



Detecting The Unseen: The Review Of Alternative Light Sources (ALS), Luminol, and Bluestar in forensic Bloodstain Analysis

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Abstract: Hidden bloodstains, invisible without special tools, are crucial evidence in solving crimes like murders. This review examines how well Alternative Light Sources (ALS), luminol, Bluestar, and other methods such as fluorescein, tetramethylbenzidine (TMB), and near-Infrared (NIR) dyes detect these bloodstains. ALS uses light at wavelengths like violet (410nm) or NIR (700-1000nm) to show blood on dark surfaces, such as black clothes or painted walls, without damaging the evidence. This makes it perfect for initial checks, as it keeps DNA safe for later tests. Luminol, a chemical that glows blue when it touches blood, can find tiny amounts diluted up to 1:5,000,000, but it needs a dark room and fresh preparation. Bluestar similar to luminol is brighter, lasts longer, and works in dim light, detecting blood up to 1:10,000,000. It's also more stable, lasting up to week after mixing. Fluorescein and TMB are less sensitive, spotting blood at about 1:10,000, but are good for confirming blood presence. New NIR dyes are highly accurate, targeting blood proteins, but aren't widely available yet. The review compares these methods based on how well they detect blood, avoid false results are easy to use and protect DNA evidence. Bluestar is the most sensitive and practical for crime scenes, while ALS is best for not harming evidence. Challenges include false positives from chemicals like bleach for luminol and Bluestar, and the high cost of ALS equipment. Compared to older studies, this review includes recent research (2013-2024) and focuses on DNA safety and tough surfaces like burned wood or washed fabrics. A new insight is that combining ALS for first checks with Bluestar for tiny traces gives the best results. Future tools like NIR dyes could make detection even better. This review suggests using ALS and Bluestar together for strong forensic investigations.

KEYWORDS:

Forensic science; Latent bloodstain detection; Alternative Light Source (ALS); Luminol; Bluestar; Fluorescein; Tetramethylbenzidine (TMB); Phenolphthalein; Near-infrared (NIR) dyes; Chemiluminescence; DNA preservation; False positives; Crime scene investigation.

INTRODUCTION

The ability to uncover hidden bloodstains at crime scenes is crucial for solving violent crimes, such as homicides, assaults, or sexual violence. These latent bloodstains, invisible to the naked eye, often hold critical evidence that can link suspects to a crime, reconstruct events, or provide DNA for identification. Unlike visible bloodstains, latent stains may be concealed by cleaning efforts, blended into dark or patterned surfaces, or altered by environmental factors like heat or moisture. Detecting these stains requires tools that are sensitive enough to find trace amounts of blood, accurate enough to avoid misleading results, and gentle enough to preserve DNA evidence for further analysis. The challenge is compounded by the variety of surfaces at crime scenes-fabrics, wood, metal, painted walls, or even burned materials- each demanding specific detection approaches. Over the years, forensic scientists have developed various methods to tackle these challenges, from light- based techniques to chemical reagents, each with its own strengths and limitations. This review explores a range of tools, including Alternative Light Sources(ALS), luminol, Bluestar, fluorescein, tetramethylbenzidine(TMB), phenolphthalein (Kastle-Meyer), and emerging near-infrared(NIR) dyes, to understand their roles in modern forensic investigations. Latent bloodstain detection has evolved significantly since the early days of forensic science. In the past, investigations relied on visual inspection or basic chemical tests, which often missed hidden stains or damaged evidence. The introduction of luminol in the mid-20th century marked a breakthrough, allowing detection of blood traces too faint to see. Luminol's glowing reaction with blood revealed stains on cleaned surfaces, but its need for darkness and potential to affect DNA sparked the development of improved reagents like Bluestar, which is easier to use in varied conditions. Meanwhile, ALS emerged as a non-destructive alternative, using specific light wavelengths to highlight bloodstains without altering them. These advancements reflect the growing need for methods that balance sensitivity with evidence preservation, especially as DNA analysis has become a cornerstone of forensic work. Today, tools like fluorescein and TMB serve as confirmatory tests, while NIR dyes represent cutting-edge options that target blood components with high precision. The significance of latent bloodstain detection extends beyond finding evidence; it directly impacts the success of criminal investigations. A single bloodstain can provide a DNA profile to identify a suspect or victim, corroborate witness statements, or reveal the sequence of events at a crime scene. However, detection methods must navigate practical challenges. For example, blood on dark fabrics or painted surfaces can be hard to spot, and cleaning attempts, such as washing or bleaching, reduce the amount of detectable blood. Environmental factors like fire or humidity can further degrade stains, making reliable detection critical. False positives-when a method reacts to substances like

bleach or plant material can mislead investigators, while methods that damage DNA can limit further testing. Forensic teams also face logistical issues, such as the need for portable, easy-to-use tools in field settings versus more complex equipment in labs. This review aims to provide a comprehensive guide for forensic practitioners by examining the most widely used and emerging tools for latent bloodstain detection. ALS, which shines light at wavelengths like UV, violet, or NIR, reveals blood without touching it, making it ideal for preserving evidence. Luminol and Bluestar, which glow when they contact blood are known for finding tiny traces,

RESEARCH METHODOLOGY

This review evaluates methods for detecting latent bloodstains, focusing on their sensitivity, specificity, ease of use, and impact on DNA preservation across various substrates. The methods include: Alternative Light Sources (ALS): ALS uses specific light wavelengths (300–1000 nm) to visualize bloodstains based on their absorption and low fluorescence. Key wavelengths include ultraviolet (UV, 300–400 nm), violet (410 nm), blue (450–480 nm), and near-infrared (NIR, 700–1000 nm). Devices like Polilight and CrimeScope with barrier filters (e.g., orange goggles) enhance contrast on dark fabrics, painted surfaces, or burned materials. Studies test ALS on cotton, wood, metal, and compromised substrates (e.g., washed or painted). Luminol: Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) reacts with hemoglobin's heme in the presence of hydrogen peroxide, emitting blue chemiluminescence (~454 nm). Applied as a spray (e.g., Grodsky formulation), it requires total darkness for visualization. Sensitivity is tested at dilutions up to 1:5,000,000 on substrates like cotton, glass, and painted drywall. Bluestar: A luminol-based reagent, Bluestar produces brighter, longer-lasting chemiluminescence (420–440 nm) and is stable for up to 7 days. It is tested on porous (e.g., filter paper, fabrics) and non-porous (e.g., glass, metal) surfaces, with sensitivity up to 1:10,000,000. Other Reagents: Fluorescein: Reduced to fluorescein, it fluoresces under blue ALS light (450–480 nm) with blood and hydrogen peroxide, with sensitivity ~1:10,000. TMB and Phenolphthalein: Colorimetric tests producing green (TMB) or pink (phenolphthalein) color changes, with sensitivity ~1:10,000, used for confirmation. NIR Dyes (e.g., SO3SQ): Bind to blood's serum albumin, fluorescing in NIR under ALS, offering high specificity but limited availability. Comparative studies (2013–2024, sourced from PubMed, Wiley, and forensic journals) assess these methods on sensitivity (minimum detectable dilution), specificity (false positives with non-blood substances), ease of use (field vs. lab applicability), and DNA preservation. Substrates include dark fabrics, burned wood, washed materials, and painted surfaces. Performance on compromised surfaces and DNA impact are key metrics. Results and Discussion

Studies reveal distinct strengths and trade-offs among detection methods. Luminol detects blood at dilutions of 1:200,000 to 1:5,000,000, performing well on porous surfaces like cotton but less effectively on non-porous glass due to runoff (Seashols et al., 2013). Bluestar achieves higher sensitivity (1:10,000,000 on glass) and outperforms luminol on porous substrates like filter paper and two-ply fabrics, with brighter, longer-lasting glow (up to 1 minute) (Łuczak et al., 2006; Yimnoon et al., 2024). ALS, using 410 nm or NIR light, detects blood up to ~1:1,000, excelling on dark fabrics (e.g., black clothing) and painted surfaces due to blood's low fluorescence, but struggles with highly diluted stains (Patel et al., 2021). Fluorescein and TMB, with sensitivities ~1:10,000, are less effective for trace detection but serve as confirmatory tests. NIR dyes show comparable sensitivity to luminol on specific substrates and high specificity by targeting serum albumin (Lee et al., 2023). Specificity varies significantly. Luminol and Bluestar risk false positives with bleach, copper, iron, or organic materials (e.g., horseradish), though Bluestar's formulation reduces some interferences (Barni et al., 2007). ALS, especially in NIR, is highly specific, minimizing substrate interference (Farrar et al., 2012). TMB and phenolphthalein are specific but require visible stains, limiting their use for latent detection. NIR dyes avoid common interferences, offering a promising alternative (Lee et al., 2023). Ease of use is a critical factor. Luminol's need for darkness and fresh preparation complicates field application and photography (Barni et al., 2007). Bluestar's stability and semidarkness compatibility make it more practical for crime scenes (Yimnoon et al., 2024). ALS requires costly equipment and training to select wavelengths, but its non-destructive nature streamlines workflows (Patel et al., 2021). Fluorescein needs ALS, adding complexity, while TMB and phenolphthalein are simpler but less versatile. NIR dyes require advanced ALS setups, limiting current use. DNA preservation is paramount. ALS is non-destructive, ensuring full DNA recovery for STR analysis (Patel et al., 2021). Luminol and Bluestar have minimal impact on DNA when applied correctly, with Bluestar causing less degradation (Jakovich, 2015). Prolonged exposure or high concentrations can damage DNA, particularly in aged samples. Fluorescein and NIR dyes preserve DNA, while TMB may interfere if over-applied. On compromised surfaces, Bluestar outperforms luminol on burned wood (5.2% vs. 13% positivity) but struggles with direct fire exposure (Connon et al., 2018). Luminol detects blood beneath up to three paint layers, with porous surfaces like drywall retaining blood longer (Castelló et al., 2009). ALS, using NIR, excels on painted surfaces and dark fabrics, preserving stain patterns (Farrar et al., 2012). On washed fabrics, Bluestar is more effective, particularly on two-ply materials (Yimnoon et al., 2024). Practical applications favor ALS for initial non-destructive screening, especially on dark or complex surfaces, followed by Bluestar for trace detection in field settings. Luminol is cost-effective but less practical due to darkness requirements. TMB and phenolphthalein are best for confirmation, while NIR dyes await broader adoption. Integrated protocols combining ALS and Bluestar optimize sensitivity and evidence preservation, a novel insight from this review (Morrison et al., 2018).

RESULTS AND DISCUSSION

DISCUSSION:

The findings highlight varied performance among techniques for locating concealed blood traces, with implications for forensic practice. Light-based detection excels on dark textiles and painted surfaces, leveraging blood's optical properties to reveal traces without alteration (Patel et al., 2021; Farrar et al., 2012). Its lower detection limit (~1:1,000) makes it less suitable for minute residues, but its non-invasive nature ensures genetic evidence remains intact, a key advantage for investigations requiring DNA analysis. Chemical agents, particularly the enhanced version, offer superior detection capabilities, identifying blood at extreme dilutions (1:10,000,000) and performing well on porous or fire-damaged materials (Yimnoon et al., 2024; Connon et al., 2018). However, their reactivity with non-blood substances risks errors, necessitating confirmatory tests (Barni et al., 2007). Practical use varies by context. The original chemical's need for complete darkness limits its field applicability, while the enhanced version's

dim-light compatibility enhances versatility (Łuczak et al., 2006). Light-based systems require costly equipment and expertise, restricting access in resource-limited settings, but their efficiency in evidence collection is unmatched (Patel et al., 2021). Confirmatory tests are simple but limited to visible traces, and emerging compounds, despite high reliability, need advanced setups, hindering current use (Lee et al., 2023). A novel approach from this review is combining light-based screening with the enhanced chemical. Initial non-invasive screening identifies visible traces, followed by chemical application for minute residues, optimizing detection and evidence protection (Morrison et al., 2018). This strategy suits diverse crime scene conditions, enhancing efficiency. Forensic teams should prioritize light-based methods for initial surveys on dark or painted materials and the enhanced chemical for trace detection on porous or cleaned surfaces. Emerging compounds show promise for future reliability but require further development. These insights guide method selection, ensuring effective investigations while safeguarding genetic evidence.

RESULT:

Results Studies from 2013 to 2024 provide data on techniques for identifying concealed blood traces, including light-based systems, chemical agents emitting light, and confirmatory or emerging compounds, tested on materials like textiles, timber, metal, and painted surfaces. Light-based detection, using devices like Polilight® or Crime Scope®, detects blood diluted to ~1:1,000 on dark textiles, such as black cotton, due to blood's distinct optical properties (Patel et al., 2021). It identifies blood beneath one or two paint layers on drywall using infrared light (700–1000 nm), preserving evidence patterns (Farrar et al., 2012). Its detection limit is lower than chemical methods for trace residues.

The primary chemical agent, applied as a spray, reveals blood diluted up to 1:5,000,000 on porous surfaces like cotton or drywall but shows reduced effectiveness on non-porous metal or glass due to liquid runoff (Seashols et al., 2013). An enhanced version of this chemical detects blood at 1:10,000,000, performing strongly on porous materials like filter paper and two-ply fabrics, with a brighter glow lasting up to 1 minute (Yimnoon et al., 2024; Łuczak et al., 2006). On fire-damaged timber, it achieves a 5.2% positive detection rate compared to 13% for the original chemical (Connon et al., 2018).

Confirmatory tests include a compound glowing under blue light (450–480 nm), detecting blood at ~1:10,000 on wood or fabric (Seashols et al., 2013). Color-changing tests, producing green or pink hues, detect blood at similar dilutions but are less effective for concealed traces (Jakovich, 2015). Emerging compounds binding to blood's protein component under infrared light detect blood at ~1:1,000, showing high reliability against contaminants (Lee et al., 2023).

Reliability tests show chemical agents reacting with non-blood substances like cleaning agents or plant materials, causing errors (Barni et al., 2007). The enhanced chemical reduces some interferences, while light-based detection, especially infrared, minimizes substrate issues (Farrar et al., 2012). Color tests are reliable for confirmation. Light-based methods cause no genetic evidence degradation, while chemical agents have minimal impact if applied briefly (Jakovich, 2015). On cleaned textiles, the enhanced chemical outperforms others, and the original chemical detects blood beneath three paint layers on porous walls (Castelló et al., 2009).

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