B52 Rapid Loop-Mediated Isothermal Amplification (LAMP) of RNA Biomarkers for Forensic Identification of Semen and Saliva

Erin K. Hanson, PhD, PO Box 162367, Orlando, FL 32816; Kelsey Neary, BS, National Center for Forensic Science, PO Box 162367, Orlando, FL 32826; John R. Waldeisen, PhD, Diassess Inc, 130 Stanley Hall, Berkeley, CA 94720; Debkishore Mitra, PhD, Diassess Inc, 130 Stanley Hall, Berkeley, CA 94720; Ivan K. Dimov, PhD, Diassess Inc, 130 Stanley Hall, Berkeley, CA 94720; Martin R. Buoncristiani, MSc, 1001 W Cutting Boulevard, Ste 110, Richmond, CA 94706; Eva M. Steinberger, PhD, 1001 W Cutting Boulevard, Richmond, CA 95804; Cristian J. Orrego, PhD, UC Berkeley, Human Rights Center, 2850 Telegraph Avenue, Ste 500, Berkeley, CA 94705; and John Ballantyne, PhD, University of Central Florida, Dept of Chemistry, 4000 Central Florida Boulevard, Orlando, FL 32816-2366*

After attending this presentation, attendees will learn how a new isothermal amplification method can be applied to mRNA profiling for the identification of forensically relevant biological fluids and tissues.

This presentation will impact the forensic science community by presenting possible alternatives to how mRNA profiling assays are performed and how these new methods may be more amenable to on-site analysis at crime scenes.

Conventional body fluid identification methods are often costly, not only in terms of time and labor required for their completion but also in the amount of sample consumed. Additionally, while methods are available for the identification of human blood and semen, none of the routinely used serological and immunological tests can definitively identify the presence of human saliva, vaginal secretions, menstrual blood, or skin. Thus, attempts to develop molecular-based approaches to body fluid identification have been made in order to provide operational crime laboratories with significantly improved specificity and sensitivity. The use of messenger RNA (mRNA) profiling has been proposed to supplant conventional methods for body fluid identification. It is based on the premise that each single tissue type is comprised of cells that have a unique transcriptome or gene expression profile. Currently, numerous biomarkers have been identified and validated for the identification of forensically relevant body fluids and tissues (blood, semen, saliva, vaginal secretions, and skin). Standard mRNA body fluid assays require laboratory equipment that is not meant to be used at crime scenes and requires hours to generate data. Thus, there is a need for the development of more rapid, simple, and inexpensive methods for mRNA profiling.

A potential alternative mRNA profiling strategy involves the use of LAMP. LAMP's advantages are primarily derived from its unique reaction mechanism. The amplification starts at 60°C to 65°C with primers annealing the target DNA strand and self-priming to form dumbbell-shaped structures that will be used in the strand displacement amplification. In this way, the primers can continuously prime, extend, and displace on their own amplicons. For the detection of RNA targets, LAMP can be combined with Reverse Transcriptase (RT-LAMP) in a single mix. The reaction can produce large quantities of DNA (up to 109 copies in under an hour), enabling naked-eye detection of amplicons with suitable colorimetric or turbidometric sensors. Product detection without aperture of the reaction tube diminishes concerns about amplified product contamination of laboratory environments, a matter of constant vigilance with current detection schemes based on the Polymerase Chain Reaction (PCR).

Here a prototype rapid LAMP of mRNA biomarkers method for the identification of semen (PRM2, TGM4) and saliva (STATH, HTN3) is described. Based on initial testing, the developed 90-minute assays (one-step closed reaction from RT to detection) permit the identification of the body fluid origin of dried semen and saliva stains and demonstrate a high degree of reproducibility (technical and biological replicates). For semen, it was determined that PRM2 was the most suitable candidate. Semen was correctly identified in 8/10 known semen samples (one false negative, one vasectomized male donor so PRM2 was not expected or observed). Specificity was demonstrated with a failure to detect PRM2 (semen) in blood, saliva, vaginal secretions, menstrual blood, or skin samples (N=15) (separation in detection time of ~40 to 55 minutes). Reproducibility was assessed through an evaluation of ten donors (three technical replicates per donor). Semen was correctly identified in nine donors (10th donor was a vasectomized male and the observed standard deviation among individual donors was as little as 0.2-3.4 minutes). While more variability was observed for saliva due to the nature of the body fluid itself, saliva (STATH) was successfully identified in samples stored at room temperature for one to two years (N=4) and samples exposed to environmental insults (heat, light, humidity, and rain) for days to up to one month (N=17), with results comparable to a Capillary Electrophoresis (CE) -based mRNA profiling multiplex. Successful detection of saliva was achieved for 38% of the compromised and environmental samples using the STATH-LAMP assays compared to 31% successful detection of STATH using CE-based detection.

The results from these initial studies indicate a possible utility of isothermal RT-LAMP assays for body fluid identification. The potential exists (and is the subject of current work) for the development of these assays on an integrated microfluidic device which would permit confirmatory body fluid identification by mRNA profiling at its point-of-use (crime scenes or in the biology screening laboratory).

Isothermal Amplification, RNA Profiling, Body Fluid Identification