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The killer outfit and timing: Impact of the fabric and time in body fluid identification and DNA profiling

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ABSTRACT

The present work aimed to study the detection, through lateral flow immunochromatographic (LFI) tests, of saliva samples over time in three different types of fabrics, as well as, the possibility of DNA isolation and characterization from the sample tubes and the cassettes. Fifty microliters of saliva (three samples/time) were deposited in denim, cotton, and polyester. Saliva was identified by SERATEC® Amylase Test and the Crime Scene version SALIVA CS, being able to detect it up to six months of deposition, although with different band intensities. Polyester showed stronger bands than cotton, probably due to its synthetic nature, and denim, as an inked fabric, showed less band intensities. Statistical analyses confirmed significant differences among fabrics, but not over time in the same type of fabric. Total DNA from the sample tubes was successfully recovered, in contrast, from the cassettes, only polyester retrieved amplifiable DNA. These findings indicated that it is possible to recover and identify saliva up to six months after deposition, also obtaining DNA. Future research will be able to expand these results, analyzing the stability of other body fluids, and the sensitivity of lateral flow immunochromatographic tests to detect them.

1. Introduction

The detection and identification of saliva at a crime scene may be crucial in establishing physical presence of someone at the scene, with the appropriate criminal repercussions [1].

There are different techniques for detection of saliva at crime scenes. Lateral flow Immunochromatographic (LFI) tests detect the presence of human salivary-α-amylase, based on antigen-antibody reactions. This is one of the major protein components in the saliva, being its levels at least 10-fold greater than that in other body fluids [2]. Thus, it is an effective marker for saliva identification. These LFI tests are widely used at crime scenes and in the lab based on its simplicity and quick result. However, there are few studies analyzing different factors that could affect the detection of saliva by these tests. An important one is the time of evidence recovery. Some crimes are discovered few hours or days after committed, others, could be revealed after months or even after cross-examination of additional evidence. It is important to assess the sensitivity of these LFI tests over time.

The present work aimed to study the identification of saliva stains and DNA recovery over time up to six months after deposition on three different types of fabrics, applying LFI tests.

2. Material and methods

2.1. The experiment

Fifty microliters of saliva (three samples per time) from two donors were deposited in three different types of fabrics: denim, cotton, and polyester. New Jersey Institute of Technology Institutional Review Board (IRB) approved the procedures related to human body fluid experimentation.

2.2. Lateral flow immunochromatographic tests

The samples were recovered by swabbing using cotton swabs (Cotton-tipped applicators sterile, wood shaft from McKesson) after 24

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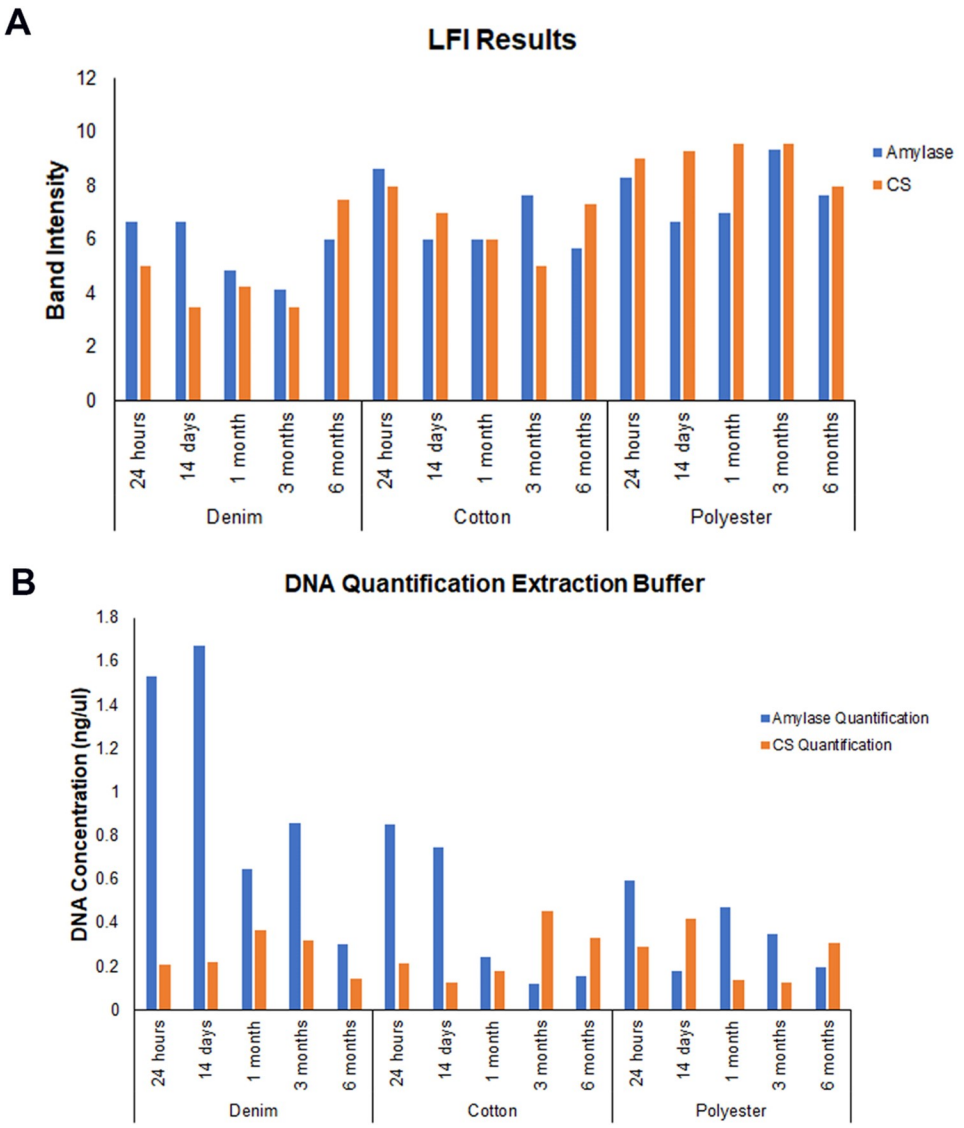

Fig. 1. A. Average of band intensity on LFI tests. **B**. DNA Quantification from the Extraction Buffer.

hours, 3 days, 7 days, 14 days, 21 days, 1 month, 2 months, 3 months, 4 months, 5 months, and 6 months, and assessed the presence of amylase through SERATEC® Amylase test for laboratory application, and the new crime scene optimized SERATEC® SALIVA CS test.

2.3. DNA extraction and quantification

DNA was extracted from the extraction buffer and sample pad applying a modification of the DNeasy Blood and tissue kit (Qiagen®, Hilden, Germany), according to our previous published protocol [3] and quantify with Qubit dsDNA HS (Thermo Fisher Scientific, Waltham, MA, USA) and Quantifiler Trio (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Statistical analyses

Statistical analyses were carried out using SPSS (IBM) and applying parametric tests and non-parametric tests.

3. Results

3.1. Lateral flow immunochromatographic tests results

It was possible to detect saliva up to six months after deposition with both tests. The intensity of the bands varied among clothing and time. According to the statistical tests, there were not significant differences on band intensities on the same clothing over time, in contrast, significant differences were found among fabrics in both tests (Fig. 1A).

3.2. Assessment of DNA yield from EB (extraction buffer) and cassettes (sample pad)

It was possible to obtain quantifiable DNA from the EB after 24 hours, 14 days, 1 month, 3 months, and 6 months per fabric and tests (Fig. 1B). In contrast, from the sample pad of the cassettes, only polyester retrieved quantifiable DNA (data not shown).

4. Discussion

The results indicated that in both types of tests and in the three fabrics, it was possible to detect saliva up to six months, with different

degrees of intensities. This is in agreement with the study of Carboni et al. [4], where, they analyzed the efficiency of LFI, the RSID™-Saliva test, to detect saliva up to ten years in envelopes, cigarette butts, and from a forensic case, envelopes aged twenty-six years, obtaining positive results in almost all samples studied. As a secondary aim in this work, DNA yield was evaluated after 24 hours, 14 days, 1 month, 3 months, and 6 months after deposition, obtaining high concentrations in all times and type of fabrics from the Extraction Buffer, in contrast to the aforementioned Carboni et al. study. However, from the sample pad, it was only possible to obtain quantifiable DNA from polyester samples, probably due to its synthetic nature.

5. Conclusions

It is possible to detect saliva up to six months after deposition with LFI tests in three different types of fabrics. Quantifiable DNA was obtained from the tests Extraction Buffer. Only Sample Pad from Polyester textiles retrieved quantifiable DNA. These findings could be useful in crimes discovered months after committed and cross-examination of the evidence, leading to both the identification of the body fluid and DNA profiling.

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Conflict of interest

The authors declare no conflict of interest.

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