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Enhancing the sexual assault workflow: Development of a rapid male screening assay incorporating molecular non-microscopic sperm identification

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ABSTRACT

Sexual assault samples are among the most difficult sample types encountered by forensic laboratories. Typically, a sexual assault sample poses multiple challenges including small quantity of male DNA and a relatively high quantity of female DNA. Differential extraction procedures are time-consuming and labor-intensive, particularly with microscopic sperm identification. A rapid upfront screening or triage assay prior to use of differential extraction procedures could ensure that male DNA is present prior to the use of full DNA workflows. Previously reported rapid male screening assays do not provide a confirmation of whether sperm are present and therefore still require the use of microscopic sperm identification. This work was focused on an improved rapid male DNA screening assay with an upfront sperm identification using mRNA profiling.

The rapid male screening assay consists of a brief lysis using only a small tip portion of a swab, to obtain both RNA (eluted) and DNA (extraction 'waste') followed by a one-step reverse transcription-high resolution melt assay for PRM2, a sperm-specific mRNA. Without additional purification, the DNA 'waste' fraction can be quantitated and used to obtain an upfront Y-STR profile of the sperm donor. The assay takes 30 min for lysis followed by ~2 h for quantitation and sperm identification.

Here, we demonstrate the specificity of the assay to detect male DNA in semen samples with no cross reactivity with other body fluids and no PRM2 detection in vasectomized males. RNA and DNA profiling results were obtained with as little as ~0.15 µl of semen in vaginal-semen admixtures. In all samples tested, Y-STR profiling results were obtained when male DNA was indicated based on quantitation results.

1. Introduction

A determination of the origin of a stain can aid investigators in better understanding the circumstances surrounding a crime. In cases such as sexual assaults, it is important to not only identify those involved but also to confirm the presence of male specific body fluids such as semen and even more specifically semen. It is typical for a preliminary screening process to be performed in order to identify the presence of these fluids. These techniques can be time consuming especially the microscope examinations needed to identify the presence of sperm. In low- or few-sperm containing samples this can take significant amounts of time and it is possible that a single sperm may not be observable. Therefore, there is a need for the development of a rapid upfront male DNA screening method to triage sexual assault evidence [1]. In order for this assay to be of even greater use, the integration of an additional upfront definitive identification of the presence of sperm would be desirable. Here, we describe the development of a rapid RNA extraction method from a swab portion that also permits recovery of gDNA from the waste fractions of this extraction. The RNA can then used in a rapid one-step reverse transcription-mRNA profiling high resolution melt assay for the identification of sperm (PRM2). The DNA can then be diluted, quantitated for support the presence of male DNA and then also obtain an upfront Y-STR profile. This is all accomplished using only a small swab-tip portion of the sample. The remaining sample can then be used for a standard differential extraction for routine DNA analysis. Successful results have been obtained from as little as ~0.15 µl of semen in vaginal-semen admixtures

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2. Materials and methods

2.1. Body fluid samples

Body fluid samples were collected from volunteers using procedures approved by the University of Central Florida's Institutional Review board. Blood, semen, saliva, vaginal secretions and menstrual blood samples were collected as previously described [2].

2.2. RNA extraction

Body fluid samples were extracted using the Dynabeads™ mRNA Direct™ Purification kit (Invitorgen™ by ThermoFisher Scientific) according to the manufacturers recommended protocol. The supernatant removed after the initial lysis and bead pelleting was saved for further DNA testing. RNA was eluted in 10 µl of 10 mM Tris−HCl (elution buffer) was washed across the beads. All RNA extracts were DNase-treated using the TURBO™ DNase kit (Invitrogen™).

2.3. One-step RT-HRM analysis for sperm identification

RNA samples were screened for the presence of sperm using the Power SYBR® Green RNA-to-C_T™ 1-Step Kit (Applied Biosystems by ThermoFisher Scientific). An 18 µl reaction mix containing RT PCR mix, unlabeled PRM2 primers, RT enzyme mix and RNase-free water was prepared per sample. Two microliters of DNased mRNA extract was added for a final reaction volume of 20 µl. The assay was run on a 7500 Real Time PCR instrument (Applied Biosystems): 48 °C 15 min; 95 °C 10 min; 40 cycles of 95 °C 15 s, 60 °C 30 s; melt analysis using the software default conditions (95 °C 15 s, 60 °C 15 s, 95 °C 15 s). The presence of PRM2 was determined by the presence of a melt peak (Tm) at ~82 °C.

2.4. DNA quantitation, Y-STR amplification and detection

All DNA extracts were diluted 1:25 and then quantitated using the Quantifiler™ Trio DNA Quantification kit (Applied Biosystems) according to the manufacturer's protocol. If male DNA was present, 0.5 ng of input male DNA was amplified using the Yfiler™ Plus PCR Amplification Kit (Applied Biosystems) using the suggested manufacturer's protocol. Amplified products (1µl) were detected using capillary electrophoresis (3130 Genetic Analyzer, Data Collection Software v3.0, GeneScan™ 600 LIZ™ size standard v2.0, POP-4™; J6; 5 s inj, 3 kV, 60 °C, 1500sec run time) and analysed using GeneMapper ID-X software (applied Biosystems).

3. Results and discussion

3.1. Single source body fluid samples

Fifteen single source semen samples were tested. Sperm (PRM2) was detected in all 15 samples. One semen sample from a vasectomized male was tested and was negative for the presence of sperm as would be expected. Additional non-semen single source body fluid samples (vaginal N = 8, Menstrual blood N = 4, Saliva N = 4 and Blood N = 4) were also tested and were negative for the presence of sperm.

Male DNA was detected in all 15 single source semen samples with concentrations ranging from 0.03 to 1 ng/µl. Y-STR amplifications were performed using 0.15 – 0.5 ng of male DNA. Full Y-STR profiles were obtained for 14 of the 15 samples, with only one allele drop-out (26/27 alleles recovered) for the remaining sample.

3.2. Vaginal-semen admixtures

Six vaginal-semen admixtures were prepared using ~1/4 vaginal swab and adding liquid semen in 10, 5, 2 and 1 µl quantities. The samples were dried overnight and tested using the above described methods. Sperm was successfully detected in a majority of the admixed samples: 6/6 (10 µl semen), 6/6 (5 µl semen), 5/6 (2 µl semen) and 4/6 (1 µl semen). As an example from one of the donor sets, PRM2 and male DNA were detected in all four samples. For the 5 and 10 µl semen samples, 300 pg of male DNA was amplified and full Y-STR profiles were obtained. For the 1 and 2 µl semen samples, only 70 pg of male DNA was available for amplification and still 23 and 18 alleles, respectively, were obtained from these samples.

Since successful results were obtained from as little as 1 µl of semen in vaginal-semen admixtures, serial dilutions of semen were made resulting in ~1.25 µl down to 0.16 µl quantities and added to ¼ vaginal samples (two donor sets). PRM2 was successfully detected in as little as 0.31 µl for one donor set and to 0.16 µl for the other sample.

The results of the admixed sample testing demonstrate the potential usefulness of the developed approach as a successful triage of sexual assault evidence. This assay permits an identification of male DNA, a non-microscopic definitive identification of sperm and the recovery of upfront Y-STR profiles. Current work is being performed to further improve assay speed.

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Declaration of Competing Interest

None.

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