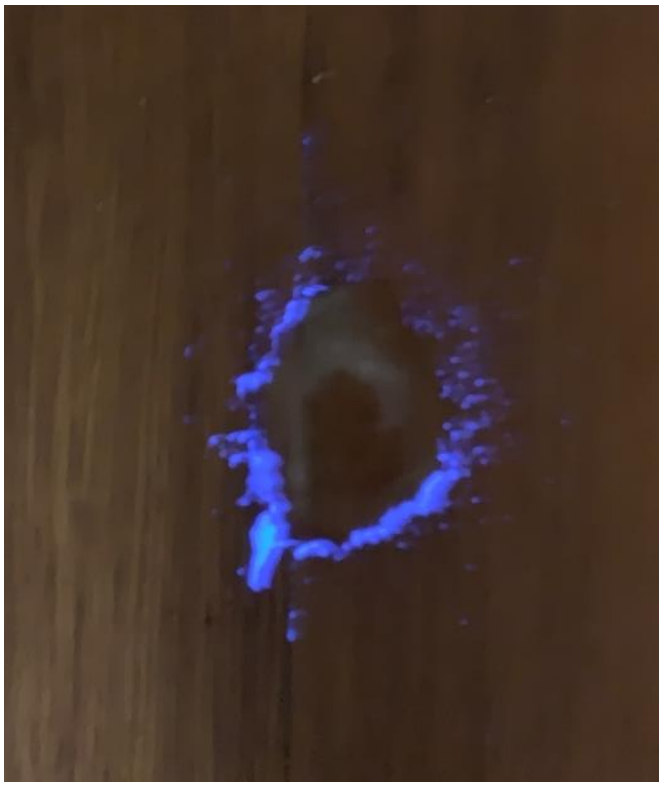


Figure 3. Fluorescence of Mrs. Meyer's disinfectant.

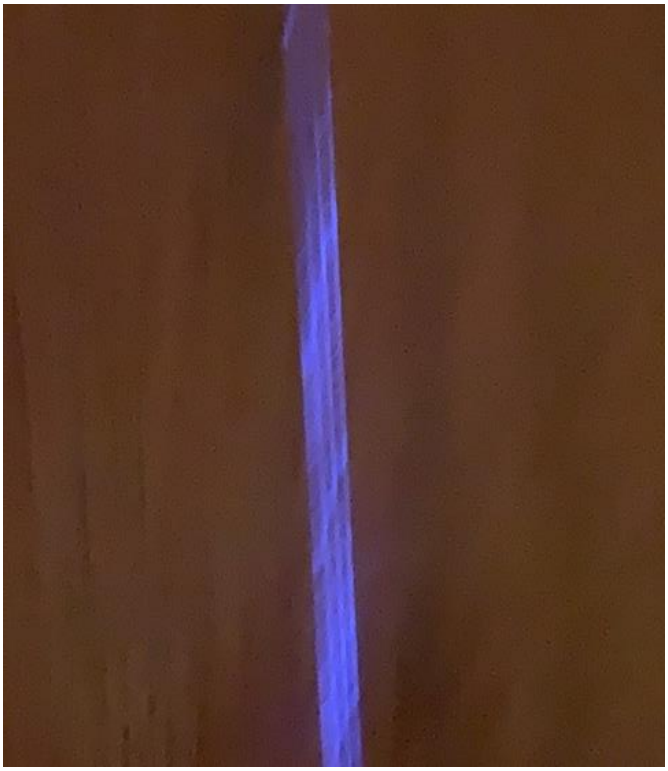
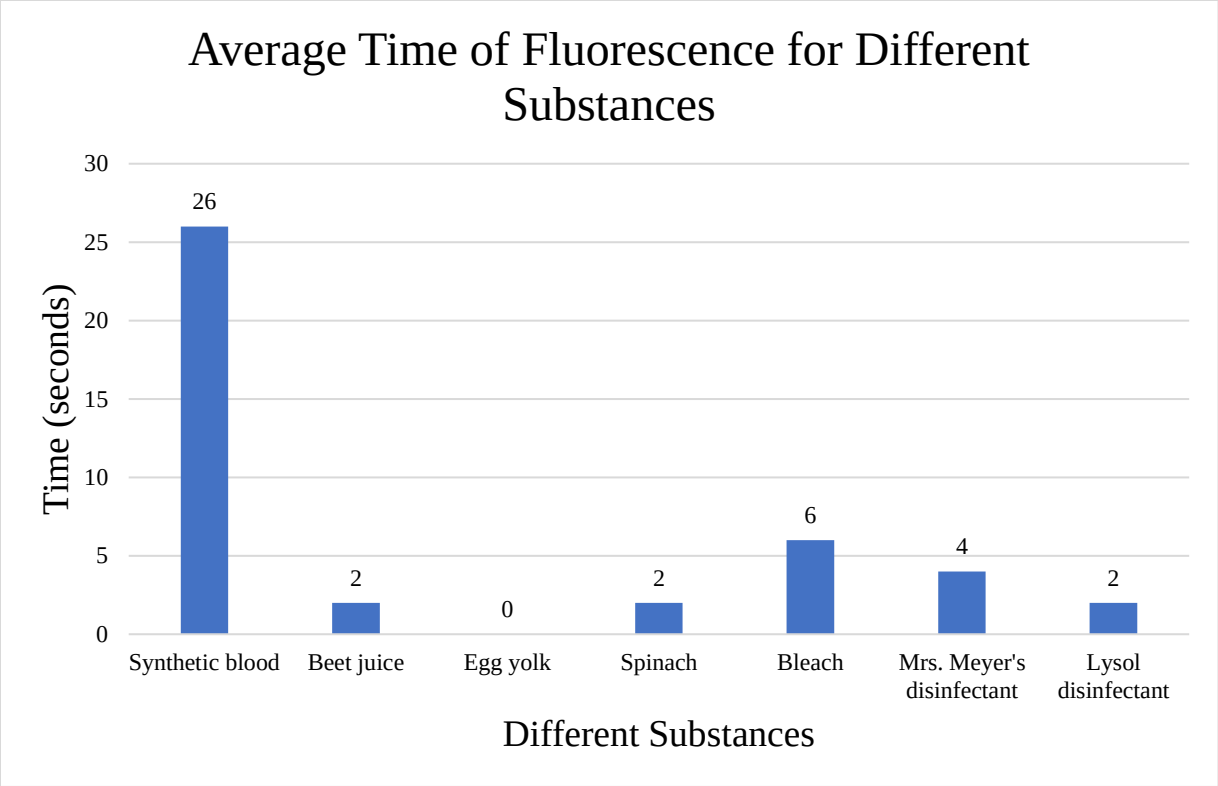


Figure 4. Average time of fluorescence.



Excluding the synthetic blood, the fluorescence of the bleach lasted the longest on average. The Mrs. Meyer's disinfectant's fluorescence lasted the second longest amount of time. The beet juice, egg yolk, and Lysol disinfectant's fluorescence lasted the third longest amount of time.

Discussion

Luminol is one of the most common presumptive blood tests used by crime scene investigators searching for latent blood (Seashols et al., 2013). It is very sensitive and can detect blood in trace amounts but is not specific to just blood (Castelló et al., 2002). Some interfering substances include soils, bleaches, metal objects, and vegetable compounds (Barni et al., 2007). In this study, the presence of iron was the focus, so the substances tested either contained iron or didn't. The experiment was testing whether the presence of iron would cause the substances to fluoresce when sprayed with luminol or not. In this experiment, the substances containing iron either had a lower level of fluorescence or none at all. The substances not containing iron had either a higher level of fluorescence or one similar to that of the substances containing iron. These results indicate that the fluorescence of the bleach, Mrs. Meyer's disinfectant, and Lysol disinfectant was not due to iron because none of them contain a high amount of iron. The low levels of fluorescence for the beet juice and spinach could possibly be due to a presence of iron or could be due to another trait since the substances not containing iron

also had the ability to produce fluorescence. Substances that are known to interfere with the luminol test are usually strong oxidants or true peroxidases (Stoica et al., 2016). This explains why the substances not containing iron also produced fluorescence. Bleach is a very strong oxidant and produced the highest level of fluorescence. The beet juice and spinach could have produced fluorescence because they both contain peroxidase (Liang et al., 2013). Hemoglobin is known to have peroxidase-like activity, which catalyzes the oxidation of the luminol (Webb et al., 2006). The results of this study refute the original hypothesis that if the substance contains iron, it will produce fluorescence when sprayed with luminol. The results still demonstrate that luminol reacts with substances other than blood. However, investigators are usually able to distinguish between the fluorescence produced by blood and the fluorescence produced by other substances because of the shape and pattern (Quickenden and Creamer, 2001). Bleach, for example, is usually used for cleaning so the fluorescence would be an even layer on the surface and would be different from the fluorescence of blood at a crime scene. Care should still be taken when using luminol because false-positives are known to occur for certain substances like the ones in this study because of their oxidizing abilities or peroxidase-like activity. The results of this study indicate that the presence of iron in a substance is not the cause of the production of fluorescence when the substance is sprayed with luminol. Therefore, luminol, while still producing some false positives, is one of the more reliable presumptive blood tests. Since blood detection is a critical part of crime scene investigation, further research could be done to possibly produce an even more reliable presumptive blood test.

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