Common Household Reagents that React with Luminol in a Similar Manner as Hemoglobin that Decrease the Reliability of Luminol

Author: Taeja Zubillaga

Editor: Alex Watros

**Abstract**: This study was conducted to identify common household reagents, such as bleach or rust, that would react with Luminol in a similar manner as hemoglobin, and thus decrease the reliability of Luminol when used at crime scenes. This experiment was conducted in a household, using 10 samples of the household reagents bleach, rust from pennies, laundry detergent, hoisin sauce, lemon juice, and urine. A small sample of blood in addition to each of the 10 samples of the reagents were placed onto a bed sheet, and liquid Luminol was sprayed over them. The luminescence, or lack thereof, of the Luminol in conjunction with each of the household reagents was then ranked on a scale of 1-4 in terms of how similarly it appeared to that of the hemoglobin. The major findings from this study, were that the bleach reacted with Luminol 95% of the time. Rust on pennies reacted with Luminol 70% of the time, whereas hoisin sauce reacted with Luminol only 17.5% of the time. Urine reacted with Luminol minimally and 7.5% of the time, and lemon juice and laundry detergent reacted with Luminol 0% of the time. Bleach and rust (Copper Oxide) were determined to have a high chance of decreasing the reliability of Luminol. Hoisin sauce, urine, lemon juice, and laundry detergents are common household reagents that do not have much bearing on the reliability of Luminol when it is used at crime scenes.

*Keywords*:Luminol, forensics, hemoglobin, luminescence, bleach.

This study explored the compound Luminol and its reliability in the presence of common household reagents. This is an important topic, as Luminol is a reagent that is used frequently by crime scene investigators, to detect trace amounts of blood. If Luminol is illuminated by substances other than hemoglobin, it results in something called a “false-positive”, which can drastically change the outcome of a proper crime scene investigation.

It is known that there are a few other reagents that can trigger the oxidation, and therefore the luminescence, of Luminol without the presence of hemoglobin. According to a study of common interferences with the forensic Luminol test for blood, conducted at the University of Western Australia, many substances in each category of reagents gave intensities of luminescence, which were comparable with the intensities of hemoglobin, when sprayed with a solution containing aqueous luminol and sodium perborate (Quickenden and Creamer 295). Some of these substances included vegetable and fruit pulps, juices, domestic and commercial oils, cleaning agents, an insecticide, and various glues, paints, and varnishes. While this was not necessarily a topic that included much published research, it was pretty well-known before the study was conducted my that bleach, urine with low traces of blood, and copper in some form would oxidize Luminol and trigger its luminescence.

“What chemical substances, that are commonly found in or around households, are most likely to falsely trigger the luminescence of the compound Luminol, and thus which of these compounds are most likely to decrease the reliability of Luminol being used at crime scenes?” This question will advance our knowledge, as a scientific community, of Luminol and how reliable it is when used in households, where it is fairly common to find things such as bleach, urine, rust, and more. The hypothesis tested was that the household reagent bleach would react with Luminol roughly 90% of the time, rust would react with Luminol roughly 60% of the time, and other substances such as detergents, acidic foods and juices, and urine would trigger the luminescence of Luminol up to 40% of the time. From this study, depending upon the results, we can begin a discussion that either Luminol is very reliable in most instances and it should continue to be used at crime scenes, or that it is overall not as reliable as once thought, and a different test for hemoglobin should be used.

**Materials and Methods**

**Preparing the Experiment**

The objects used in this study were Luminol and blood, as well as the household reagents of Clorox bleach, rust from corroded pennies, Gain laundry detergent, Kikkoman hoisin sauce, Realemon lemon juice, and a urine sample from myself. The Luminol was a mixture of 2 grams of powdered Luminol (C8H7N3O2) and 200mL of deionized water. The household reagents came in a 25mL sample as they were, from an unopened bottle. For the rust treatment, one corroded penny per trial was used. The study was conducted in my house, on a bedsheet that was designed to most similarly represent the fabric of clothing or bedsheets, which are present at many crime scenes. 25ml of solution was placed on the bed sheet. Luminol was sprayed on each testing site for a total of 10 trials.

**Control and Variables of the Experiment**

The control in this study was a few drops of blood from a pricked finger. The treatments that were administered were 10 separate trials of 25mL each of the household reagents bleach, laundry detergent, hoisin sauce, lemon juice, and urine, as well as 10 separate trials containing one corroded penny. The variables measured were how many trials out of 10 the Luminol was oxidized by the particular household reagent, and how similarly each trail “ranked” in terms of similarity to that of Luminol and hemoglobin, on a scale of 0-4.

**Protocol and Experimental Procedure**

The protocol for this study is as follows:

1. Gather 10 samples of 25mL each of the household reagents needed for the experiment: bleach, rust (from corroded pennies), laundry detergent, hoisin sauce, lemon juice, and urine.
2. Create a chart to record data, with 10 slots for 10 trials of each of the substances.
3. Smear 25mL of each of the samples, or in the case of the pennies place 1 penny per trial, on a bed sheet for testing.
4. Spray each sample with the substance Luminol, and allow enough time for the samples to “illuminate” if it does.
5. “Rank” each of the first test of samples on a scale of 0-4, in regard to how illuminated the substance is. The rankings are classified as follows:

0: The household reagent did not illuminate at all.

1: The household reagent was very dimly illuminated in some instances, though it was difficult to decipher and was very inconsistent.

2: The household reagent was illuminated, but only somewhat.

3: The household reagent illuminated clearly and was brighter and more obvious than that of the previous level.

4: The household reagent illuminated very clearly and obviously, and it was hard to distinguish this level of luminescence from that of hemoglobin.

1. After recording the data for each of the substances, place another sample significantly below the previous on the bedsheet.
2. Repeat steps 3-5 another 9 times, so that each reagent was tested with the Luminol a total of 10 times.
3. Compare the data, noting the percentage at which “ranking” each substance was illuminated, for each of the 10 trials. For example, if bleach had a ranking of 4 for 6 out of the 10 trials, bleach had a ranking of 4 60% of the time.

**Analyzing Data**

To analyze the data and answer the proposed research question, The total rankings for each of the household reagents were added up and compared. The highest “score” a particular reagent could get was a 40, as the highest ranking is a 4 and there are 10 total trials. The lowest “score” would be a 0. Once the total scores were added up, they were then divided by 40 and multiplied by 100, to end with a percentage. For the purposes of this study, that percentage represented the likelihood that the presence of the particular household reagent would trigger the luminescence of Luminol and decrease its reliability, when used at crime scenes. For example, if bleach had a total score of 30, (30/40)\*(100) = 75%, so it can be assumed that in this particular experiment, the presence of bleach would trigger the luminescence of Luminol roughly 75% of the time, significantly decreasing its reliability when used at crime scenes.

**Results**

Clorox bleach and rust by far had the highest likelihood of decreasing the reliability of Luminol when used at crime scenes.

***Table 1: Luminescence Rankings***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Trial Number** | **Clorox Bleach** | **Rust on Pennies** | **Gain Laundry Detergent** | **Kikkoman Hoisin Sauce** | **Realemon Lemon Juice** | **Urine (mine)** |
| **Trial 1** | 4 | 2 | 0 | 0 | 0 | 0 |
| **Trial 2** | 3 | 3 | 0 | 0 | 0 | 1 |
| **Trial 3** | 4 | 4 | 0 | 0 | 0 | 1 |
| **Trial 4** | 4 | 2 | 0 | 1 | 0 | 0 |
| **Trial 5** | 4 | 3 | 0 | 2 | 0 | 0 |
| **Trial 6** | 4 | 2 | 0 | 1 | 0 | 0 |
| **Trial 7** | 4 | 4 | 0 | 1 | 0 | 0 |
| **Trial 8** | 4 | 3 | 0 | 0 | 0 | 0 |
| **Trial 9** | 3 | 3 | 0 | 2 | 0 | 0 |
| **Trial 10** | 4 | 2 | 0 | 0 | 0 | 1 |

About 80% of the trials of bleach ranked a 4, signifying the household reagent illuminated very clearly and brightly. Out of a possible total of 40, the luminescence of Luminol and bleach scored a 38, or 95%. Figure 1 is a visual representation of the luminescence of bleach and Luminol. About 20% of the trials of rust ranked a 4, 40% ranked a 3, and 40% ranked a 2. Out of a possible total of 40 the luminescence of Luminol and rust scored a 28, or 70%. About 30% of the trials with the reagent urine ranked a 1, and 70% of the trials ranked a 0. Out of a possible total of 40, the luminescence of the urine and Luminol scored a 3, or 7.5%. About 20% of the trials of hoisin sauce ranked a 2, 30% ranked a 1, and 50% ranked a 0. Out of a possible total of 50, the luminescence of hoisin sauce and Luminol scored a 7, or 17.5%. 100% of the trials of both laundry detergent and lemon juice ranked a 0. Out of a possible total of 40 both of these reagents scored a 0.



**Fig. 1.**Photograph of spots of bleach on a bedsheet sprayed with Luminol, taken on an iPhone camera.

**Discussion**

Given the results, the conclusion can be made that household reagent bleach will trigger the luminescence of Luminol roughly 95% of the time and almost always decrease Luminol’s reliability when used at crime scenes. The reagent rust will trigger the luminescence of Luminol roughly 70% of the time and significantly decrease Luminol’s reliability when used at crime scenes. The reagent urine will trigger the luminescence of Luminol 7.5% of the time and it may, in some instances such as when traces of blood are present, have some effect on Luminol’s reliability when used at crime scenes, though it is not highly likely. The household reagent hoisin sauce will trigger the luminescence of Luminol 17.5% of the time, and it is not probable that it will decrease Luminol’s reliability when used at crime scenes. Lastly, given a score of 0% for both reagents, it is not probable that either laundry detergent or lemon juice would react with and decrease the reliability of Luminol when used at crime scenes.

These findings do agree with what others have shown, in regard to the reagents bleach, rust, urine, and lemon juice. Numerous studies, such as one conducted at the University of Valencia, agree that in the presence of household bleach, presumptive tests such as Luminol are invalidated (Castello et. al 1). Other research has shown that pennies readily oxidize Luminol and luminesce, as will urine if traces of blood are present. The laundry detergent reagent that I tested did not, however, agree with some other findings that say chemicals in detergents and fabric softeners can illuminate Luminol. The results from the experiment showed that Gain laundry detergent, when sprayed with Luminol, illuminated 0% of the time. The difference between the here results and those of others, could be due to the varying chemicals in the specific brand of laundry detergent used, and the fact that it lacked an oxidizing agent.

Given these conclusions, our new understanding of Luminol and its reliability in the presence of common household reagents, is that it is not as reliable in some instances as we may have previously thought. The luminescence of Luminol is not hemoglobin-specific and thus, it is easily manipulated by other chemicals that act as an oxidizing agent. Many investigative practices are moving to the use of BlueStar, so this would be another topic of exploration. A similar study could be conducted to determine if other presumptive tests such as BlueStar are easily manipulated by other reagents that may be present at crime scenes, or if they are more precise and react only to hemoglobin.

**References**

**Butler, J., Chaseling, J., & Wright, K. 2019.** A comparison of four presumptive tests for the detection of blood on dark materials.*Journal of Forensic Sciences, 64*(6), 1838-1843.

**Cassidy, B.M., Lu Z., Martin, J.P., Tazik, S.K., Kellogg, K.W., DeJong, S.A., and S.L. Morgan. 2017.** A quantitative method for determining a representative detection limit of the forensic luminol test for latent bloodstains.*Forensic Science International (Online), 278*, 396-403.

**Castelló, A., Francés, F., and Verdú, F. 2009.** Bleach interference in forensic luminol tests on porous surfaces: More about the drying time effect. Talanta, Volume 77, Issue 4, Pages 1555-1557.

**Howard, D., Chaseling, J., and Wright K. 2019.** Detection of blood on clothing laundered with sodium percarbonate.*Forensic Science International (Online), 302*.

**Oldfield, C., Morgan, R.M., Miles, H.C., and J.C. French. 2018.** The efficacy of luminol in detecting bloodstains that have been washed with sodium percarbonate and exposed to environmental conditions, Australian Journal of Forensic Sciences, 50(4), 345-354.

**Quickenden, T.I. and J.I. Creamer. 2001.** A study of common interferences with the forensic luminol test for blood. Luminescence 16(4):8-295.

**Stoica, B.A., Bunescu, S., Neamtu, A., Bulgaru-Iliescu, D., Foia, L., & Botnariu, E.G. 2016.** Improving luminol blood detection in forensics.*Journal of Forensic Sciences, 61*(5), 1331-1336.

**Toluwase, H.F. 2020.***In-silico investigation of luminol, its analogues and improved mechanism of chemiluminescence for blood identification beyond forensics*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

**Competency, Forensic.** “Review: Improving Luminol Blood Detection in Forensics.” *Florida Forensic Science*, Forensic Competency Http://Floridaforensicscience.com/Wp-Content/Uploads/2016/12/Forensic-logo1-1030x153.Png, 12 July 2019, [www.floridaforensicscience.com/improving-luminol-blood-detection-forensics/](http://www.floridaforensicscience.com/improving-luminol-blood-detection-forensics/).

**Interest, Post author By Compound, et al.** “Crime Scene Chemistry – Luminol, Blood & Horseradish.” *Compound Interest*, 19 Oct. 2016, [www.compoundchem.com/2014/10/17/luminol/](http://www.compoundchem.com/2014/10/17/luminol/).