Forensic Science International 278 (2017) 396–403

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

A quantitative method for determining a representative detection limit of the forensic luminol test for latent bloodstains☆

Brianna M. Cassidy, Zhenyu Lu, Jennifer P. Martin, Shawna K. Tazik, Katie W. Kellogg, Stephanie A. DeJong, Elle O. Belliveau, Katherine E. Kilgore, Samantha M. Ervin, Mackenzie Meece-Rayle, Alyssa M. Abraham, Michael L. Myrick*, Stephen L. Morgan*

Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 20 June 2017
Accepted 27 June 2017
Available online 5 July 2017

Keywords:
Luminol
Blood
Bloodstain
Limit of detection
Chemiluminescence
Experimental control

ABSTRACT

The luminol test has been used for over 60 years by forensic investigators for presumptive identification of blood and visualization of blood splatter patterns. Multiple studies have estimated the limit of detection (LD) for bloodstains when luminol is employed, with results ranging from 100× to 5,000,000× dilute. However, these studies typically have not identified and controlled important experimental variables which may affect the luminol LD for bloodstains. Without control of experimental parameters in the laboratory, variables which affect the potential of presumptive bloodstain test methods remain largely unknown, and comparisons required to establish new, more powerful detection methods are simply impossible. We have developed a quantitative method to determine the relationship between the amount of blood present and its reaction with luminol by measuring, under controlled conditions, the resulting chemiluminescent intensity with a video camera, combined with processing of the digital intensity data. The method resulted in an estimated LD for bloodstains on cotton fabric at ~200,000× diluted blood with a specific luminol formulation. Although luminol is the focus of this study, the experimental protocol used could be modified to study effects of variables using other blood detection reagents.

© 2017 Elsevier B.V. All rights reserved.

The initial task of a forensic crime scene investigator is to recognize items that might have evidentiary value. Blood is among the most commonly encountered bodily fluids encountered by forensic investigators. The advent of trace DNA amplification has increased the importance of blood detection. However, if bloodstains have been diluted by deliberate washing of the substrate or by environmental exposure to rain or submersion in water, detection can be compromised [1]. Latent stains, those invisible to the naked eye, may result if only trace amounts of blood remain. If bloodstains are present but not identified, crucial evidence might be overlooked as illustrated by the Damilola Taylor case [1]. Therefore, if there is reason to believe blood might be present, a presumptive test is often performed, with positive results followed by confirmatory tests [2–4].

Luminol (3-aminophthalhydrazide) is among the most sensitive blood detection reagents available for forensic investigation and has been employed for decades [2,3,5]. Luminol solutions for bloodstain detection are typically alkaline and contain hydrogen peroxide as an oxidizing reagent. Ferric heme groups in blood catalyze oxidation of luminol by decomposition of hydrogen peroxide present in the luminol solution [5]. The chemiluminescent reaction path of luminol has been studied for over 50 years. Studies have described the general reaction mechanism to involve the oxidation and excitation of luminol resulting in the excited state dianion intermediate, 3-aminophthalate, that upon return to ground state emits a broad spectrum of light centered around 425 nm (Fig. 1) [5,6–8]. White et al. identified 3-aminophthalate as the light emitting species in the luminol reaction by matching its

☆ The authors declare no competing financial interest.

* Corresponding author.
E-mail addresses: cassidbm@email.sc.edu (B.M. Cassidy), lu29@email.sc.edu (Z. Lu), marti567@email.sc.edu (J.P. Martin), tazik@email.sc.edu (S.K. Tazik), witherka@gmail.com (K.W. Kellogg), dejongs@email.sc.edu (S.A. DeJong), bellivea@email.sc.edu (E.O. Belliveau), kek.kilgore@yahoo.com (K.E. Kilgore), ervinsm@email.sc.edu (S.M. Ervin), meeceray@texas.edu (M. Meece-Rayle), alyssaa@email.sc.edu (A.M. Abraham), myrick@mailbox.sc.edu (M.L. Myrick), morgansl@mailbox.sc.edu (S.L. Morgan).

http://dx.doi.org/10.1016/j.forsciint.2017.06.031
0379-0738/© 2017 Elsevier B.V. All rights reserved.

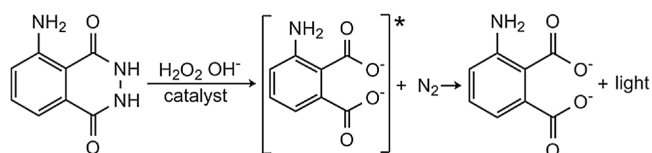

Fig. 1. The luminol light-emitting reaction involves oxidation of luminol and excitation of an intermediate species which emits light upon returning to ground state.

fluorescence spectrum to the chemiluminescent spectrum of luminol [7]. However, further details involving the light-emitting pathway of luminol remain speculative and reaction intermediates have not been completely characterized [5,9–11].

Luminol applied to bloodstains has been shown not to negatively affect subsequent DNA analysis of bloodstains [6,12–15]. However, luminol has been reported to denature blood enzymes, with consequent effects on the biochemical profile [11]. Grispino and Laux emphasize that patent bloodstains should never be contaminated with presumptive blood detection reagents [11,15].

Absolute and relative sensitivities of presumptive tests for blood have been studied for over 60 years [16]. Some studies compare non-luminol based techniques to luminol-based techniques [2,13,14,16,17], while other studies compare the sensitivity of different luminol formulations [9,18]. However, results are inconsistent. For example, Bluestar® (a commercialized luminol formula) was reported to outperform a luminol solution prepared according to a police department's crime lab protocol [18]. Patel and Hopwood tested five luminol formulations and found Bluestar® Magnum to have greater sensitivity than other formulations on both porous and non-porous surfaces [9]. Seashols et al. modified a luminol solution previously suggested by Grodsky [16] and found it to perform similarly to Bluestar® for all tested cases, except when tested on linoleum [17]. The range of published luminol LDs for bloodstains spans nearly five orders of magnitude from 100× to more than 5,000,000× dilute bloodstains.

Lack of agreement among estimated LDs of presumptive tests for bloodstains is of concern to the forensic community [9,13,19,20]. Cox attributes the large range of reported LDs to variations in substrates, sample preparation methods, reagent concentrations, and result interpretations [20]. Recently, DeJong et al. suggested inconsistencies of reported LDs are due to the absence of blank measurements, lack of quantitative detection methods, and lack of data validation, among other reasons [19]. In this manuscript, we identify several factors that could affect the response of luminol to dried bloodstains and control them—the methods by which blood is measured, blood dilutions are made, replicate bloodstain samples are made, luminol is prepared, luminol is stored, luminol is applied to bloodstains, and the method by which chemiluminescent response is detected. We estimate a best case LD of ~200,000× diluted blood on cotton. The outcome of this work is a method for measuring the reaction of bloodstains with luminol to enable accurate and reproducible determination of LDs. The techniques provided here can also be used in further experiments to show how variations in the factors we have controlled degrade the luminol LD.

1. Experimental section

1.1. Substrate preparation

The substrate used in this study, 8 oz. 100% Cotton Twill Wingfoot (Milliken, Oakbrook, IL), was cut into 5″ × 5″ swatches. Each swatch was sonicated in 100 mL of methanol (ACS grade, Sigma Aldrich, CAS: 67-56-1, St. Louis, MO) for one hour and hung to dry overnight in a fume hood. These samples are simply referred to as "cotton substrate" in the following text.

1.2. Stain barrier application

Determination of a LD depends heavily on the ability to create reproducible samples. Previous luminol LD studies report depositing bloodstain solutions in measured aliquots. However, the more dilute a blood solution, the further the solution spreads when applied to a substrate (Fig. 2, top). Van Dalan deposited 300 μL of varying dilutions of porcine blood solutions on cotton and reported substantial increases in spot size with dilution increase [21]. This phenomenon, which complicates calculation and relationship of the mass of blood solids per amount of substrate and introduces an element of randomness from sample to sample, has been previously confirmed using mouse blood dilutions on the same cotton substrate used in this study [19].

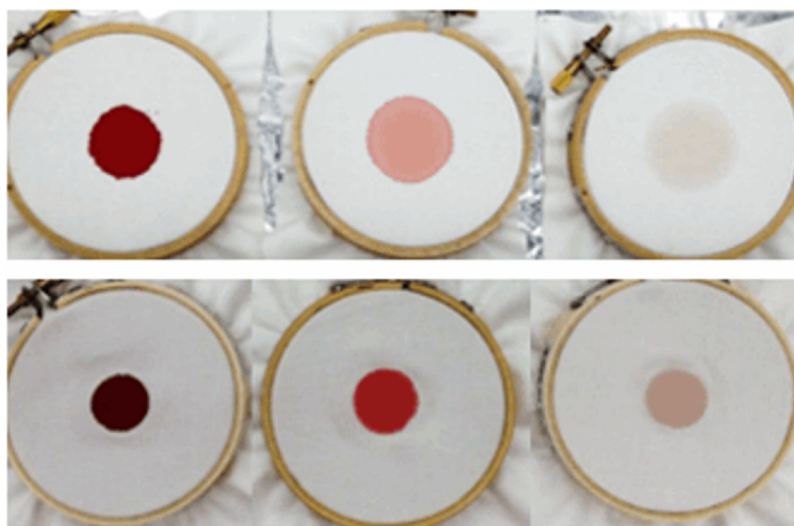

Fig. 2. Blood solutions (left to right: whole blood, 10× diluted blood and 100× diluted blood) deposited as separate 100 μL aliquots on cotton substrate suspended in 3″ embroidery hoops. Top: The diameter of the resulting stain is dependent on the dilution applied, which ultimately compromises the ability to compare quantitatively one stain dilution to another. Bottom: Blood solutions are restricted by PVC stain barriers to occupy the same, reproducible area of fabric.

Download English Version:

https://daneshyari.com/en/article/6462263

Download Persian Version:

https://daneshyari.com/article/6462263

Daneshyari.com