The Sensitivity of Luminol to Common Household Produce Regarding Forensic Use

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Abstract. 3-Aminophthalhydrazide, later coined luminol in 1934 by Ernest H. Huntress, is a well-known chemical used in the detection of blood at a crime scene. For almost a century, it has been utilized as a presumptive for blood in scenes of crimes and laboratories. While luminol is useful as it is extremely sensitive to the presence of hemoglobin and hematin, there are also known false positives that can skew the interpretation of a crime scene. As a result, an experiment was conducted in which common household fruits and vegetables were sprayed with a luminol mixture to test if they would result in a false positive identification for blood. In this study, the produce was separated into their individual parts: skin, leaves, and flesh, and juiced. Each of these parts were then sprayed independently with a luminol mixture and observed in darkness to check if chemiluminescence was present. The results indicated that out of the nine different types of produce tested (navel orange, lime, red grapefruit, beet, radish, kale, spinach, rainbow swiss chard, broccoli), only radishes reacted with the luminol mixture. However, since the duration of the luminescence and the intensity of the luminescence differed heavily from the duration and intensity of luminescence from a luminol and blood reaction, it is highly unlikely that radishes will result in a false positive identification for blood at the scene of a crime. Thus, none of the nine different types of produce tested were deemed likely to result in a false positive identification at the scene of a crime. This study is a foundation for the extent of false positives on household fruits and vegetables sprayed with luminol; however, several questions need a wider array of the Brassicaceae family to check if more than just radishes react to luminol.

Key Words: *3-Aminophthalhydrazide, luminol, chemiluminescence, forensics*

The use of luminol has proven to play an integral part in the analysis of crime scenes for almost 100 years. From its synthesis in 1902 to its first use as a presumptive blood test in 1937, the use of luminol has proven to be an effective way to identify traces of blood at the scene of a crime (Grispino, 1990). In a 1939 study by Moody and Proescher, luminol was able to detect hematin in a $1:10^8$ dilution of blood. A 1986 study by Thornton showed that the luminescence of luminol in a $1:10^4$ of blood was detectable by the human eye. An infrared starlight scope could be used to detect the luminescence of luminol in a dilution of blood of $1:10^6$ to $5:10^6$ (Grispino, 1990). However, it has been noted in several studies that the presence of copper, iron, ferricyanide, manganese peroxide, and hypochlorites have resulted in false positives (Gundermann, 1965; Schneider, 1970; Gaennsslen, 1983; Wei and White, 1971; Roswell and White, 1978). This raised concerns about the overall accuracy of luminol for forensic use.

This experiment aims to alleviate some of these concerns by subjecting some common household produce to luminol to see if any of these results in a false positive identification. These different types of produce would come from different taxonomic families to observe whether produce from the same family would yield similar results. This experiment will test whether the produce reacts with luminol, and it will also contain a comparison of each reaction to how luminol reacts with blood.

Materials and Methods.

All nine different types of produce were sourced from the produce section of the Walmart Supercenter (643 N Harvey Mitchell Pkwy. Bryan, TX 77807). One bunch of beets, one bushel of rainbow swiss chard, and one bushel of spinach were used to represent the Amaranthaceae family. One head of broccoli, one bunch of radishes, and one bushel of kale leaves were used to represent the Brassicaceae family. One navel orange, one lime, and one red grapefruit were used to represent the Rutaceae family.

The luminol mixture used in this experiment was made up of luminol (The Best Chemicals, California, USA), washing soda (Church & Dwight Co., Inc., Ewing, New Jersey), hydrogen peroxide (Better Living Brands LLC, Pleasanton, California), and distilled water (Better Living Brands LLC, Pleasanton, California).

The controls used in this experiment were distilled water (Better Living Brands LLC, Pleasanton, California) (negative) and bleach (Industrias AlEn, Los Treviño Santa Catarina, Nuevo Neon, Mexico).

Experimental Design. Distilled water and bleach were used to act as the controls for this experiment. For the negative control, approximately 10 mL of distilled water was sprayed with the luminol mixture to test for luminescence. For the positive control, approximately 10 mL of bleach was sprayed with the luminol mixture to test for luminescence.

The luminol mixture was sprayed over the individual parts of each type of produce to test for luminescence. The skin was separated from the flesh for the beets, radishes, navel orange, lime, and red grapefruit. The leaf was separated from the midrib for the rainbow swiss chard, spinach, and kale. The flowery head was separated from the stalk on the broccoli. Approximately half of these were set aside to be juiced with a Juice Extractor (Kitchen Living, HL-2098A, 01/14, 90952). This was done on setting 2, the second-lowest setting. Each separate piece of produce was juiced and put in a separate cup. The juicer was cleaned in between each use. The luminol mixture was made by mixing 5 g luminol, 20 g washing soda, 360 mL distilled water, and 360 mL 3% hydrogen peroxide. The luminol and washing powder were added first, with care taken in mixing the powders well. The distilled water was added in small amounts, then the 3% hydrogen peroxide in small amounts. This mixture was then transferred into a spray bottle to mimic spraying luminol around a crime scene to check for blood spatter. The separated pieces of the produce were lined up on a table and sprayed individually with the luminol mixture. For Part A of the experiment, the skins of the navel orange, lime, red grapefruit, beet, and radish were used. For Part B, the flesh of the navel orange, lime, red grapefruit, beet, and radish were used. For Part C, the leaves of the kale, spinach, and rainbow swiss chard were used. For Part D, the flowery head and stalk of the broccoli were used. For Part E, the juice of all nine pieces of produce was tested. A timer was started as the first piece of produce was sprayed. Approximately 10 mL of each juice was used. The lights were then turned off to check for luminescence. The lights were turned back on once the timer ended for the last piece of produce still glowing. This procedure was then repeated for the cups of juice. The majority of the data collected for this experiment was qualitative because there was no access to resources to measure the intensity of the chemiluminescence reactions. The luminescence was analyzed visually along with a record of how long the reaction persisted.

Results.

While running the controls, it was noted that water displayed no luminescence when sprayed with the luminol mixture. Bleach displayed a bright blue light that lasted less than a second when mixed with the luminol mixture.

Out of the nine pieces of produce that were sprayed with the luminol mixture, only one of them showed any signs of luminescence. This was the radish from the Brassicaceae family. After running Part, A (**Table 1**) Trial #1, skins of the navel orange, beet, lime, red grapefruit, and radish with the luminol mixture, it was noted that there was no visible reaction. After running Part A, Trial #2, there was no noticeable reaction of any of the skins. For Part A Trail #3, there was no visible reaction between the skins of the navel orange, beet, lime, red grapefruit, and radish, and the luminol mixture.

Table 1. Reaction Observations for Part A

Produce Type	Reaction Present?	Observations
Navel Orange	Trial 1: No Trial 2: No Trial 3: No	N/A

Table 1. Reaction Observations for Part A

Lime	Trial 1: No Trial 2: No Trial 3: No	N/A
Red Grapefruit	Trial 1: No Trial 2: No Trial 3: No	N/A
Beet	Trial 1: No Trial 2: No Trial 3: No	N/A
Radish	Trial 1: No Trial 2: No Trial 3: No	N/A

For Part B (**Table 2**) Trial 1, after spraying the flesh of the navel orange, beet, lime, red grapefruit, and radish with the luminol mixture, only the radish displayed a chemiluminescence reaction. This was an initial medium intensity white/light blue glow that persisted for 1 hour 24 minutes and 13 seconds. This glow was not nearly as strong as the bright blue luminescence seen in the positive control. In Part B Trial 2, the flesh of the radish showed an initial medium intensity white/light blue glow similar in intensity to that of Part B Trial 1 that persisted for 1 hour 10 minutes and 54 seconds. In Part B Trial 3, the flesh of the radish showed an initial medium intensity white/light blue glow similar in intensity to that of Part B Trial 1 and Trial 2 that persisted for 1 hour 14 minutes and 20 seconds.

Table 2. Reaction Observations for Part B

Produce Type	**Reaction Present?**	**Observations**
Navel Orange	Trial 1: No Trial 2: No Trial 3: No	N/A

Table 2. Reaction Observations for Part B

Lime	Trial 1: No Trial 2: No Trial 3: No	N/A
Red Grapefruit	Trial 1: No Trial 2: No Trial 3: No	N/A
Beet	Trial 1: No Trial 2: No Trial 3: No	N/A
Radish	Trial 1: Yes Trial 2: Yes Trial 3: Yes	Trial 1: Medium intensity, white/blue glow, persisted for 1 hour 24 minutes 13 seconds Trial 2: Medium intensity, white/blue glow, persisted for 1 hour 10 minutes 54 seconds Trial 3: Medium intensity, white/blue glow, persisted for 1 hour 14 minutes 20 seconds Average Time: 1.274722222 hours

Part C (**Table 3**) Trial 1, when spraying the leaves of the spinach, kale, and rainbow swiss chard with the luminol mixture, there was no observable luminescence present. This lack of luminescence was also observed in Part C Trials 2 and 3.

Table 3. Reaction Observations for Part C

Produce Type	Reaction Present?	Observations
Kale	Trial 1: No Trial 2: No Trial 3: No	N/A
Spinach	Trial 1: No Trial 2: No Trial 3: No	N/A

Table 3. Reaction Observations for Part C

Rainbow Swiss Chard	Trial 1: No Trial 2: No Trial 3: No	N/A

For Part D (**Table 4**) Trial 1, when spraying the flowery head and the stalk of the broccoli with the luminol mixture, there was no observable luminescence present. This lack of luminescence was also observed in Part D Trials 2 and 3.

Table 4. Reaction Observations for Part D

Produce Type	Produce Part	Reaction Present?	Observations
Broccoli	Flowery Head	Trial 1: No Trial 2: No Trial 3: No	N/A
	Stalk	Trial 1: No Trial 2: No Trial 3: No	N/A

For Part E (**Table 5**), after spraying the juice of the navel orange, red grapefruit, lime, beet, radish, broccoli, kale, spinach, and rainbow swiss chard with the luminol mixture, only the juice of the radish displayed a chemiluminescence reaction. There was an initial medium intensity white/light blue glow similar in intensity to that of the trials run in Part B that persisted for 53 minutes and 13 seconds.

Table 5. Reaction Observations for Part E

Produce Type	Reaction Present?	Observations
Navel Orange	No	N/A
Lime	No	N/A
Red Grapefruit	No	N/A
Beet	No	N/A

Table 5. Reaction Observations for Part E

Radish	Yes	Medium intensity, white/blue glow, persisted for 53 minutes 13 seconds
Kale	No	N/A
Spinach	No	N/A
Rainbow Swiss Chard	No	N/A
Broccoli	No	N/A

Discussion.

The results of the reactions between the luminol mixture and the various produce, is of great importance for forensics. It is evident that only radishes reacted with the luminol mixture. This means that it is highly unlikely that any parts of the navel orange, lime, red grapefruit, beet, broccoli, kale, spinach, or rainbow swiss chard found on a crime scene will result in a false positive for blood when utilizing luminol. Even though radishes (flesh and juice) do react with luminol to produce a long-lasting glow, it is unlikely that radishes found at the scene of a crime will result in a false positive for blood when utilizing luminol. This is because the reaction between blood and luminol results in a high intensity white/blue glow that lasts for approximately one minute (Rogiski da Silva, Agustini, Lopes da Silva, Frigeri, 2012) while the reaction between radishes (flesh and juice) and luminol results in a medium intensity white/light blue glow that lasts, on average, 1.275 hours (**Table 2**). Even if Bluestar Forensic, known for increasing the duration of the luminescence (Dilbeck, 2006), is used instead of the luminol mixture, the difference in luminosity between the reaction with blood and the reaction with radishes is enough to eliminate the chance of a false positive identification.

In future experiments, it would be beneficial to test a wider array of the Brassicaceae family to check if more than just radishes react to luminol. It would also be advantageous to test different common food items such as meats and liquor for more false positives. If this protocol were to be run through again, it would be recommended to obtain multiple limes, grapefruits, and navel oranges, more than one bunch of radishes and beets, and multiple bushels of kale, spinach, and rainbow swiss chard since there was not enough to reasonable perform multiple trials of Part E. Using Bluestar alongside the luminol mixture would provide a comparison for the accuracy of each product and would supply useful information on how susceptible each product is to false positives. It would also prove favorable to use a camera to record the luminol reactions as having one person wait for over an hour to check for the luminescence of a piece of produce can result in a major risk of human error.

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