Advanced Rapid Identification of Vaginal Fluid with miRNA based LFA Technology

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Introduction

Body fluid identification is a crucial component of forensic investigations, helping to establish key details about crime. A typical investigation consists of two primary components: Suspect identification and Crime Identification. While suspects are determined by investigative leads and human identification, the presence and nature of crime is based on stain identification. Presence or absence of specific body fluids in strategically collected stain samples may support or refute the victim's and suspect's account of events steering the investigation in right direction. Hence, considerable amount of effort is made for correct body fluid identifications.

Traditional methods for identifying body fluids rely on chemical tests and staining techniques. While these methods have been widely used, they often lack specificity, require longer skilled work and expert interpretations. Current advanced methods for accurate body fluid analysis include: RNA marker analysis, DNA methylation analysis, Proteomics, and Microbiome Analysis. However, the amount of resources needed for these methods make their utility time and cost inefficient.

Another rapid and cost-efficient method for identifying body fluids is based on LFA (Lateral Flow Assay) which identify unique markers of body fluids based on antigen-antibody interaction. Commercial tests are available for detecting fluids like blood, semen and saliva but nothing similar exists for vaginal fluid detection. The main challenge in case of vaginal fluid is the absence of unique protein markers for reliable detection.

Methods

Molecular Beacon Technology

Molecular beacons (MBs) are specially designed hairpin like structures of nucleic acids, in this case DNA (Figure. 1). The two ends of single-stranded DNA (shown in green) are complementary to each other hence forming a loop due to Watson-crick hybridization. The loop (shown in orange) is complementary to a target single stranded nucleic acid. Additionally, a detection molecule like Gold nanoparticle is conjugated to complementary sequence of one end of the MB while it's other end is modified to attach to the surface.

In presence of a target molecule, the loop becomes double stranded disrupting the loop structure and exposing one end of the beacon. The gold-conjugated DNA can now bind to the MB creating a double stranded end generating a visual signal. For control, only the small complementary sequence of the gold-conjugated DNA is attached to the surface which generates a visual signal even in absence of any target molecule.

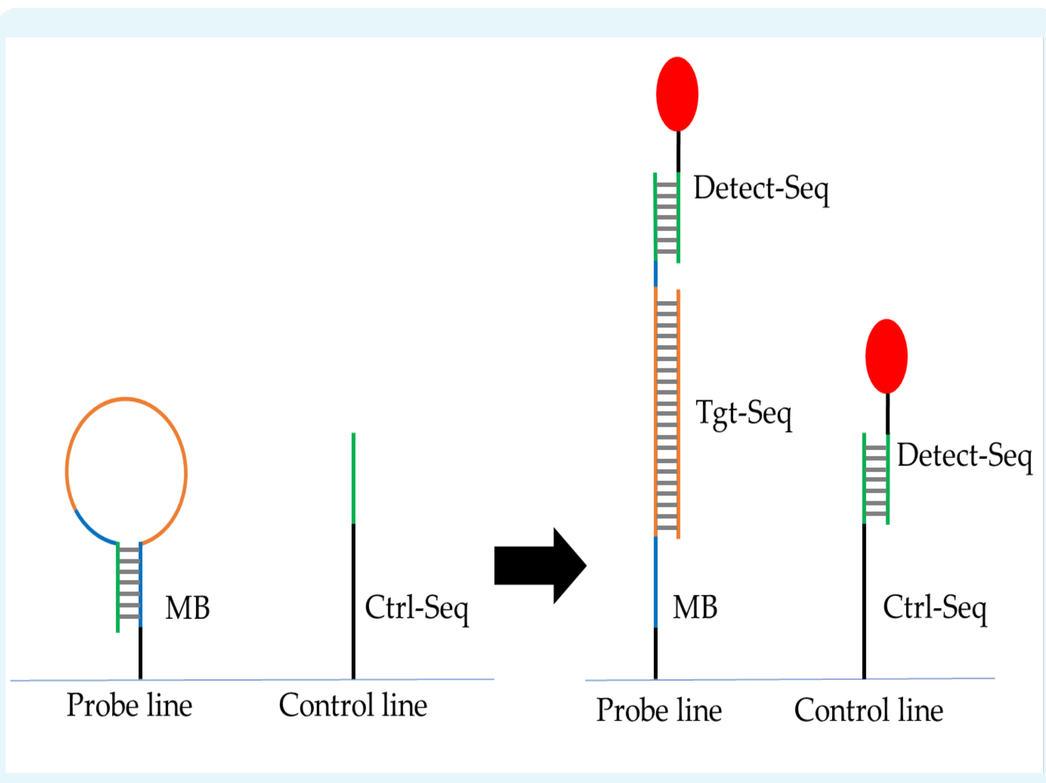

Figure. 1: Scheme of the NALFA. Two 5' NH2-modified DNA oligos have been immobilized on the probe line (Molecular beacon MB2) and the control line (Ctrl-Seq) of the surface of lateral flow strip. Detect-Seq coupled to a particle or Cy5-dye (red) should hybridize always to Ctrl-Seq, but only to MB in the presence of Tgt-Seq in the sample solution.

Results

Molecular Beacons were tested for detection limit (LOD) on a traditional lined LFA. However, the detection limit was 20 pmol which is too high for typical biological samples. Hence, miniaturized LFA was designed for Vaginal fluid detection. Figure 2 shows the improved LOD of 1 pmol. Figure 3 depicts the specificity of MBs to detect vaginal fluid. Temperature sensitivity of the MB based NALFA was tested as shown in Figure 4.

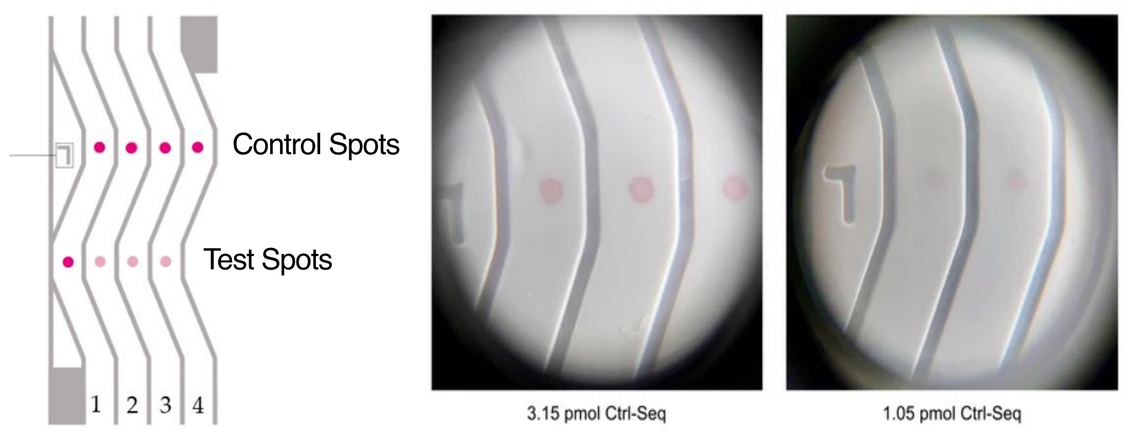

Figure. 2: A miniaturized LFA was created using special structured membranes which helped in lowering the limit of detection of miRNA in a sample to only 1 pmol as shown in the 20x magnified images of the structured membranes (right).

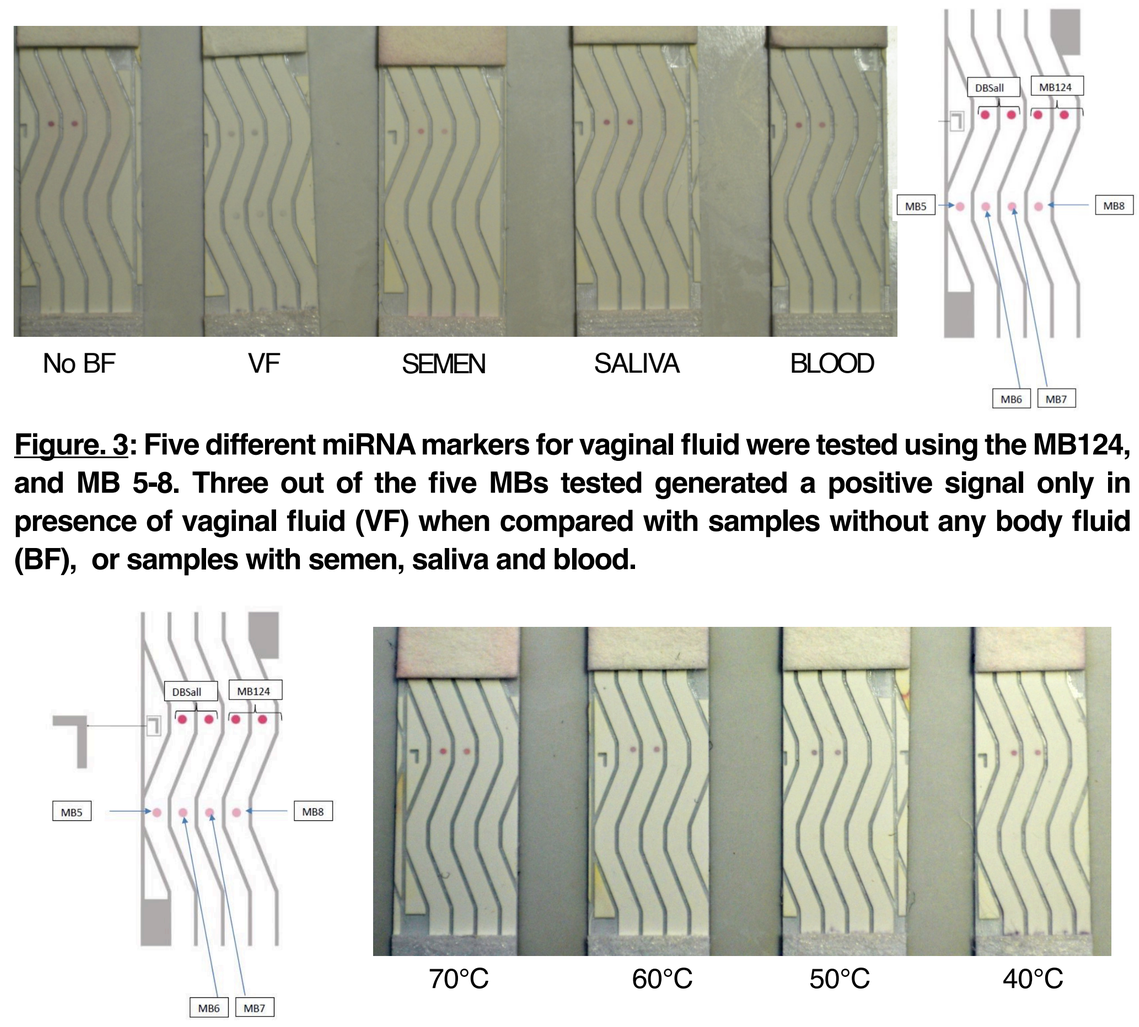

Figure. 3: Five different miRNA markers for vaginal fluid were tested using the MB124, and MB 5-8. Three out of the five MBs tested generated a positive signal only in presence of vaginal fluid (VF) when compared with samples without any body fluid (BF), or samples with semen, saliva and blood.

Figure. 4: Because hair-pin like structures are known to be temperature sensitive, the functionality of these tests were tested at different temperatures. The tests showed no unspecific binding on test spots up to 70 degree Celsius.

Conclusions

1. Miniaturized multiplex NALFA (Nucleic Acid based Lateral Flow Assay) provides rapid and cost-effective solution to detect vaginal fluid in forensic samples.

2. Protein-free nature if these LFAs results in improved stability. Results show that these tests are reliable at temperatures up to 70 degree Celsius.

3. The limit of detection of the presented NALFA is 1 pmol which provides higher reliability and superior sensitivity.

4. Designed to hybridize with unique RNA or DNA markers, these NALFAs reduce the risk of false positives.

References

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2. Javani, A., F. Javadi-Zarnaghi, and M.J. Rasaee, A multiplex protein-free lateral flow assay for detection of microRNAs based on unmodified molecular beacons. Anal Biochem, 2017. 537: p. 99-105.

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