Luminescence 2001;16:295–298
DOI: 10.1002/bio.657

ORIGINAL RESEARCH

A study of common interferences with the forensic luminol test for blood

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Received 5 January 2001; accepted 14 March 2001

ABSTRACT: A wide range of domestic and industrial substances that might be mistaken for haemoglobin in the forensic luminol test for blood were examined. The substances studied were in the categories of vegetable or fruit pulps and juices; domestic and commercial oils; cleaning agents; an insecticide; and various glues, paints and varnishes. A significant number of substances in each category gave luminescence intensities that were comparable with the intensities of undiluted haemoglobin, when sprayed with the standard forensic solution containing aqueous alkaline luminol and sodium perborate. In these cases the substance could be easily mistaken for blood when the luminol test is used, but in the remaining cases the luminescence intensity was so weak that it is unlikely that a false-positive test would be obtained. In a few cases the brightly emitting substance could be distinguished from blood by a small but detectable shift of the peak emission wavelength. The results indicated that particular care should be taken to avoid interferences when a crime scene is contaminated with parsnip, turnip or horseradish, and when surfaces coated with enamel paint are involved. To a lesser extent, some care should be taken when surfaces covered with terracotta or ceramic tiles, polyurethane varnishes or jute and sisal matting are involved. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: forensic science; presumptive blood test; chemiluminescence; luminol; interfering catalysts

INTRODUCTION

The forensic luminol test for blood has been known for over 60 years, since its discovery by Walter Sprecht (1) in 1937. The test involves spraying suspected blood samples with a standard mixture of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) dissolved in alkaline, aqueous solution containing either dilute hydrogen peroxide or sodium perborate (2). Traces of human blood as small as 1 ng can be detected (3) by the pale blue chemiluminescence emitted when they come into contact with the forensic luminol mixture. Visual rather than instrumental detection of the luminescence is usually used and the test is regarded as presumptive rather than definitive, due to the range of interfering substances that can trigger the emission of the blue chemiluminescence.

Much has been written about the fact that the luminol test is not specific to blood. Sodium hypochlorite (bleach) (4, 5), plant peroxidases (4–6), and iron or copper compounds (3–6) have all been reported as producing visible chemiluminescence when exposed to the luminol solution. However, these publications provide only a qualitative study of these interferences.

A special feature of the luminol test that makes it somewhat more useful then the other three common presumptive tests for blood (the benzidine, phenolphthalein and leuco-malachite colour tests) (4) is the ability of the luminol test to highlight the presence of scattered, very small droplets of blood by the individual 'sparkles' of blue chemiluminescence each droplet produces. Interfering substances that are solids will generally produce a different spatial distribution of luminescence, as will surfaces that are coated homogeneously with interfering substances (e.g. some varnishes and paints). Interviews with forensic practitioners who use the luminol test sometimes point out that an experienced practitioner can sometimes distinguish interfering substances from blood in this manner.

Nevertheless, the question of interferences with the luminol test for blood are of increasing importance because of the proliferation of new floor and wall coatings and of many new domestic and industrial products (e.g. insecticides) that are routinely sprayed on surfaces in such environments. In view of such problems, it is surprising that there are few quantitative studies of interferences with the luminol test that may occur in industrial and domestic situations. A recent paper (2) from the present research group has shown that sodium hypochlorite (a common component of domestic and industrial bleaches) produces substantial interference

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Table 1. Spectral measurements showing vegetable and fruit catalytic interferences with the luminol test for blood detection[†]

Catalytic sample	Mean peak position/nm[‡]	Mean peak shift from Hb (150 g/L)/nm	Mean peak intensity/ arbitrary units	Visibility to the naked eye
150 g/L Hb Solution	438 ± 2	Not applicable	65 ± 14	Strong
Turnip (smear)	425 ± 6	13 ± 6	36 ± 11	Moderate
Turnip (pulp)	435 ± 3	3 ± 4	175 ± 74	Strong
Turnip (juice)	429 ± 3	9 ± 4	9.6 ± 6.8	Weak
Parsnip (smear)	433 ± 5	5 ± 5	4.4 ± 1.8	Barely visible
Parsnip (pulp)	430 ± 5	8 ± 5	118 ± 9	Strong
Horseradish (pulp)	435 ± 3	3 ± 4	49 ± 26	Strong
Carrot (pulp)	433 ± 4	5 ± 4	23 ± 4	Moderate
Carrot (juice)	430 ± 8	8 ± 8	3.8 ± 1.3	Barely visible
Onion (pulp)	439 ± 4	1 ± 4	16 ± 14	Moderate
Pumpkin (pulp)	434 ± 5	4 ± 5	8.9 ± 4.4	Weak
Pumpkin (juice)	436 ± 3	2 ± 4	4.0 ± 1.4	Barely visible
Potato (smear)	419 ± 10	19 ± 10	2.1 ± 0.3	Not visible
Potato (pulp)	430 ± 3	8 ± 4	7.6 ± 3.4	Weak
Potato (juice)	421 ± 6	17 ± 6	2.2 ± 0.7	Not visible
Tomato (pulp)	427 ± 5	11 ± 5	5.3 ± 3.1	Barely visible
Watermelon (pulp)	415 ± 10	23 ± 10	2.5 ± 0.1	Not visible
Banana (smear)	414 ± 9	24 ± 9	2.2 ± 1.0	Not visible

Hb, haemoglobin.
[†] The following substances produced no detectable chemiluminescence on the application of the luminol solution: horseradish (smear, juice), carrot (smear), onion (smear, juice), pumpkin (smear), tomato (smear, juice), watermelon (smear, juice), banana (pulp), apple, beetroot, canned beetroot, lemon, orange.
[‡] 95% confidence interval in the mean of four replicates.

with the luminol test, as it catalyses* the formation of chemiluminescence which is easily as bright as that caused by haemoglobin. This is a particularly serious issue, as attempts to clean up a crime scene and remove a trace of blood may well involve the use of commercial and household bleaches. Fortunately, it was shown in that work that there is a significant spectral shift of the luminol chemiluminescence catalysed by sodium hypochlorite compared with that catalysed by haemoglobin. Unfortunately, this spectral shift is not sufficient to produce significant colour changes during visual observations, but would require the use of special spectroscopic equipment to prove the spectral shift.

One earlier paper by Kraul and Meyer (7) does quantitatively address the issue of spectral shifts in the luminol emission when interfering substances are involved, but the study was very limited and only involved catalysis by rust (Fe_2O_3) and several types of human blood. There appear to be no other quantitative forensic measurements of interferences with the luminol test.

The aim of the present project was provide a comprehensive, quantitative study of the common interferences with the luminol test for blood, which may be found in domestic and industrial situations.

METHODS

The luminol test solution was prepared using the following chemicals; 0.1 g luminol (Aldrich), 5.0 g Na_2CO_3 (Ajax), 0.7 g $NaBO_3.4H_2O$ (Aldrich) and 100 mL distilled water.

The human haemoglobin (Sigma) samples were prepared as described by Quickenden and Cooper (2). The vegetable, household and industrial samples were smeared by rubbing a cut surface of the vegetable on a filter paper, or pulped or juiced using a Tiffany (Australia) two-speed blender. In each case, each sample was applied to a sheet of Whatman 541 hardened ashless filter paper, which was placed in a light-tight compartment and viewed via the photomultiplier tube.

The light detection equipment used was that described by Quickenden and Cooper (2), with two alterations. First, adjustments were made to the microprocessor, decreasing the scan period to ca. 2.5 s. This eliminated the need for any decay correction of the chemiluminescence scans. Second, adjustable gain settings ($\times 5$, $\times 20$, $\times 50$, $\times 100$) were placed on the operational

*Substances such as haemoglobin, potassium ferricyanide and cupric ions, which greatly enhance the intensity of the luminescence from an alkaline solution of luminol containing either hydrogen peroxide or sodium perborate, are often referred to as '*catalysts*' for the reaction. However, there is generally little evidence that they are true catalysts in the purist sense, i.e. that they enhance the reaction rate but remain unchanged at the end of the reaction. It is quite difficult to find studies of the luminol reaction that show the latter. Nevertheless, the forensic literature (and much of the other literature on the luminol reaction) commonly uses the term '*catalyst*' and this terminology will be retained in the present paper.

Table 2. Spectral measurements showing household and industrial catalytic interferences with the luminol test for blood detection*

Catalytic sample	Mean peak position/nm†	Mean peak shift from Hb (150 g/L)/nm	Mean peak intensity/ arbitrary units	Visibility to the naked eye
150 g/L Hb solution	438 ± 2	Not applicable	65 ± 14	Strong
Machine oil (Ampol®)	403 ± 10	35 ± 10	2.0 ± 0.2	Not visible
Enamel paint (Dulux®)	429 ± 3	9 ± 4	229 ± 42	Strong
Polyurethane paint (Berger®)	435 ± 3	3 ± 4	6.0 ± 3.0	Barely visible
Jute natural matting	428 ± 6	10 ± 6	7.3 ± 0.8	Weak
Sisal natural matting	434 ± 1	4 ± 2	7.7 ± 2.7	Weak
Terracotta tile	447 ± 1	9 ± 2	13.7 ± 3.0	Weak
Stone tile	418 ± 4	20 ± 4	3.0 ± 1.5	Not visible
Ceramic tile	424 ± 4	14 ± 4	4.0 ± 3.1	Barely visible
Hard vinyl flooring	416 ± 8	22 ± 8	1.8 ± 0.8	Not visible

Hb, haemoglobin.
* Each error is the 95% confidence interval in the mean of four replicates.
† The following substances produced no detectable chemiluminescence on application of the luminol solution: gloss acrylic paint (Taubman®), matte finish paint (Dulux®), flat oil-based paint (Top Dek®), vinyl floor polish (Ormonoid®), jarrah flooring, soft-backed vinyl, coir natural matting, industrial strength cleaner (CLR), lubricating spray (WD40®), bearing grease (Rocol®Sapphire), common insecticide (Confidor®), linseed oil, gear oil (Castrol®), weed killer (Zero®), motor oil (Castrol®), engine coolant (Valvoline®), mineral turpentine, silicone (Selleys®), adhesive (Araldite®).

amplifier, thus increasing the maximum sensitivity of the equipment 100-fold.

As described by Quickenden and Cooper (2), each sample was placed in the light-tight compartment and sprayed with the luminol test solution. The resultant chemiluminescence was passed through a calibrated circular graded interference filter, which was rotated by a microprocessor-driven motor, thus monochromating the emitted light before it was detected by a photomultiplier tube, operating in DC mode. The photocurrent from the tube was passed through a load resistor and the potential difference generated was applied to the inverting operational amplifier, an analogue-to-digital converter, and was finally recorded on a digital computer.

RESULTS AND DISCUSSION

Table 1 shows all of the spectroscopic data obtained for each vegetable sample during the course of the research. As can be seen from the results, many of the vegetables, in one form or another, did not produce a detectable amount of chemiluminescence when exposed to the luminol test solution. Of those that did, it was not possible to separate all of the vegetable samples from the undiluted (150 g/L) haemoglobin samples, which represent undiluted human blood, on the basis of mean peak shift.

The mean peak intensities of the majority of vegetable samples were very low compared with those of the haemoglobin-catalysed chemiluminescence. This meant that all but three of the samples tested could be distinguished from haemoglobin on the basis of their very low chemiluminescence intensities compared with those of a haemoglobin sample of similar proportions. The exceptions to this were the turnip, parsnip and horseradish pulps, which all had comparable intensities to the human haemoglobin.

A number of papers (8, 9) suggest that 'fresh potato juice' produced a strong chemiluminescence on application of luminol solution. However, the potato juice stains analysed produced only a barely detectable chemiluminescence that was not visible to the naked eye. It should be noted that the samples were tested some 18 h after preparation, making them less than fresh. Also, the papers cited talk of potato juice whereas the samples tested in this case were dried stains of potato juice.

It should be noted that while all the vegetable samples were tested at least 18 h after preparation, they still looked more like the vegetables they represented as opposed to blood (that is to say that a parsnip pulp sample looked like a sample of pulped parsnip). With the samples in this condition it was easy to distinguish between these samples and haemoglobin stains, purely via their physical appearances. The smear and juice stains were slightly more ambiguous, but they still did not resemble a bloodstain to any great extent. That coupled with the fact that the recorded intensities of all the juice and smear samples were visibly lower than those recorded for the human haemoglobin, meant that similar-sized stains could be easily distinguished by the naked eye.

Table 2 contains all the spectroscopic data we obtained for household and industrial substance-catalysed luminol chemiluminescences. As it shows, nine of the tested substances produced a level of chemiluminescence detectable with the present equipment. The machine oil (manufactured by Ampol®) produced a level of chemi-

luminescence, in the presence of the luminol solution, which was just detectable on the spectroscopic equipment-but was not visible to the naked eye. It was noted that the machine oil could be distinguished from the human haemoglobin due to a considerable difference in mean peak positions.

The concerning result from the household and industrial interferences tested was the production of visible chemiluminescence when the luminol test solution was applied to the samples of polyurethane varnish, enamel gloss paint, jute and sisal matting and terracotta or ceramic tile (while the stone tile and the hard-backed vinyl flooring did produce detectable chemiluminescence, this luminescence was not visible to the naked eye).

The mean chemiluminescence intensity produced by enamel paint exceeds that of the haemoglobin and it is identical to the naked eye. While the other chemiluminescence intensities produced were far less visible to the naked eye, they were still visible and are consequently still problematic. This has serious implications, as these substances are commonly used to coat floors, walls and other surfaces, and these surfaces are often found at crime scenes. In terms of mean peak positions, only the polyurethane paint did not have a noticeable shift from the mean peak position of the haemoglobin.

These results further show that the testing of blanks at a crime scene is an important part of blood detection. There is obviously a need for future work in looking at the possible interfering effects of surfaces and their coatings on the luminol chemiluminescence blood detection test.

CONCLUSIONS

It can be seen that the majority of interfering substances tested during the course of this research can be distinguished from human haemoglobin because they produce considerably lower chemiluminescence intensities on application of the luminol solution. The exceptions to this are turnip, parsnip and horseradish pulps, and care should be taken when applying the luminol test in the possible presence of these substances.

Also, forensic investigators should note that chemiluminescence was produced when the luminol solution was sprayed on a surface of jute matting, sisal matting, terracotta tile, ceramic tile, or a surface coated with polyurethane paint or enamel gloss paint. Further research into the possible interference to the luminol test by various domestic surfaces and surface coatings is necessary, and this result indicates the need for forensic investigators to test blanks at a crime scene.

Acknowledgements

The authors would like to thank the Forensic Division of the Western Australia Police Service, in particular Superintendent Dick Milner, Senior Sergeant Ken Sanderson and Sergeant Vic Webb, for their helpful discussions and for providing information on their approach to forensic blood detection.

REFERENCES

1. Sprecht W. Die Chemiluminesenz des Hämins, ein Hilfsmittel zur Auffindung und Erkennung forensisch wichturger Blutspuren. *Angew. Chem.* 1937; **50**: 155–157.
2. Quickenden TI, Cooper PD. Increasing the specificity of the forensic luminol test for blood. *Luminescence* 2001; **16**: 251–253.
3. Grodsky M, Wright K, Kirk PL. Simplified preliminary blood testing: an improved technique and a comparative study of methods. *J. Crimin. Law Criminol. Police Sci.* 1951; **42**: 95–104.
4. Yeshion TE. The forensic application of luminol as a presumptive blood test. In *Proceedings of the 6th International Symposium on Bioluminescence and Chemiluminescence 1990*, Stanley PE, Kricka LJ (eds). Wiley: Chichester, 1991; 379–384.
5. Shiro G. Collection and preservation of blood evidence from crime scenes. In http//police2.ucr.edu/blood1.html 1998.
6. Lytle LT, Hedgecock DG. Chemiluminescence in the visualisation of forensic bloodstains. *J. Forens. Sci.* 1978; **23**: 550–562.
7. Kraul R, Meyer HH. Ist der Nachweis von Blutspuren durch 3-Amino-phthalsäurehydrazid ein kennzeichnendes Verfahren? *Angew. Chem.* 1941; **54**: 213–215.
8. Albrecht HO. Uber die Chemiluminescenz des Aminophthalsaurehydrazids. *Z. Phys. Chem. (Leipzig)* 1928; **135**: 321–330.
9. James SH, Eckert WG. *Interpretation of Bloodstain Evidence at Crime Scenes* CRC Press: Boca Raton, FL; 1999.

