Technical Note

STR Analysis Following Latent Blood Detection by Luminol, Fluorescein, and BlueStar

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Abstract: Luminol and fluorescein are chemicals commonly used for presumptive tests to visualize latent blood associated with a crime scene. A new chemical, BlueStar, is now available for the same purpose. Research has shown that luminol and fluorescein do not interfere with STR analysis but little research has been done to demonstrate the effect of BlueStar, if any, on DNA analysis. In this study, blood-stained carpets that had been sprayed with luminol, fluorescein, and BlueStar were swabbed and the swabs were submitted for STR analysis. Full profiles at the 13 core CODIS STR loci were obtained from swabs from each carpet, demonstrating that BlueStar, like luminol and fluorescein, does not inhibit STR analysis.

Introduction

Blood left at a crime scene can not only give an indication of foul play, but it can also have the potential to reveal the identity of the person who left it. In most instances where blood is visible, DNA analysis will be possible. However, if the blood is latent, not only must it be found but the method used to find the blood must not interfere with DNA analysis.

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Two chemicals, luminol and fluorescein, are widely used to detect latent blood [1]. Research has shown that luminol and fluorescein do not affect STR typing of DNA [1, 2]. These chemicals differ in other ways, however. Luminol must be applied in absolute darkness for visualization, whereas fluorescein can be observed in partial darkness. The fluorescence in the fluorescein reaction also reportedly lasts longer than the luminescence created by luminol [1]. Fluorescein, however, requires much more time and resources to prepare than luminol. While laboratories weigh the pros and cons of each method to determine which to use, an additional choice is now available. This new chemical, called BlueStar Forensic, also detects latent blood, but its makers claim an ease of use and long-lasting reaction that rivals luminol and fluorescein [3]. Like fluorescein and luminol, the manufacturers of BlueStar also advertise that it does not interfere with DNA analysis [3]. To date, the author is not aware of published data comparing BlueStar, fluorescein, and luminol in terms of their effect on DNA analysis. As a result, this study was undertaken to determine whether BlueStar interferes with STR analysis and whether there are any significant differences among the three as far as their effect on STR analysis.

Materials and Methods

Sample Preparation

Blood was drawn from a known donor. Some of the blood was applied to a sponge that was then pressed against the sole of a shoe. The shoe was pressed onto butcher paper several times until blood was faintly visible on the paper. Blood was also applied to a golf club and machete, which were then wiped with a cloth. The golf club and the machete were then rinsed with water until no more blood was visible on either item. A drop of blood was applied to the donor's hands and the hands were rubbed together. The blood-stained items were pressed evenly onto pieces of 24" x 18" indoor/outdoor red carpet until it appeared no more blood could be transferred from each item to the carpet. The pieces of carpet were cut and labeled bluestar, luminol, and fluorescein. The carpets were allowed to dry for at least 5 days before continuing.

Reagent Preparation

Luminol and BlueStar were prepared and used according to the manufacturers' directions just prior to use. Fluorescein was prepared and used according to the San Diego Sheriff's Crime Laboratory (SDSO) protocol just prior to use. Each reagent was sprayed evenly over its respective carpet in a darkened room until a visible reaction was observed. The luminol and BlueStar reactions were observed with the naked eye, whereas the fluorescein reaction was observed with orange goggles while illuminating the carpet with an Omnichrome forensic light source at 450 nm. Each carpet was allowed to dry for at least 24 hours.

Presumptive Blood Testing

To aid in interpreting the DNA results, the Kastle-Meyer presumptive blood test was used on three areas of each carpet according to SDSO protocol. Seven of the nine areas tested gave a positive result, and one area on both the luminol and fluorescein carpets gave a negative result. All nine areas tested were swabbed for DNA analysis.

DNA Extraction

The entire swabs were placed in separate 1.5 mL tubes. An unused swab was placed in a tube as a control. A cutting of a known blood stain used as a positive control and a reagent blank were also prepared. Digest buffer (500 µL) and 15 µL 10 mg/mL proteinase K were added to each tube and left to digest at least 2 hours at 56 ºC. After digestion, the substrates were spun down in spin baskets to remove any remaining liquid and removed from the tubes.

The DNA was extracted according to SDSO protocols for purification of DNA by organic extraction and ultrafiltration with Centricon 100 (Millipore Corporation, Bedford, MA). The DNA was quantified using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Human DNA was detected on all nine swabs.

DNA Amplification

The extracted DNA was amplified using the AmpFlSTR Profiler Plus and AmpFlSTR COfiler PCR Amplification Kits (Applied Biosystems, Foster City, CA) using SDSO protocols. Positive and negative amplification controls were added to the samples. All samples were amplified on an Applied Biosystems 9600E Thermal Cycler (Applied Biosystems, Foster City, CA) under the following conditions:

95 ºC 11 minute hold

28 cycles of the following:

94 ºC 1 minute melt

59 ºC 1 minute anneal

72 ºC 1 minute extend

60 ºC 45 minute hold

Capillary Electrophoresis and Analysis

Samples were prepared for capillary electrophoresis and typed by an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) according to SDSO protocols. Allele sizes were determined using GeneScan Analysis v. 3.1.2 software and the Local Southern size calling method. Alleles were designated using Genotyper v. 2.5.2 software (Applied Biosystems, Foster City, CA).

Results and Discussion

Full profiles were obtained from all carpet swabs with the exception of a swab from the luminol carpet which gave a negative Kastle-Meyer result but during quantitation showed a small amount of DNA that fell below the range of the Quantifiler standard curve. Because the amount of DNA detected was so low, it was concentrated down and amplified without requantifying it (so as to save as much sample for amplification as possible). There are possible explanations for the negative Kastle-Meyer result and presence of DNA on the luminol carpet. It is possi-

ble the Kastle-Meyer test was not sensitive enough to detect the small amount of DNA recovered. It is also possible that a larger area of the carpet was swabbed for DNA analysis than was swabbed for the presumptive blood test. In any event, the DNA detected during quantitation on this sample was not enough to produce any type of genetic profile after amplication.

These possibilities may also explain why a swab taken of the fluorescein carpet also gave a negative Kastle-Meyer result but through DNA analysis produced a full profile. For this particular sample, however, the quantity amplified was well within the typing range (~1 ng).

Because full profiles resulted from carpets treated with all three chemicals, there does not appear to be a significant difference among luminol, fluorescein, and BlueStar when it comes to affecting STR analysis under these experimental conditions.

There are several limitations to this study for which follow-up research is recommended. This study did not address the sensitivity of each latent blood detection method. Rather, undiluted blood was added in amounts that ensured there was enough blood for STR analysis. This study also did not address the range of substrates that may be encountered at crime scenes, such as walls or linoleum, to see how each substrate affects the analysis.

With research on BlueStar as limited as it is, the goal of this study was to confirm that luminol and fluorescein did not interefere with STR analysis and to see how BlueStar compared. The results confirm that luminol and fluorescein do not interfere with STR analysis and further demonstrate that BlueStar also does not interfere with STR analysis.

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