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RNA/DNA co-analysis from human saliva and semen stains – Results of a third collaborative EDNAP exercise

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ABSTRACT

A third collaborative exercise on RNA/DNA co-analysis for body fluid identification and STR profiling was organized by the European DNA Profiling Group (EDNAP). Twenty saliva and semen stains, four dilution series (10–0.01 μl saliva, 5–0.01 μl semen) and, optionally, *bona fide* or mock casework samples of human or non-human origin were analyzed by 20 participating laboratories using an RNA extraction or RNA/DNA co-extraction method. Two novel mRNA multiplexes were used: a saliva triplex (HTN3, STATH and MUC7) and a semen pentaplex (PRM1, PRM2, PSA, SEMG1 and TGM4). The laboratories used different chemistries and instrumentation and a majority (16/20) were able to successfully isolate and detect mRNA in dried stains. The simultaneous extraction of RNA and DNA from individual stains not only permitted a confirmation of the presence of saliva/semen (i.e. tissue/fluid source of origin), but allowed an STR profile of the stain donor to be obtained as well. The method proved to be reproducible and sensitive, with as little as 0.05 μl saliva or semen, using different analysis strategies. Additionally, we demonstrated the ability to positively identify the presence of saliva and semen, as well as obtain high quality DNA profiles, from old and compromised casework samples. The results of this collaborative exercise involving an RNA/DNA co-extraction strategy support the potential use of an mRNA based system for the identification of saliva and semen in forensic casework that is compatible with current DNA analysis methodologies.

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1. Introduction

The analysis of cell-specific mRNA expression is a confirmative method for the identification of body fluids [1–24], as opposed to conventional immunological and enzymatic tests, most of which either lack specificity or cannot be used to identify all forensically relevant biological fluids. The suitability of mRNA profiling assays with forensic samples, such as old and environmentally compromised samples, has been demonstrated [25–28]. Previously, two collaborative exercises were performed by the European DNA Profiling Group (EDNAP – http://www.isfg.org/EDNAP) in order to evaluate the robustness and reproducibility of mRNA profiling for blood identification: (1) evaluation of three blood-specific markers (HBB, SPTB and PBGD) using singleplex reactions [29]; (2) evaluation of seven blood-specific markers using two multiplex systems, a 'high sensitivity' duplex (HBB, HBA) and a 'moderate sensitivity' pentaplex (ALAS2, CD3G, ANK1, PBGD and SPTB) [30]. Most laboratories, some of which had no prior experience with RNA, were able to successfully isolate and analyze RNA from the provided samples. While sensitivity varied between laboratories, the method proved to be reproducible and sensitive using different analysis strategies [29,30].

A third collaborative exercise was organized by the Institute of Legal Medicine, University of Zürich, Switzerland, on behalf of EDNAP in order to test forensically suitable saliva and semen markers. In a preliminary study performed by the Florida and Zürich laboratories, various saliva and semen mRNA markers described in the literature [3,6,8,9,13,15,18] as well as from unpublished data (Table 1) were evaluated in terms of sensitivity, specificity and performance with casework samples. In this study, 7 saliva and 11 semen markers were tested, of which 3 saliva and 5 semen markers were deemed most suitable for forensic use (Table 1). For this exercise, the following saliva and semen multiplexes were developed and provided to the participating laboratories: (1) a saliva triplex including the markers HTN3, STATH and MUC7 and (2) a semen pentaplex allowing the detection and differentiation of sperm (PRM1, PRM2) and seminal plasma (PSA, SEMG1 and TGM4), the latter of which is necessary for the identification of semen from azoospermic men. The exercise included two rounds of testing: In the original exercise (part 1), 5 laboratories encountered technical problems (reduced sensitivity, no result at all, contamination of negative controls and spectral pull-ups). As a consequence, a second exercise (part 2) was arranged in order identify the source of these problems, and potentially to obtain improved results. Each part of the exercise included the analysis of 10 mock casework samples, saliva and semen dilution series and, if available, optional extra casework samples from the participating laboratories. In addition to the provided samples analyzed by all laboratories, a set of human tissues and animal saliva samples were tested with the multiplexes by 4 laboratories to complement the specificity testing of the markers.

2. Materials and methods

2.1. Samples and materials provided

The organizing laboratory (Institute of Legal Medicine, University of Zürich, Switzerland) sent 2 sets of samples to the participating laboratories: part 1 included stains 1–10 (saliva, semen, non-saliva/semen and non-human stains) and dilution series A (5–0.01 μl semen) and B (5–0.01 μl saliva); part 2 included stains 11–20 (saliva, semen, non-saliva/semen and non-human stains) and dilution series C (10–0.05 μl saliva) and D (5–0.01 μl semen). Fresh saliva samples were collected from 10 individuals for 12 stains/dilution series (different donors were used for parts 1 and 2 except for one donor who was used in both parts) and were deposited onto different carrier materials including cotton swab/pads, recycling tissue, paper and glass slides. Additionally, quarter pieces of chewed chewing gums and licked plastic spoons were provided. Semen samples from 9 individuals were used to prepare 10 stains/dilution series (different donors were used for parts 1 and 2 except for one donor who was used in both parts). The semen, which had been frozen for up to 25 years, was deposited onto different carrier materials including cotton swab/pads, white textile, toilet paper and latex gloves. In addition, blood samples (2 donors), vaginal swabs (1 donor) and buccal swabs from a dog and a cat were used as non-saliva/semen and non-human stains. For the dilution series, saliva and semen samples were diluted in 0.9% NaCl to a final volume of 5 μl per sample and placed on swabs. The laboratories

Table 1
List of evaluated mRNA markers for the identification of saliva and semen.

RNA marker	Protein	Ref.	
Saliva			
HTN3	Histatin 3	6	good candidates
STATH	Statherin	6	
MUC7	Mucin 7	*	
PRB1-3	Proline-rich proteins	*	not sensitive
PRB4	Proline-rich protein	8	
SPRR2A	small proline-rich protein 2A	13	not specific
KRT13	Keratin 13	13	
Semen			
PRM1	Protamine 1	3,6	good candidates
PRM2	Protamine 2	3,6	
TGM4	Transglutaminase 4	18,*	
PSA/KLK3	Prostate Specific Antigen, Kallikrein 3	9,15	
SEMG1	Semenogelin 1	9,15	
SPANXB	SPANX family member B	*	inconstistent results during singleplex testing
HSFY	Heat Shock Transcription Factor, Y-Linked	*	
SPAM1-v2	Sperm Adhesion Molecule	*	
ZPBP	Zona Pellucida Binding Protein	*	
ODF1	Outer Dense Fiber of Sperm Tails 1	*	
BPY2/VCY2	Basic Charged, Y-linked, 2	*	

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