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Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence

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ABSTRACT

We present a rapid alkaline lysis procedure for the extraction of DNA from sexual assault evidence that generates purified sperm fraction extracts that yield STR typing results similar to those obtained from the traditional organic/dithiothreitol differential extraction. Specifically, a sodium hydroxide based differential extraction method has been developed in a single-tube format and further optimized in a 96-well format. The method yields purified extracts from a small sample set (~2–6 swabs) in approximately 2 h and from a larger sample set (up to 96 swabs) in approximately 4 h. While conventional differential extraction methods require vigorous sample manipulation to remove the spermatozoa from the substrate, the method described here exploits the propensity of sperm to adhere to a substrate and does not require any manipulation of the substrate after it is sampled. For swabs, sample handling is minimized by employing a process where the tip of the swab, including the shaft, is transferred to the appropriate vessel eliminating the need for potentially hazardous scalpels to separate the swab material from the shaft. The absence of multiple handling steps allows the process to be semi-automated, however the procedure as described here does not require use of a robotic system. This method may provide forensic laboratories a cost-effective tool for the eradication of backlogs of sexual assault evidence, and more timely service to their client agencies. In addition, we have demonstrated that a modification of the procedure can be used to retrieve residual sperm-cell DNA from previously extracted swabs.

1. Introduction

Escalating demands on forensic laboratories coupled with laborious methods used in the analysis of sexual assault evidence have contributed to an increasing backlog of evidence from sexual assault cases. As a result, the ability to examine sexual assault evidence immediately after the event is a capability few laboratories have. This is especially true for suspectless sexual assaults, despite the ability to search national databases like CODIS. The need to increase throughput in DNA typing laboratories has generated a great deal of interest in alterative DNA extraction procedures, including alkaline lysis procedures with blood, semen, saliva [1,2], fingernails [3], head hair [4] and formalin fixed paraffin embedded tissue [5]. The benefits of an alkaline lysis extraction method include a simple procedure with decreased extraction time and minimal reagent costs. This would seem to make alkaline lysis a perfect tool for decreasing the backlog of evidence collected in sexual assault cases [6]. However, the more complex nature of sexual assault evidence, that typically includes mixtures of spermatozoa and female epithelial cells, prevents a simple, single-step alkaline lysis procedure from being used with these types of samples. Traditionally, the separation of sperm cell DNA from non-sperm cell DNA has been achieved by a differential extraction method [7], in which non-sperm cells are first lysed with a sodium dodecyl sulfate (SDS)/proteinase K solution. The intact sperm cells are separated from the non-sperm cell lysate using a series of centrifugation and wash steps, after which the sperm cell portion is lysed in a SDS/proteinase K/DTT solution. In the last step of the differential extraction procedure, the non-sperm-cell and sperm-cell lysates are extracted independently to achieve final separated fractions. While the efficiency of this last step in the differential extraction procedure has improved due to the use of automated DNA extraction methods, the initial steps required to separate the non-sperm and sperm cell fractions have been less amenable to automation and typically rely on manual processes that are labor and time intensive [8–12]. Furthermore, the traditional method is dependent on the removal of the spermatozoa from the substrate prior to their lysis, which has been estimated to yield 10–40% recoveries of the spermatozoa from the substrates [13,14]. Alternative methods of generating profiles from sperm cell DNA contained in sexual assault evidence include modified differential extraction methods [15–21] and laser

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microdissection [22], but each is dependent on the removal of the sperm cells from the substrate prior to sperm cell lysis.

In this article, we report the development of an alkaline differential extraction method that rapidly yields purified sperm fraction lysates with minimal sample handling. Non-sperm lysates are generated by heating the substrate in a mild alkaline solution (0.1 N NaOH), followed by neutralization and enzymatic digestion steps to remove residual non-sperm DNA from the substrate. Sperm fraction lysates are subsequently generated by heating the substrate in 1 N NaOH, which are then concentrated and purified using silica columns. This method was developed using mock sexual assault evidence swabs that were prepared by transferring semen onto buccal swabs collected from a female contributor, and has been successfully used with authentic post-coital swabs that have been stored frozen as indicated by the California Medical Protocol for Examination of Sexual Assault and Child Abuse Victims [23]. Additionally, swabs that have previously been extracted using a standard differential extraction method have been successfully re-extracted with this alkaline lysis procedure.

2. Materials and methods

2.1. Samples

Post-coital swabs, semen and saliva samples were donated by laboratory staff. Pre-quantified, high molecular weight, human genomic DNA was obtained from Promega, Madison, WI (Male-#G1471), Applied Biosystems, Foster City, CA (TaqMan® Control DNA) and ATCC, Manassas, VA (HL60).

2.2. Preparation and sampling of mock sexual assault evidence swabs

Simulated sexual assault evidence (SAE) swabs were prepared by transferring semen onto buccal swabs collected from female contributors using sterile cotton swabs (Puritan Medical Products, Guilford, ME), which were allowed to air-dry at room temperature for a period of 2–4 h prior to storing frozen at −20 °C [23]. All SAE swabs were sampled by cutting the tip off with dissecting scissors or breaking the tip off with a clean, gloved hand and/or a Kimwipe to yield a section of the swab tip and shaft that was less than or equal to ~1.5 cm in length.

2.3. Alkaline lysis

All alkaline lysis steps were conducted in dry bath incubators with NaOH solutions prepared by dissolving NaOH pellets (Sigma, St. Louis, MO) in DEPC treated molecular biology grade water (Rockland Immunochemicals, Gilbertsville, PA) or sterile water. Single-tube (1.5 mL microcentrifuge tube (Dynalab, Rochester, NY)), lysis steps were carried out in VWR Heat Block 1 incubators (VWR, West Chester, PA) with 10 mm heat block inserts as indicated in Fig. 1a. 96-Well lysis steps were conducted in Boekel Digital Dry Bath Incubators (Boekel Scientific, Festerville, PA) fitted with heat block inserts (Promega) and custom, VP 741 I, 96-well plate heating block inserts (V&P Scientific, San Diego, CA) using color coded Abgene AB-0932, 2.2 mL, 96-well plates (Thermo-Fisher Scientific, Pittsburgh, PA) for each step as indicated in Fig. 1a.

2.4. DNase digestion

DNase digestion was completed with DNase I (Invitrogen, Carlsbad, CA) in a 1× DNase I Reaction Buffer (20 mM Tris–HCl (pH 8), 2 mM $MgCl_2$, 50 mM KCl) as indicated by the manufacturer.

Specifically, each reaction was carried out at 37 °C in dry bath incubators for 1–1 h in a solution consisting of 0.1 U/μL DNase I and 10× DNase I Reaction Buffer diluted in DEPC-treated molecular biology grade water to bring the solution to a 1× reaction buffer concentration.

2.5. Concentration and clean-up of lysates

Sperm fraction lysates were concentrated and cleaned up by microdialysis filtration with Centricon® YM-100 devices (Millipore, Billerica, MA) or by silica-binding with NucleoSpin XS columns or NucleoSpin-96 tissue plates (Macherey-Nagel, Bethleham, PA), which are referred to as "C100", "NSXS" and "NS96T", respectively, in Tables 2 and 3. Centricon® YM-100 devices were used following neutralization with 2 M Tris base (pH 7.5) and dilution in TE^{-4} buffer (10 mM Tris, pH 7.5; 0.1 mM EDTA) with repeated TE^{-4} buffer rinses, as indicated in Table 2. NucleoSpin XS columns/NucleoSpin-96 tissue plates were loaded after neutralization with glacial acetic acid, dilution of the lysates in TE^{-4} buffer in 2 mL microcentrifuge tubes/4 mL 96-well pyramid bottom plates (E&K Scientific, Santa Clara, CA) and the addition of NT or NTC binding buffer (provided by Macherey-Nagel). The silica columns/96-well plates were washed with ethanol based B5 buffer (provided by Macherey-Nagel) with the waste collected in 2 mL microcentrifuge tubes/1.1 mL, 96 deep well plates (E&K Scientific). The DNA was eluted with TE^{-4}, Tris, BE elution buffer (provided by Macherey-Nagel), or sterile H_2O into capless 1.5 mL microcentrifuge tubes/1/2 skirt PCR plates (E&K Scientific) and heated in 90 °C dry bath incubators to drive off residual ethanol and further concentrate the samples, as indicated in Tables 2 and 3 and indicated in Fig. 1b.

Non-sperm fraction lysates generated in these studies did not require concentration. However, these samples have been successfully concentrated and purified using microdialysis devices (as indicated above) and silica-binding devices (as described in Fig. 1c), for samples containing few non-sperm cells or where premature lysis of the spermatozoa occurred. Additionally, the NucleoFast 96-well microdialysis plates (Macherey-Nagel) have been successfully employed with neutralized non-sperm fraction lysates and repeated TE^{-4} buffer rinses.

2.6. Standard procedure for differential extraction of mock sexual assault evidence swabs

Standard differential extractions of sperm and non-sperm fractions were performed on swab substrates that had been excised from the shaft with sterile scalpels using a dithiothreitol-based digestion protocol (DTT) [7] with Centricon® YM-100 concentration of the aqueous layer from the phenol/chloroform extraction.

2.7. DNA quantification

Extracts were quantified with a previously in-house developed quadruplex qPCR assay (nuTH01–nuSRY–nuCSF–IPC) on an Applied Biosystems 7500 Real Time PCR system (7500 SDS software v 1.3) [24].

2.8. STR genotyping

The AmpF*l*STR® Identifiler™ PCR Amplification kit (Applied Biosystems) was used for STR genotyping. Unless otherwise noted and whenever possible, 1 ng template quantities of autosomal DNA were targeted in the 25 μL PCRs that were performed according to vendor instructions on a GeneAmp® 9700 PCR thermal cycler (Applied Biosystems). STRs were resolved and detected on an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems) according to vendor instructions. Electrophoresis data were analyzed using