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ORIGINAL RESEARCH

Attempted cleaning of bloodstains and its effect on the forensic luminol test

Jonathan I. Creamer,[1,2] Terence I. Quickenden,[1]* Leah B. Crichton,[1] Patrick Robertson[1] and Rasha A. Ruhayel[1]

[1]Chemistry, M313, School of Biomedical and Chemical Sciences, Centre for Forensic Science, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[2]Centre for Forensic Science, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

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ABSTRACT: The forensic luminol test has long been valued for its ability to detect trace amounts of blood that are invisible to the naked eye. This is the first quantitative study to determine the effect on the luminol test when an attempt is made to clean bloodstained tiles with a known interfering catalyst (bleach). Tiles covered with either wet or dry blood were tested, and either water or sodium hypochlorite solution (bleach) was used to clean the tiles. As expected, the chemiluminescence intensity produced when luminol was applied generally decreased with the number of times that a tile was cleaned with water, until the chemiluminescence was neither visible nor detectable. However, when the tiles were cleaned with bleach there was an initial drop in chemiluminescence intensity, followed by a rise to a consistently high value, visibly indistinguishable from that of blood. Examination of bleach drying time suggested that any interfering effect becomes negligible after 8 h. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: forensic science; presumptive blood testing; chemiluminescence; luminol; bloodstains

INTRODUCTION

The forensic luminol test involves the emission of luminol chemiluminescence (CL) in the presence of haemoglobin. It is used at crime scenes as a presumptive test for blood. The major advantage of the luminol test is its high sensitivity—it can detect nanogram traces of blood (1) that are invisible to the naked eye—which makes it up to 20 times more sensitive than any other blood detection test (2).

The limitation with luminol and other blood detection tests is their lack of selectivity. Substances other than blood (2–6) can catalyse the CL reaction that is the central component of the luminol test. One commonly reported interfering catalyst (2–6) is sodium hypochlorite solution (bleach), a common ingredient in the majority of cleaning agents around most homes and industries. Curiously, there is a lack of quantitative papers on the effect of bleach-based cleaners on bloodstains tested with luminol.

Criminals often attempt to clean up blood spills after committing a violent crime, so it is important to know what effect attempted cleaning has on luminol test results. In the present study we apply haemoglobin stains to ceramic tiles and *quantitatively* observe the effects of repeated washing with water or bleach, on the CL intensity when luminol is applied to the tile. The effect of bleach drying time on luminol CL is also observed.

*Correspondence to: T. I. Quickenden, Chemistry, M313, School of Biomedical and Chemical Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.
Email: tiq@chem.uwa.edu.au

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METHODS

Standard luminol solution was prepared as described by Creamer *et al.* (5). The dilute (15 g/L) haemoglobin solution consisted of 0.15 g haemoglobin (Sigma) dissolved in 10 mL 0.2 mol/L NaOH. The bleach solution used for the tiles was diluted to a concentration of ~ 60 g/L NaClO, while the drying experiments were performed with 125 g/L and 10 g/L NaClO (all dilutions in water).

Glazed terracotta tile was used as the test surface. Each tile was sprayed with haemoglobin solution using a hand-held sprayer, and either tested immediately, in the case of the 'wet' stains, or left to dry for 1 h. A single wipe across the tile surface with a paper towel soaked in either water or bleach constituted one 'clean'. The direction of each subsequent clean was parallel to the first and this pattern continued for all cleaning steps.

The bleach samples for the drying experiments were prepared as described by Creamer *et al.* (5), and left to dry for 0, 2, 8 and 16 h.

The procedure for measuring CL intensity was as described by Quickenden and Creamer (4). CL for each tile was re-measured after each stage of cleaning. One

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researcher tested all the tiles and then two different researchers repeated the same experiments independently.

RESULTS AND DISCUSSION

Before staining with haemoglobin, blank experiments were carried out by spraying each tile with luminol after it had been wiped with water. The same experiment was repeated after the tiles were wiped with bleach. The tiles wiped with water exhibited negligible background CL (2.0 ± 0.6 arbitrary units, a.u.), not visible to the naked eye. The tiles wiped with bleach, showed a considerable and highly visible level of CL (192 ± 25 a.u.). This was not a surprise, as emission of CL has been reported previously when solutions of bleach have been added to luminol solution (2–6).

Figs 1 and 2 depict the changes in CL intensity when haemoglobin-stained tiles were cleaned with either water or bleach-soaked cloths, respectively. Each point is the mean of 12 replicates.

The two replicate plots of the CL from haemoglobin-stained tiles cleaned with water were very similar. The CL intensities decreased with the number of cleaning steps. After 14 steps, the CL produced was less than could be observed with the naked eye. The haemoglobin stains that were left to dry for 1 h were slightly harder to remove, which presumably caused the occasionally higher intensity readings, although the effect was minor.

The haemoglobin-stained tiles cleaned with bleach-soaked cloths presented very different results. The initial clean obviously removed more haemoglobin than the water, as can be seen by the larger intensity drop. Thereafter, the build-up of bleach caused an increase in CL, until the intensities levelled off at very similar values to those originally observed for haemoglobin. The dried haemoglobin stain showed a smaller initial intensity drop, presumably because it was more difficult to remove.

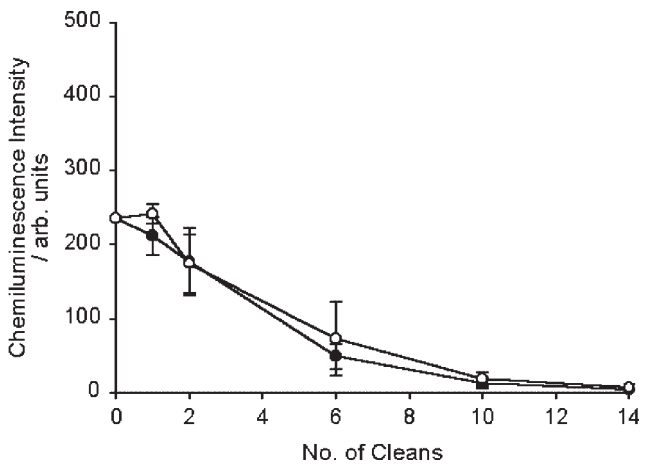

Figure 1. The effect of cleaning bloodstains on the resultant chemiluminescence from the luminol test. ●, wet blood and water; ○, dry blood and water.

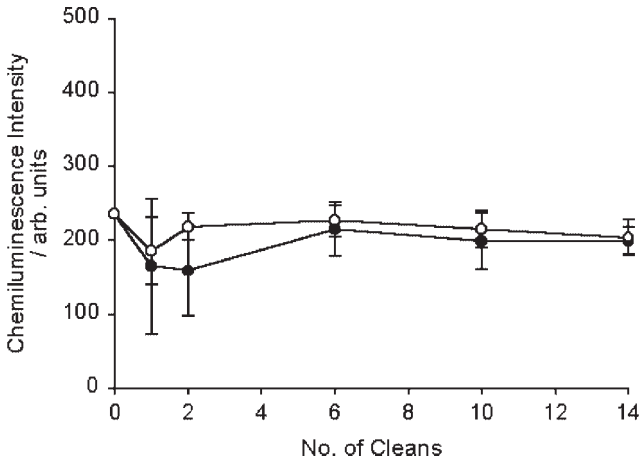

Figure 2. The effect of cleaning bloodstains on the resultant chemiluminescence from the luminol test. ●, wet blood and commercial bleach; ○, dry blood and commercial bleach.

Table 1. Effect of drying time on luminol chemiluminescence from bleach stains*

Drying time (h)	125 g/L NaClO (a.u.)	10 g/L NaClO (a.u.)
<1	80 ± 21	135 ± 38
2	184 ± 23	126 ± 32
8	–	11.7 ± 2.3

* No chemiluminescence was observed after 16 h for either bleach concentration.

Table 1 shows the effect on CL intensity when luminol is applied to a bleach-stained surface after various drying times. While bleach solutions contain stabilizers, they are volatile, decomposing and evaporating reasonably quickly, as observed. While the bleach stains initially catalysed considerable CL, any interference became negligible after 8 h.

This is the first quantitative study to determine the effect on the luminol test when bloodstained tiles are cleaned with a known interfering catalyst (bleach). It is shown that cleaning a glazed tile surface with bleach-based cleaners produced levels of luminol CL indistinguishable from that of haemoglobin, thus compromising the evidentiary value of the bloodstain. It is noted, however, that bleach interference dissipates after ~ 8 h.

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