IDENTIFICATION OF BODY FLUIDS BY MRNA ANALYSIS WITH MINION NANOPORE SEQUENCING

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The identification of body fluids on evidence items in a criminal investigation can be vital to understanding the nature of a crime. Although crime labs can confirm the presence of body fluids like semen and blood on a piece of evidence using traditional serological techniques, these tests can be laborious and consume much of what may already be a very small sample. Furthermore, many crime labs are not able to confirm the presence of saliva or vaginal secretions or differentiate peripheral blood from menstrual blood. Identifying these fluids is important in oral or digital assaults, when the presence or absence of semen is not informative within the context of the assault, or when menstrual blood must be differentiated from blood caused by trauma. Tests for these body fluids are presumptive only, so there is a need in the field of forensics for robust and reliable tests that can confirm the presence of various body fluids that may be present on a sample.

Due to the unique patterns of gene expression in different cell types, different body fluids contain distinct messenger RNA (mRNA) molecules, which can be analyzed to generate mRNA profiles for confirmatory identification of body fluids. Various highly specific and sensitive mRNA markers have been identified for various body fluids. Although capillary electrophoresis methods have been used in the past, more studies are using next-generation sequencing technologies due to the high throughput. The MinION by Oxford Nanopore Technologies is a new, portable, and relatively inexpensive sequencer in comparison to other next-generation sequencers. Because this sequencer is new, its ability to sufficiently sequence forensic type samples is not fully known.

The purpose of this study was to explore a rapid method for identifying body fluids from forensic samples. Because the MinION DNA sequencer is very small, inexpensive, and has a rapid data turnaround in comparison to other next-generation DNA sequencers, it would be ideal for the processing of evidence in crime labs. To examine the reliability of this method, semen, saliva, peripheral blood, menstrual blood, and vaginal fluid from 8 different donors per body fluid were analyzed with the MinION sequencer using a multiplex PCR that was developed to target two specific genes per body fluid, including two housekeeping gene targets as endogenous controls. Additionally, dilution and mixture studies were performed to assess the sensitivity and specificity of this method. This study explored the ability of this new sequencer to work in a forensic setting and determine the robustness, reliability, and reproducibility of the results of analysis of various body fluid samples.