A5 Comparison of Six Latent Bloodstain Reagents for Sensitivity, Specificity, and Impact on DNA Yield and Quality

Sara E. Bitner, MSF, 1520 Penn Ave, Pittsburgh, PA 15222; Amanda Dargay, BS, 104 Cobblestone Dr, Pittsburgh, PA 15239; and Amanda Neis, BS, 321 S Maple Ave, Apt 6, Greensburg, PA 15601*

After attending this presentation, attendees will benefit from scientific observations on a variety of latent bloodstainenhancing reagents based on their sensitivity, specificity, longevity of reaction, ease of use, as well as impact on downstream DNA analysis.

This presentation will impact the forensic science community by providing aid in selecting latent bloodstaintesting methods that allows for high-quality results, while also aiding in efficiency of the laboratory. Because backlogs are a constant companion to the forensic science laboratory, efficiency is essential in proper casework management.

Due to the ease of use as well as the enhancements made by the manufacturers which allows for greater longevity in the reaction, the manufactured reagents will provide a suitable alternative to the traditional recipes many laboratories currently prepare in-house.

While blood is typically the most visually apparent physiological fluid present at a crime scene, or on items of evidence in a forensic laboratory, there are situations in which the blood is latent, or not visible to the naked eye. In these cases, some form of enhancement is required for the visualization and eventual collection of the bloodstains. The latency of the blood is often due to efforts to clean or remove the blood. As a result, any latent blood present at a crime scene has been thoroughly diluted. Additionally, there are often chemicals present in the area that is being tested that can potentially stymie future serological, chemical, or DNA testing. A latent blood test to aid the forensic scientist in locating the bloodstains must be sensitive, so that it can detect these low levels of blood, as well as specific, so that it properly identifies blood and not one of the many potential cleaning agents or other chemical and natural insults that could cause false positive or false negative results. A pitfall of latent bloodstain tests is the ephemeral quality of the reaction; the optimum reagent would present a reaction that could be easily viewed and potentially photographed without requiring further applications of reagent. Finally, the ideal reagent should not impact the downstream DNA testing that is required for identification of the source of the blood.

A wide variety of manufactured reagents, as well formulas for preparation of in-house reagents, are available to the forensic scientists. Six latent bloodstain reagents were selected for comparison. The reagents (luminol, fluorescein, fluorescein with thickener, Hemascein®, Tink's Starlight Bloodhound™, and BlueStar®) were applied to a variety of blood dilutions, substrates, and blood mixed with chemical and physiological fluid insults. The results of the testing, as well as observations on longevity of the results and ease of preparation, were noted. A selection of the blood dilutions was sampled for DNA analysis, to determine the impact on DNA yield and DNA quality. The selected samples were extracted on a variety of platforms, to monitor any variability that might result between an organic extraction method versus automated, silica-based extraction methods. Additionally, the impact on yield was measured through the quantitation of the DNA extractions. Finally, the quality of the DNA obtained from the tested samples was determined through the amplification and subsequent genetic analysis via capillary electrophoresis.

The manufactured reagents were observed to exhibit qualities commensurate or exceeding those of the in-house formulas. As a result, the manufactured reagents, while sometimes more expensive, provide an ease of use and quality of results that makes them a suitable, if not superior, alternative to the in-house reagents.

Latent Blood, Luminol, DNA

 ** Presenting Author*