H35 Development of the Discrimination Procedures Between Nasal Secretion and Saliva by Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) and Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

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Learning Overview: After attending this presentation, attendees will understand the applicability of rRT-PCR and ELISA procedures on nasal secretion- and saliva-characteristic markers for the discrimination of these body fluids through analysis of specificities and sensitivities of these procedures.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing reliable information on the source of human DNA through the identification of body fluids, which are often left at crime scenes but are difficult to distinguish from each other.

In forensic casework, nasal secretions could be a good source of DNA for the identification of individuals. There are, however, few procedures to identify nasal secretions. Further, saliva that is often left at crime scenes could prove sexual assault. However, presumptive and confirmative tests for saliva on the basis of α-amylase can cross-react with other body fluids, such as nasal secretions. In previous studies on messenger RNA (mRNA) - based identification of body fluids, some nasal secretion- and/or saliva-characteristic genes have been reported; however, end point detection of these genes may be difficult to discriminate from each other because of their insufficient specificity and detectability. In addition, there are no reports of protein marker(s) for the identification of nasal secretions. Furthermore, ELISA detection of Statherin (STATH), commonly used as a saliva marker, cross-reacted with nasal secretions.

Therefore, the goal of this study was to develop more specific procedure(s) for discrimination between nasal secretions and saliva for forensic purposes. Candidate molecules for the identification of nasal secretion (bactericidal permeability increasing protein fold containing family A member 1 ((BPIFA1), STATH) and saliva (STATH, histatin 3 (HTN3)), and proline-rich protein HaeIII subfamily 2 (PRH2)) were selected, and RT-PCR and indirect ELISA procedures were developed to determine these mRNA and protein expression levels quantitatively.

Expression levels of candidate genes were determined in various body fluids and discrimination criteria for nasal secretions and saliva were determined based on quantitative results of multiple markers. In addition, a flowchart to discriminate among nasal secretions, saliva, and other body fluids was proposed and evaluated on various forensic samples. Similarly, the specificity and sensitivity of ELISA detection of candidate proteins were determined using various body fluids, and a positive threshold value was set for each candidate marker. Then, applicability of ELISA procedures to the forensic casework was investigated using simulated casework samples.

As a result of real-time RT-PCR analysis, BPIFA1 was highly but incompletely expressed in nasal secretions and expressed in semen and vaginal fluids in trace levels. STATH was expressed in almost all the nasal secretion and saliva samples but not detected in other body fluids analyzed in this study. HTN3 was specifically expressed in saliva samples as reported previously. Unexpectedly, only a few saliva samples showed positive results in RT-PCR analysis for PRH2 though it was reported as a specific protein marker for saliva. Using determined discrimination criteria and the proposed flowchart, nasal secretions and saliva were successfully discriminated among various body fluids analyzed in this study.

The results of ELISA analysis showed that BPIFA1 was specific to nasal secretions. Besides, STATH was detected in both nasal secretion and saliva samples in accordance with previous reports and results of gene expression analysis. PRH2 was specifically detected in most saliva samples despite low gene expression levels in saliva. Development of ELISA detection of HTN3 was not achieved because suitable antibodies were not found in this study.

In conclusion, for the discrimination of nasal secretions and saliva among various body fluids, BPIFA1, STATH, and HTN3 could be effective markers for RT-PCR. On the other hand, BPIFA1, STATH, and PRH2 could be useful as protein markers for ELISA. Suitable procedures could be selected depending on the sample condition and sample type because environmental tolerance between mRNA and protein was thought to be different.

Nasal Secretion, Saliva, Body Fluid Identification