B1 A Novel Colorimetric Isothermal Amplification Method for Cost-Effective, Rapid Body Fluid Identification

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Learning Overview: After attending this presentation, attendees will have been introduced to a simple isothermal amplification method with colorimetric analysis, the use of novel messenger RNA (mRNA) targets for differentiation of body fluids, and how the integration of these two scientific advances applies directly to forensic body fluid identification. The information will present a novel alternative to current methods used in forensic laboratories and literature.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a simple, yet specific, alternative method for body fluid identification that can be easily integrated into the forensic workflow, is automated to minimize user intervention, and has value for adapting to other areas of forensic science and/or newer technology platforms.

Loop-Mediated Isothermal Amplification (LAMP) is ideal for qualitative assays because of its high specificity using two to three pairs of primers, generates multi-sized amplicon, and successful amplification can be read out colorimetrically. This study shows this technique can be directly applied to an important forensic application—body fluid Identification (bfID). Accurate presumptive and confirmatory tests are essential for gaining contextual information for crime scene investigators. False positive results are not uncommon with biochemical-based tests demonstrating a lack of specificity. In addition, many methods are known to be destructive to the sample and/or inhibit downstream procedures. Last, the analyst determines if the identification tests are positive or negative based on a color result, but this result is subjective due to differences that can occur from person to person.

This study demonstrates data on a LAMP method that is amenable to rapidly identifying body fluids. The panel includes venous blood, semen, and saliva and vaginal fluid detected with a colorimetric indicator and automated image analysis. The method is designed for easy implementation into the current forensic case workflow. Using mRNA targets for bfID, a high level of tissue- and human-specificity are derived from the use of multiple primer sets in the LAMP assay. The simplicity of this analytical method will minimally disrupt forensic labs performing DNA analysis, and the nature of the isothermal amplification massively reduces the complexity of instrumentation needed. Due to the use of phenol red in the reactions, an enclosed system was built with static lighting to analyze the reactions via camera or mobile phone. After an image is captured, it is analyzed for the hue value of each reaction, thus eliminating subjectivity from the analysis. This simple integrated system allows for minimal hands-on time with automated heating and image capture and analysis. Together the method and instrument show high promise as a forensic identification method.

Isothermal Amplification, Body Fluid Identification, Colorimetric

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