A138 On-Site Body Fluid Identification

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After attending this presentation, attendees will understand the development and design of short, fluorescently-labeled, single-stranded DNA probes, known as molecular beacons to detect tissue-specific ribonucleic acids as a possible on-site confirmatory test for human body fluid type.

This presentation will impact the forensic science community by outlining a universal, cost-effective, confirmatory assay to determine the

body fluid content of the three most common biological stains (blood, saliva, and semen), as well as determining if the stain is human in origin, while still processing a crime scene. With such an assay in use, exclusion of non-evidentiary specimens from collection, processing, and DNA profiling can represent a significant savings in terms of both time and money.

Collection and processing of potential evidentiary samples at a crime scene is critical to the successful resolution of an investigation. At present, various presumptive and confirmatory tests are available to assist the crime scene investigator in selecting which specimens to process. Most presumptive tests can be performed at the crime scene itself, but many of the confirmatory tests must be performed at the crime laboratory. There is a great need for a universal confirmatory test for biological evidence left behind at the scene of a crime. Such an assay would assist crime scene investigators to determine whether to collect a specimen for further processing or ignore the stain as being non-evidentiary, thus saving time and money. The success, sensitivity, and popularity of DNA profiling has frequently resulted in the collection of a large number of samples at many crime scenes, resulting in increased costs to investigate a crime and often a considerable backlog in the processing of such samples. The presented assay has the potential to reduce the number of samples collected at a crime scene and could eventually have a positive impact on reducing evidence backlogs.

It was hypothesized that by designing molecular beacons to hybridize with RNAs that are specific for blood, saliva, or semen, a multiplex reaction could be created to determine the presence of each type of body fluid, or combination thereof, in a biological stain. Within this multiplex reaction, human-specificity is also determined by probing human-specific RNA sequences. With advances in technology that allow for fluorescence spectrophotometers powered by laptop computers and short RNA extraction methods that can be performed at room temperature, the advantage of portability to a crime scene is gained.

After an extensive literature search, candidate RNAs were identified and confirmed to be tissue-specific. Traditional PCR primers that encoded areas of interest on chosen RNA molecules were used to validate the candidate sequences as either tissue-specific or human-specific. The forward primer was then used to create the molecular beacon hairpin probe. The molecular beacons were then tested on biological samples of different ages.

Aspects of the development of the tissue-specific molecular beacons will be discussed in detail for the body fluids of blood, saliva, and semen. Various parameters of the assay will be discussed including detection limits (both in terms of minimum sample size, as well as over what time frame the assays are valid) and intrapersonal variability. The design of future experiments will be discussed.

Whether used in the field as originally intended, or within a crime laboratory to replace multiple current confirmatory tests, the assay has great promise. Once additional research and validation studies have been performed, the presented technique would provide a less time-consuming and more cost effective assay for confirming body fluid type.

Body Fluid Identification, Tissue-Specific RNA, Molecular Beacons

 ** Presenting Author*