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FORENSIC STAIN IDENTIFICATION BY RT-PCR ANALYSIS AND CONSEQUENT DEVELOPMENT OF A NEW DNA EXTRACTION METHOD

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In an age of countless scientific advances in molecular biology, DNA profiling has proven itself an invaluable tool in solving crimes. The potential exists, however, for the tissue origin of the suspect DNA to be called into question. For example, a semen stain containing suspect DNA can have far more serious consequences than a saliva stain. The collection of mRNAs made in any cell is unique to that cell type; thus, a differentiation could be made using mRNA as a fluid- or tissue-specific determinant. This poster describes results to date using real-time PCR to determine the specificity, sensitivity and discriminatory limits of our real-time assays, as well as the stability of mRNA over time. A major aim of this project is to work towards multiplexing the real-time PCR assays once mRNAs are identified that clearly define specific types of stains. One method that we are utilizing is the PlexorTM System from Promega. This system allows the multiplexing of four to six mRNAs in one assay, thus reducing the amount of sample needed and time of analysis. Our initial focus was to design a blood-semen stand alone assay, since our initial studies have identified mRNAs that are specific for these fluids. Therefore, we have designed PlexorTM primers sets that detect two blood-specific and two semen-specific mRNAs in order to ascertain whether blood and/or semen is present in a stain. In the future, we hope to greatly increase mRNA multiplexing using a Luminex™ bead based assay system. This approach allows multiplexing of up to 100 different gene expression assays, which will support the detection of mRNAs from fluids or tissues (brain, heart, liver, intestine, kidney, skin, muscle, adipose) which we are in the process of collecting. One of our goals is to improve RNA extraction, so that minimal amounts of sample are used, as well as to potentially obtain both DNA and RNA; thus eliminating the need for two separate extractions. We have compared RNA yields from numerous commercially available RNA extraction kits, as well as several “home-brew” protocols published in various journals. During this process, we found a method reported for plant DNA/RNA extraction that was very proficient for DNA extraction. Although this technique does not yet extract ample amounts of RNA, we felt it was important to investigate and optimize as a fast and inexpensive DNA extraction protocol. We have validated the method for CODIS samples (blood and buccal cells on FTA paper) and standards in order to switch from our automated DNA extraction system which is time consuming and costly in reagents and supplies. Additionally, our alternative phenol/chloroform extraction is not only laborious, but involves hazardous chemicals that require numerous safety precautions and special disposal. Although the new approach is not automated at this point in time, we hope to be able to automate or streamline it in the future. Nevertheless, it will decrease the time it takes to extract samples and significantly reduce the cost and safety concerns associated with DNA extraction. Studies are currently underway to determine whether the new method can be further refined for simultaneous DNA and RNA extraction, as well as validating it for casework samples.