B4 The Use of Proximity Ligation Assays Coupled With Real-Time Polymerase Chain Reaction (PCR) as a Detection Method for Proteins Within Blood for Forensic Analysis

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After attending this presentation, attendees will better understand how Proximity Ligation Real-Time PCR (PLiRT-PCR) can be utilized as a rapid confirmatory assay for the presence of blood in a biological sample.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR can be a very effective tool for throughput body fluid identification. Furthermore, the assay utilizes technology already present in forensic laboratories; thus, no new equipment is necessary. This research effort focuses on the identification of blood and is meant to complement, eventually in a single assay, PLiRT-PCR-based confirmatory assay for sperm/semen and saliva.

Blood evidence can be found at the scene of many different types of violent crime, including murder, rape/sexual assault, robbery, assault, domestic and stranger violence, and crimes involving injuries. Presumptive tests for the presence of blood performed at the crime scene are often followed by confirmatory tests in the laboratory, not only to confirm the sample is blood, but also that the blood is human. Presumptive tests are generally very sensitive but lack in specificity, while confirmatory tests, although very specific, are generally less sensitive. PLiRT-PCR possesses both characteristics.

PLiRT-PCR combines the specificity of an immunological reaction with the sensitivity of PCR and only requires a minimal amount of sample in order to be effective. A small cutting of the sample or small drop of diluted sample can be utilized for the assay, minimizing sample consumption for downstream processes (i.e., generating a DNA profile) should it prove to be human blood. An additional advantage of PLiRT-PCR is the potential for high throughput sample processing. In fact, this can be performed on a 96-well plate format and plate set up could potentially be performed by an automated liquid handling platform.

Samples are first cut from a swab or diluted from a sample of neat suspected human blood and lysed in a prepared lysis buffer to expose the proteins within the cells. Once lysed, the sample is exposed to specific antibodies that are targeting the proteins of interest. This binding reaction allows the antibodies to bind with the desired proteins for detection. The antibodies have different DNA segments attached to them to create proximity probes. After the binding step, the sample goes through a ligation period. Probes close to each other and with the complementary oligonucleotide strand will be hybridized. This new DNA strand is then amplified and can be measured by quantitative PCR (qPCR).

This presentation will demonstrate how PLiRT-PCR has the potential to be successfully implemented in the laboratory workflow of forensic laboratories for the identification of human blood. This presentation will reveal how sensitive and human specific PLiRT-PCR can be through the analysis of known samples provided by The George Washington University. This method can be used with samples that have a mixture of fluids or only the suspected blood.

Although there are other confirmatory tests for the presence of blood, PLiRT-PCR is a powerful alternative as it can be performed in as little as a few hours and with such sensitivity and specificity that it can replace other methods

of detection. PLiRT-PCR consumes only a small amount of the original sample and can utilize many instruments already in use in forensic laboratories.

Blood, Proximity Ligation PCR, Hemoglobin