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Research paper

Evaluating the efficacy of DNA differential extraction methods for sexual assault evidence

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ABSTRACT

Analysis of sexual assault evidence, often a mixture of spermatozoa and victim epithelial cells, represents a significant portion of a forensic DNA laboratory's case load. Successful genotyping of sperm DNA from these mixed cell samples, particularly with low amounts of sperm, depends on maximizing sperm DNA recovery and minimizing non-sperm DNA carryover. For evaluating the efficacy of the differential extraction, we present a method which uses a Separation Potential Ratio *(SPRED)* to consider both sperm DNA recovery and non-sperm DNA removal as variables for determining separation efficiency. In addition, we describe how the ratio of male-to-female DNA in the sperm fraction may be estimated by using the *SPRED* of the differential extraction method in conjunction with the estimated ratio of male-to-female DNA initially present on the mixed swab. This approach may be useful for evaluating or modifying differential extraction methods, as we demonstrate by comparing experimental results obtained from the traditional differential extraction and the Erase Sperm Isolation Kit (PTC©) procedures.

1. Introduction

The standard DNA differential extraction for sexual assault evidence employs a two-step process: an initial lysis of non-sperm cells and removal of their DNA (non-sperm fraction), followed by the lysis and extraction of DNA from sperm cells (sperm fraction). The success of the procedure depends on the efficiency of sperm DNA recovery as well as the efficiency of removal of non-sperm DNA, with an ideal procedure resulting in a sperm fraction extract containing very little, or no, non-sperm DNA. Sperm fraction extracts containing non-sperm DNA may result in mixed DNA typing profiles which can complicate interpretation. Typical sexual assault vaginal swab samples contain large amounts of female DNA which can be far in excess of the sperm DNA. Currently employed extraction methods vary in their efficiency of sperm DNA recovery and non-sperm DNA removal from the sperm fraction [1,2].

The standard two-step lysis differential extraction procedure utilized by many forensic practitioners was first described in 1985 [3]. The primary typing method employed at that time, Restriction Fragment Length Polymorphism (RFLP), required approximately 5 μg of DNA for successful typing [4]. Current typing methods operate at a sensitivity level of about 100 pg of DNA, thus swab samples containing a vast excess of female to male DNA can potentially provide useful information, providing the male component can be successfully isolated from the female component. This increased sensitivity has created a need for improved separation capability when limited sperm DNA is present.

Attempts at improving the efficiency of the differential extraction have often focused on maximizing sperm cell DNA recovery [5–9], which is understandable given this is the component of interest and the ultimate target of the procedure. Nevertheless, the sensitivity of detection and ultimate success of the genotyping depend greatly on the efficient removal of the non-sperm DNA to reduce carryover into the sperm DNA fraction. This critical step has received some attention [1,10–15], but there is still a primary focus on sperm DNA recovery, and we are unaware of any quantitative studies that address the impact of increasing or decreasing the relative efficiency of either. Moreover, laboratories may make an assumption that non-sperm DNA removal has been optimized for a specific protocol or that any method to facilitate greater efficiency of removal may likely result in collateral sperm DNA loss.

Our goal here is to present a semi-quantitative approach for evaluating the relative impact of both variables, the efficiency of

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sperm recovery and the efficiency of non-sperm DNA removal, on the capability of a differential extraction method to achieve a successful genotyping result of the sperm DNA component. This approach uses a straightforward calculation, a *Separation Potential Ratio for the Extraction Differential* ("*SPRED*"), to weigh both variables to assess the efficacy of a differential extraction method. As we will discuss, the *SPRED* ratio is especially useful for comparing various methods in order to provide insights into their relative strengths and limitations, especially for low-sperm or high epithelial cell samples. Our discussion will focus on comparing three differential extraction procedures to evaluate their relative efficacies for genotyping the sperm DNA.

2. Materials and methods

2.1. Samples

Mock sexual assault swabs were prepared by adding diluted semen to semen-free vaginal or buccal swabs from female contributors. Volunteers donated tissue with informed consent.

2.1.1. 0.2 μL semen on vaginal swabs

Vaginal swabs were collected simultaneously in groups of four from a single donor and divided so as to keep the epithelial-cell content roughly equivalent for each of the differential extraction methods that were examined for this study. A total of 36 vaginal swabs were collected. Each method received equal numbers of swabs sampled first, second, etc. No more than three groupings of vaginal swab collections were performed on the same day. Previously frozen neat semen was diluted in phosphate buffered saline (PBS) to a concentration of 0.004 μL neat semen/μL from which 50 μL (the equivalent of 0.2 μL of semen) was immediately applied to each swab. Swabs were air-dried overnight or longer (up to 2 weeks) at room temperature before processing.

2.1.2. 0.1 μL semen on vaginal swabs

Duplicate vaginal swabs were collected simultaneously and split across two methods for comparison. Previously frozen neat semen was diluted in PBS to a concentration of 0.002 μL neat semen/μL from which 50 μL (the equivalent of 0.1 μL of semen) was immediately applied to each swab. Swabs were air-dried overnight before processing.

2.1.3. 1.0 μL semen on buccal swabs

Two buccal swabs were collected individually from the same donor. Previously frozen neat semen was diluted in PBS to a concentration of 0.02 μL neat semen/μL from which 50 μL (the equivalent of 1.0 μL of semen) was immediately applied to each swab. Swabs were air-dried overnight and then stored at −20 °C for 1 month before processing.

2.2. Differential extraction (DE)

Separation of non-sperm DNA and sperm DNA was performed using two differential extraction (DE) approaches. Side-by-side experiments using whole swabs were performed with two versions of the standard DTT-based differential-lysis extraction procedure and with Paternity Testing Corporation's (PTC©, Columbia, Missouri) Erase Sperm Isolation Kit, hereafter referred to as Erase. After the separation steps, purification of all samples was performed manually with the PrepFiler® Forensic DNA Extraction Kit (Applied Biosystems™, Foster City, California) using 5 μl or 3 μl of 1 M DTT for the sperm or non-sperm fractions, respectively, according to the manufacturer's instructions. Final extract volumes were 50 μL.

2.2.1. Erase Sperm Isolation Kit (Paternity Testing Corporation©)

The Erase procedure uses selective degradation with a DNase to remove the residual female DNA in the sperm fraction in place of the repeated washing and re-pelleting steps employed by the standard approach [1,14,15]. Samples were processed according to the manufacturer's instructions. Swabs were incubated in a 400 μL solution of the proprietary Erase extraction buffer and proteinase K for 1 h at 56 °C in a Labnet 1550 VorTemp™ Shaking Incubator at 900 rpm. Following incubation, swabs were placed in spin baskets supplied with the Erase Kit, centrifuged at 13,300 rpm for 5 min after which the swabs and spin baskets were discarded. The supernatant (non-sperm fraction) was removed leaving behind ~50 μL of sperm fraction. Solutions 1 (salts) and 2 (nuclease) were added to the sperm fraction volume and mixed by pipetting up and down repeatedly. The ~70 μL was then transferred to a new tube and incubated for 15 min at 37 °C. Following incubation, 10 μL of solution 3 (EDTA, DTT) was added and incubated for 15 min at 56 °C.

2.2.2. Standard differential

Swabs were placed in a solution of 400 μL of extraction buffer (10 mM Tris; 10 mM EDTA; 100 mM NaCl; 2% SDS) and 10 μL ProK (20 mg/mL) and incubated for 1 h at 56 °C in a Labnet 1550 VorTemp™ Shaking Incubator at 900 rpm. Following incubation, swabs were placed in Spin-X® spin baskets (Product # 9301, Corning® Life Sciences, Tewksbury, Massachusetts), centrifuged at 13,300 rpm for 5 min after which the swabs and spin baskets were discarded. Sample tubes were then placed in a QIAcube Differential Wash Station (QIAGEN, Hilden, Germany) where the non-sperm fraction was removed and four sequential 750 μL PBS washes of the sperm fraction were performed robotically using a custom protocol leaving ~30 μL volume after each wash.

2.2.3. Standard differential with two initial lysis steps

The first initial lysis was performed exactly as described above, discarding the swab and basket. The non-sperm fraction was then manually removed, leaving ~30 μL of sperm fraction to which a fresh 400 μL of extraction buffer (10 mM Tris; 10 mM EDTA; 100 mM NaCl; 2% SDS) and 10 μL ProK (20 mg/mL) were added. These samples were then incubated again for 1 h at 56 °C in a Labnet 1550 VorTemp™ Shaking Incubator at 900 rpm. To control for the additional dilution ("wash") step that occurred for these samples, additional swabs were treated in exactly the same way, except in place of SEB/ProK, 410 μL of PBS was added. These samples remained on the benchtop at room temperature until the second initial lysis step was completed. The samples were then placed in the QIAcube Differential Wash Station and processed robotically as described above.

2.3. Quantification and STR typing

Quantification was performed using an in-house developed qPCR quadruplex assay (nuTH01-nuSRY-nuCSF-IPC) [16] with the Applied Biosystems™ 7500 Real Time PCR system. Total human DNA for the STR amplification was determined using the autosomal (nuTH01) target quantification values.

STR amplification was performed with 1 ng template (or less if <1 ng was available) in a 25 μL reaction volume using the AmpF*l*STR® Identifiler® Plus PCR Amplification Kit (Applied Biosystems™) for 28 cycles on the GeneAmp 9700 PCR thermal cycler (Applied Biosystems™). In preparation for injection and electrophoresis, 1 μL of PCR product was added to 8.5 μL of formamide and 0.5 μL of GeneScan™ 600 LIZ® dye Size Standard v2.0 (Applied Biosystems™). Products were separated and detected on the Applied Biosystems® 3500 Genetic Analyzer using the manufacturer's recommended run conditions. HID files