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| **Author(s):** | **Kevin S. Sweder, Ph.D., Michael A Marciano, M.S** |
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# An Automated Dielectrophoretic-Based Single Cell Separation Technique to Improve Laboratory Efficiency, Mixture Deconvolution and Combat Sample Inhibition

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Awardee Address:
113 Bowne Hall
Syracuse University
Syracuse, NY 13244-1200

**DUNS:**
**Fed EIN:**

***Co-PI and Technical Point of Contact:***
Michael A Marciano, M.S.
Office: 315-443-5279
Fax: 315-443-4040
Email: mamarcia@syr.edu

**Signature** 

***PI***
Kevin S. Sweder, Ph.D.
Office: 315-443-3396
Email: kssweder@syr.edu

**Signature** 

***Submitting Official and Administrative Point of Contact:***
*Amy Graves*
*Office: 315-443-9360*
*Fax:315-443-9361*
ajgraves@syr.edu

The use of the DEPArray™ and the companion Menarini Silicon Biosystems cell lysis DNA extraction kit will impart improvements on the currently used includes improvements in sensitivity and specificity while positively affecting laboratory efficiency. In addition to its application in sexual assault cases, the process of enrichment, capture and purification, will permit single cells (or few cells) to be captured and recovered, enabling the processing of low template samples without the need for quantitation. Furthermore, we expect the dielectrophoretic cages that trap the individual cells will help purge the sample substrate and residual inhibitors from the cells and allow a cleaner extraction, thus leading to less inhibition in downstream analyses.

The DEPArray not only avoids the time and expense of qPCR but also provides DNA quantities that are more accurate than any other method available today. Standard 9.4 of the FBI-QAS states, "The laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification." (Quality Assurance Standards for Forensic DNA Testing Laboratories Effective September 1, 2011). The method of quantification is not specified, therefore, simply identifying the number of cells using the DEPArray™ does constitutes as DNA quantitation. This quantitation calculation becomes exceedingly simple; knowing that human (haploid) spermatozoa contain 3.3pg of DNA and human diploid cell nuclei contain 6.6pg of DNA, the cells and or cell nuclei can be simply counted. [1]

This study involved over 100 DEPArray™ runs, including those focused on training, optimization and experimental samples described in this paper. The preparation of samples for the DEPArray™ v2.0 run were batched in groups of four and total hands-on preparation time is 1.25 h. The instrument is capable of running a single sample in a given instrument run; each run and subsequent analyses required approximately 1-1.5 h of hands-on time.

Table 1: The time required for DEPArray™ v2.0 sample processing and data interpretation.

| Step | Hands-on time | Total time |
|---|---|---|
| Cell-substrate release | 45 m / 4 sample | 2-24 h |
| Cell staining/ fixation | 45 m / 4 sample | 2.5 h |
| Instrument prep | 30 m / sample | 30 m / sample |
| Routing / recovery | 30 min / sample | 3 h / sample |

Note, the touch DNA study was executed as proposed in the original submission however, the resulting analyses of the data revealed that the optimization of this protocol required additional attention. The current data indicate that the method does not improve "touch" DNA analyses. We believe that the DEPArray™ will be a useful and potentially disruptive tool in touch DNA analyses however, the approach must be further modified. Due to the additional time required to perform this optimization, our efforts were concentrated on completing the sexual assault sample studies.

Samples

Three sample sets were used in this study:

(1) Internally generated mock samples consisting of dilutions of epithelial cells (saliva) with sperm-positive semen, and dilutions of epithelial cells (saliva), whole blood and sperm-positive semen. Samples were created using six varying dilutions of neat semen to buccal epithelial cells (1:1, 1:5, 1:10, 1:100, 1:1000, 1:10,000) and three dilutions of neat semen to whole blood to buccal epithelial cells (1:1:1, 1:1:10, 1:1:100). The diluted samples were dispensed directly onto Dacron swabs (Fitzco) and cotton underwear cuttings. Each dilution was made in duplicate using semen samples from two contributors (T4333 and T3806). Two replicates of each dilution (using both semen samples) was prepared for analysis on the DEPArray™ and the differential extraction pipeline (Table1S).

Table 1S: Dilution calculations used for internal mock sample set**. A.** Neat semen and PBS volumes used for Dacron swab substrates. **B.** Neat semen and Epithelial cell volumes used for cotton underwear substrates. **C.** Neat semen, whole blood, and epithelial cell volumes used for swab and cotton underwear substrates. All dilutions were pipetted directly onto substrates. Dilution set **A.** was added to buccal swabs.

**A.**

| Dilution | Neat Semen (µL) | PBS (µL) |
|---|---|---|
| 1:1 | 25 | 25 |
| 1:5 | 8 | 40 |
| 1:10 | 32 | 288 |
| 1:100 | 8 of 1:10 | 72 |
| 1:1,000 | 8 of 1:100 | 72 |
| 1:10,000 | 8 of 1:1000 | 72 |

**B**.

| Dilution | Neat Semen (µL) | Epithelial Cells (µL) |
|---|---|---|
| 1:1 | 25 | 25 |
| 1:5 | 8 | 40 |
| 1:10 | 32 | 288 |
| 1:100 | 8 of 1:10 | 72 |
| 1:1,000 | 8 of 1:100 | 72 |
| 1:10,000 | 8 of 1:1000 | 72 |

**C.**

| Dilution | Neat Semen (µL) | Whole Blood (µL) | Epithelial Cells (µL) |
|---|---|---|---|
| 1:1:1 | 25 | 25 | 25 |
| 1:1:10 | 5 | 5 | 50 |
| 1:1:100 | 1 | 1 | 100 |

The semen/saliva/blood dilutions that were produced in this study were made using three different contributors. Casework scenarios involving these 3 fluids would likely consist of two contributors however three were used in this sub-study permitting a quantitative analysis of the performance of the DEPArray™ and differential extraction-mediated separation of sperm cells from both epithelial and white blood cells.

*(2) Proficiency test samples*. Four single source semen samples on cotton swabs single obtained from the Onondaga County Center for Forensic Sciences.



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*(3)* Post-coital samples. The post-coital samples consisted of vaginal swabs collected in duplicate at 12, 24, 48, 72 and 96 hours post-coitus. Samples were collected from volunteers and in accordance Institutional Review Board guidelines.

All samples were processed using Silicon Biosystems kits *(1)* Sample preparation - DEPArray™ Forensic Sample Prep Kit (contains all cell staining reagents), *(2)* Instrument preparation - DEPArray Manipulation Buffer (SB115), DEPArray™ A300K DS V2.0 Cartridge (REF 300K25) and *(3)* DNA extraction kit - SBLysePrep™ Kit (REF SBLYS).

The DEPArray™ sample preparation and instrument run procedures consisted of four distinct steps: (1) Cell-substrate release - samples (swabs or cuttings) were incubated on a thermomixer for 2-24 hours to release cells from the substrate. (2) Cell staining and fixation - cells are concentrated via centrifugation and stained using stain-antibody conjugates specific for epithelial cells (Fluorescein - FITC channel), sperm cells (Allophycocyanin - APC channel), white blood cells (Phycoerythrin-PE channel), and all nuclei were stained using dapi (4',6-diamidino-2-phenylindole). (3) Instrument preparation – A sample is individually washed and added to a DEPArray cartridge. This procedure currently requires Eppendorf Research plus 20-200µL and 100-1000µL pipettes. (4) Routing and recovery – target cells are identified using the Cell Browser (software version 2.1.0) and are selected for recovery into tubes specified by the user using the Recovery Manager software.

Largely, the manufacturer recommended protocol was followed; some changes were made to further optimize the procedure to better address a forensic workflow and to enrich for the presence of sperm rather than white blood cells or epithelial cells. These changes included a reduction of the initial cell-substrate release volumes from an initial volume of 8mL of running buffer in a 15mL polypropylene conical tube to 750µL running buffer in a 1.5mL microcentrifuge tube. Cell-substrate release incubation periods were increased from 30 minutes to 2 - 24h depending on sample type and thermomixer speeds were increased from 300 rpm to 500 rpm. Specifically, proficiency test samples were incubated for two hours and mock and post-coital samples were incubated overnight (12-24 hours). The post-coital samples were incubated a second time for an additional two hours and the incubation speed was increased to 800 rpm. Post incubation centrifugation times and speeds were changed from 30m at 200 x g to 10m 1000 x g. In addition, following cell-substrate release and prior to cell staining, a 10µM size exclusion filter (Sysmex CellTrics®) was used to enrich the presence of sperm (heads 4-5 µm x 2.5-3.5µm)[2] - through decreasing the amount of epithelial cells (28-108 µm) [ 3]. This additional filter step included three washes to avoid cell loss. Furthermore, prior to cartridge loading, if a noticeable epithelial cell pellet was present, the mock and post-coital samples were filtered again using the manipulation buffer.

The mock and post-coital samples were incubated for 1.5 hours in a digest buffer (pH 7.5, 1M Tris-HCl pH 8, 0.5M EDTA, 5M NaCl, 10ml 20% SDS, 86 mL sterile deionized water). Preprocessing of samples, included for one hour incubation at 56ºC of the swabs or cuttings, suspended in a mixture of digest buffer and 10mg/ml Proteinase K. After incubation and removal of swabs/cuttings, samples were suspended and washed three times with digest buffer and one final wash with nuclease free water. Epithelial and Sperm fractions were then processed using the DNA IQ™ Casework Pro Kit and Maxwell® 16 (Promega). Sperm fractions were suspended in 18mg/mL Proteinase K, 1-Thiogylcerol and Casework Extraction Buffer and incubated a second time for 30 minutes at 56ºC. Prior to automated extraction; a lysis buffer was added to

each sperm and epithelial cell fraction. Following the extraction, samples were concentrated using DNA Fast Flow Microcon centrifugation filters (Millipore). The concentrated samples were subsequently quantitated using Plexor HY Human and Male DNA Quantification (Promega) and Roche's Light Cycler 480II using the manufacturer's recommended procedure (Promega TM302).

All samples were amplified on the Life Technologies Veriti® Thermal Cycler using Promega Powerplex Fusion 6c human DNA amplification kit (Promega TMD045); half-volume reactions were used, with all other manufacturers' recommendations were followed (29 cycles). Fragment analyses were carried out using a ThermoFisher Scientific 3500xL Genetic Analyzer (POP-4 and 36cm capillary array); subsequent software analyses were performed using GeneMarker HID v 2.8.2 (SoftGenetics, LLC).

Single sperm recoveries were analyzed differently than two or three sperm cell recoveries due to the expectation of haploid profiles. The average peak heights for single sperm recoveries were calculated by averaging the peak heights of all peaks present without factoring in allelic or locus dropout. The average peak heights for two and three sperm cells were calculated by taking the mean height of all peaks present. If a locus was heterozygous the mean peak height of the sister alleles was calculated and included in the sample average peak height. Potentially homozygous alleles were included in the average without dividing by two as is customary. This is because it is unclear whether both homozygous alleles were present or there was dropout of one of the two alleles. Note, peaks in stutter position but above the recommended stutter threshold had the peak heights corrected by removing the average expected stutter [4]. Allele dropout was not calculated in the average peak height calculations because it is unclear if a missing allele was dropout or if the allele was not present in haploid type. If we suspected aneuploidy was present, these loci were not included in the average peak height calculation but were included in the total allele count per sample. Alleles in stutter position that were expected in the profile and were above the stutter threshold were calculated as aneuploidy alleles. The average peak height calculations provide an indication of the peak heights that can be expected at any locus where signal is present, lacking dropout. The observed and expected allele counts complement the average peak height calculations, providing a quantitative metric for the level of allele and locus dropout present in a sample.

The post-coital and mock dilution samples for both the DEPArray™ and differential extraction methods were analyzed in the same manner. The peak height associated with the average reverse stutter percentage was removed from all alleles in stutter position. The mean sample peak heights for single source samples were calculated in a standard manner, sister allele peak heights at heterozygous loci were averaged and homozygous alleles were divided by two. If allelic dropout was observed a peak height of zero rfu was included in the average. However, locus dropout was removed from the average calculation. The average peak heights calculated per contributor in mixture samples were calculated in the same manner as above however only using the expected, unshared alleles present. If the contributors shared a heterozygous allele, only the unshared heterozygous allele was calculated in the sample average. For major contributor total allele count, all the present expected alleles were counted. The minor total allele count was calculated by the presence of unshared, unique alleles specific to the minor contributor. If a sample had less than a 2 to 1 ratio of contributors each unique expected allele was counted as well as all expected present alleles, including the shared alleles. If aneuploidy

was present, specifically at Y-STR alleles, these alleles were included in the sample total allele count but the loci were not calculated in the sample average.

## Results

*Sensitivity*

The sensitivity of the DEParray™ was evaluated using groups of one, two and three cell recoveries, consisting of 19 single sperm cell recoveries, 17 two sperm cell recoveries and 14 three sperm cell recoveries. The average proportion of alleles present in each of the one, two and three sperm cell recoveries was 0.296 ± 0.188, 0.302 ± 0.310 and 0.661 ± 0.131, respectively (Figure 1). As expected, there is an inverse relationship between the number of sperm cells and the occurrence of locus dropout. Average locus dropout across the one, two and three sperm cell recoveries was 0.456 ± 0.299, 0.410 ± 0.740 and 0.103 ± 0.074, respectively. The standard deviations of the one and two sperm recoveries are large, albeit not unexpected, due to the elevated allele dropout expected at 3.3pg and 6.6pg of template DNA. The maximum number of alleles dropping out or not observed in the combined haploid profile in the three sperm recoveries was 28, with a minimum of six alleles dropping out. Similarly, average peak heights increased as cell number increased, 102.2 ± 55.1 rfu for 1 sperm cell, 129.5 ± 89.7 rfu for two sperm cell recoveries and 155.3 ± 102.5 for three sperm cell recoveries (Figure 1). The comparison metrics for the 2-sperm cell recoveries are affected by three samples that fully dropped out and had one sample that had only one allele present. The remaining 75% of the 2-sperm cell recoveries have at least 16 alleles present at a minimum of 11 loci. Note, the average peak heights were calculated from loci where there was no dropout, representing the expected peak heights when interpretable data is obtained.

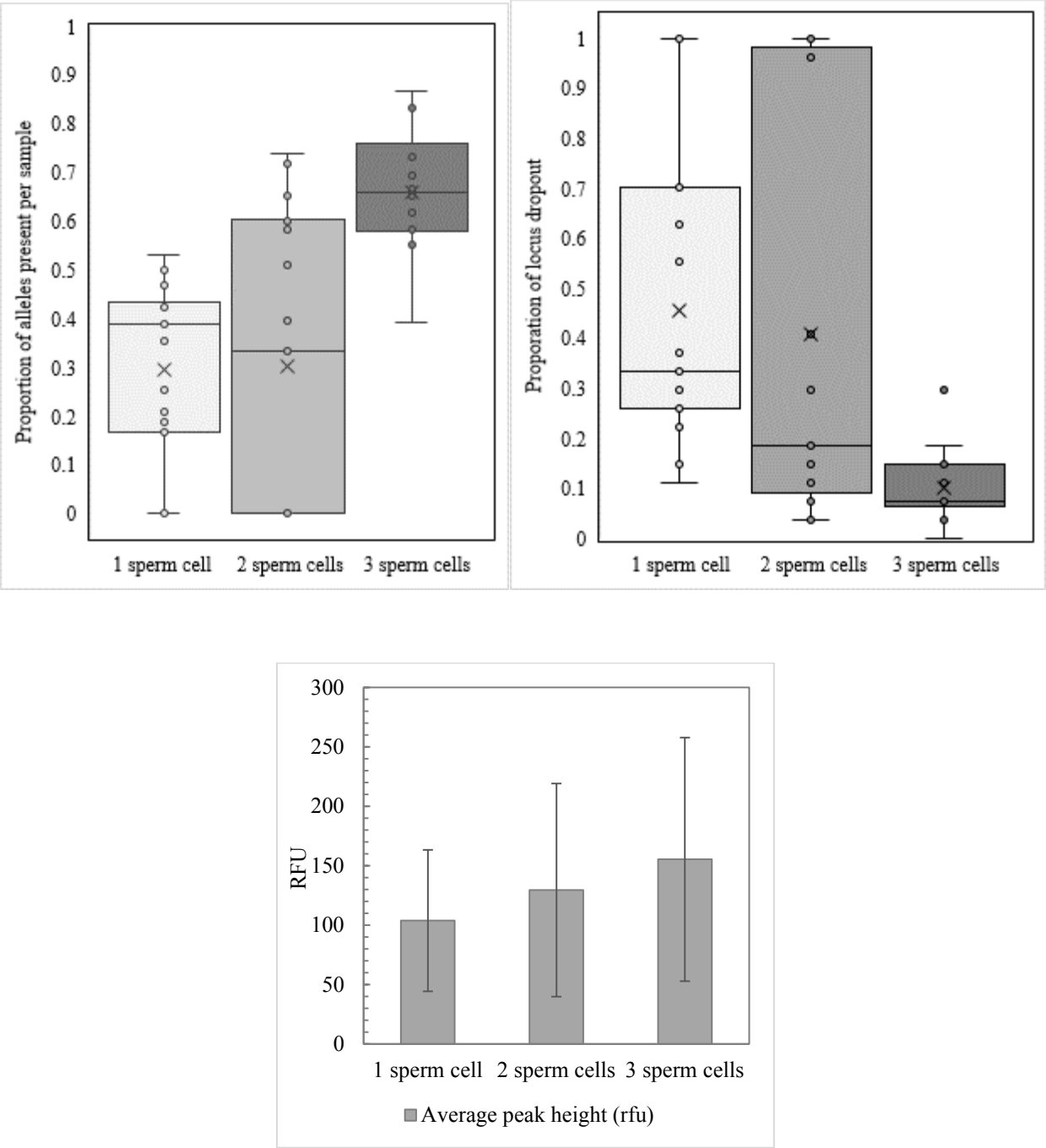


*Figure 1: The average proportion of alleles (top left) and locus dropout (top right) across one (n=19), two (n=15) and three (n=16) sperm cell recoveries and subsequent amplification with PowerPlex Fusion 6c. The average peak across one (n=19), two (n=15) and three (n=16) recoveries and subsequent amplification with PowerPlex Fusion 6c (bottom).*

We believe aneuploidy (chromosomal duplication) or partial aneuploidy (partial chromosome duplication such as a translocation) was identified in three of the 19 single sperm samples (15.8%) (Figure 2 and 3). Any single sperm cell sample exhibiting a heterozygous peak out of stutter position was identified as likely aneuploidy or partial aneuploidy. This phenomenon was

observed in single sperm recoveries from PT2301-2312 had four instances at D22S1045, D1S1656, Penta E and FGA, PT2404-2422 at D1S1656 and T3806 at D13S317. These instances had heterozygous peaks that were not in stutter position and all peaks are within a minimum of 39% of one another. These loci were not included in the calculation of average peak heights but were included in the count of the total alleles per sample.

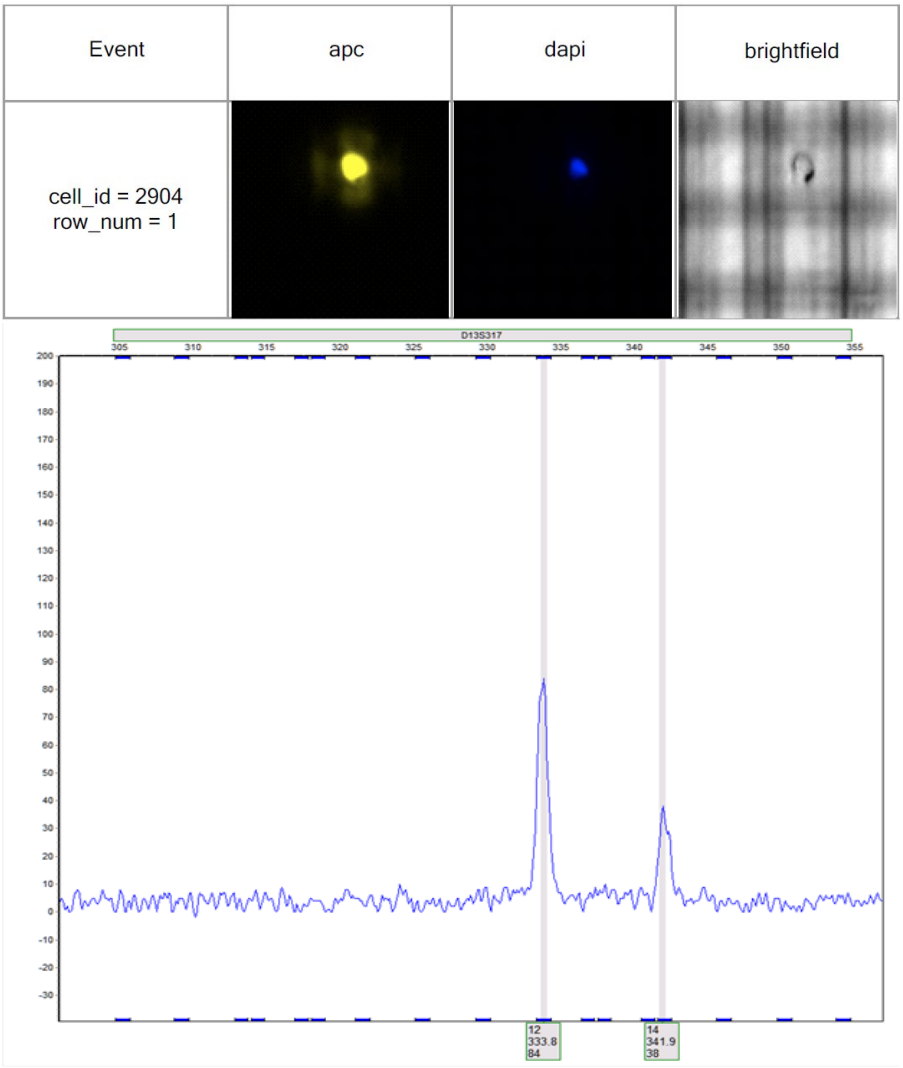


Figure 2: A single sperm cell (top) recovered from the 1 to 10 T3806 cutting sample, shows aneuploidy or partial aneuploidy at the D13 S317 locus on the resulting electropherogram (bottom). apc - human spermatozoa specific, fitc - epithelial cell specific, dapi – nucleic acid/nucleus)

*Post-Coital Samples*

Our analysis approach focused on maximizing the quality of the male profile while minimizing the presence of the epithelial cell donor. Simply, we sought to collect a maximum number of sperm at the cost of potentially co-recovering an epithelial cell that may have been



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collocated with sperm cells. This approach would be similar to the approach used during the course of casework analysis because of the limited nature of many evidentiary samples.

Spermatozoa were identified and recovered from the DEPArray™ processed samples at each post-coital time point from 12 hours to 96 hours. Mixtures were present in 4/5 samples in both the DEPArray™ processed- and differentially extracted samples. However, the female contributor had an average of five fewer alleles present per sample when using the DEPArray ™ (in the samples where the male donor was the major contributor). The minor contributor in the DEPArray™ processed samples was below 121rfu while the male contributors average peak heights are between 321 and 967 rfu.

The 24h sample was a single source male profile with an average peak height of 573.7 ± 288.5. The sperm donor was also the major contributor in the 12h, 48h and 72h DEPArray™ processed samples at ratios of 12.1:1, 8.6:1 and 2.7:1, respectively (Table 2A). The average peak heights of the male donor in the 12h, 48h and 72h samples was 966.8 ± 463.9 441.4 ± 184.6 321.0 ± 127.6, respectively. Used in conjunction with the male to female ratio this would permit a straightforward deconvolution of the individual donors. The female contributor was the major contributor in the 96h DEPArray ™ sample, with a 10 to 1 ratio. We were able to definitively identify one sperm cell in the 96h sample (Figure 3) resulting in the presence of 14 alleles exclusively attributed to the male donor (average peak height of 49.3 ± 28.6). We attribute the female profile to epithelial cell or other debris that was co-located with the sperm or identified as possible sperm. Events that were identified as possible sperm were collected in an effort to obtain as much genetic information considering the low number of sperm expected at 96h post-coitus.

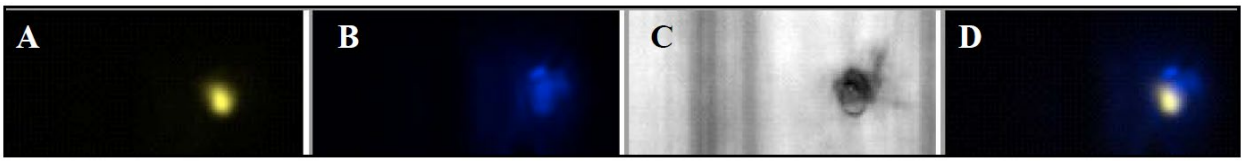


Figure 3: DEPArray™ image gallery showing the sperm cell identified in the 96h post-coital sample. (A) sperm-specific APC channel, (B) nucleic acid/nucleus-specific DAPI channel, (C) bright field and (D) sperm/nucleus channel overlay.

The differentially extracted samples did not perform as optimally as the counterparts processed using the DEPArray™ workflow. The 12h differentially extracted sperm fraction was a single source male profile however the female donor was the major contributor in both the 72h and 96h sperm fraction with ratios of 2.7 and 5.1 to 1 and male contributor average peak heights of and 88.7 ± 78.4, respectively (Table 2B). The minor (male) component in the 72h sample had 67% (32/48) of the expected alleles present with an average peak height of 868.7 ± 320.6 rfu. Similarly, the male donor was represented by 21 of 48 expected alleles (43%) with and average peak height of 88.7 ± 78.4 rfu. The male donor was the major component in the sperm fractions of the 24h and 48h samples, with ratios of male to female of 10.0 and 1.1 to 1, respectively.

The female donor was the major contributor in all differentially extracted epithelial cell fractions. The male donor was present at low levels in the 12h, 24h, 72h and 96h, with average peak heights under 84 rfu and female to male ratios of 8.6, 25.1, 99.0 and 54.7 to 1, respectively

(Table 2C). For the DEPArray™, all of the epithelial cell recoveries for the post-coital study were single source female profiles (Table 3). This clearly demonstrates the ability to obtain epithelial cells in the absence of sperm cells when the case type dictates.

Table 2: Results of post-coital sample analyses processed using the DEPArray™ (A), and differential extraction (B)-sperm fraction and (C)-epithelial fraction. * The observed minor alleles and mean peak heights were calculated using unshared alleles to more accurately represent the level of contribution. Image galleries of the cell recoveries can be found in supplementary information Table 3S.

(A)

| Sample | Total recovered sperm | Estimated template amplified (ng) | Major contributor | Major alleles present | Major average peak height (rfu)* | Minor contributor | Minor contributor alleles present* | Minor contributor mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|
| Post-coital 12h | 55 | 0.18 | PC-Male | 48/48 | 966.8 ± 463.9 | PC-Female | 4/40 | 79.6 ± 52.8 | 12.1 |
| Post-coital 24h | 36 | 0.12 | PC-Male | 48/48 | 573.7 ± 288.5 | - | - | - | - |
| Post-coital 48h | 32 | 0.11 | PC-Male | 48/48 | 441.4 ± 184.6 | PC-Female | 14/40 | 51.3 ± 32.5 | 8.6 |
| Post-coital 72h | 51 | 0.17 | PC-Male | 46/48 | 321.0 ± 127.6 | PC-Female | 19/40 | 120.4 ± 89.0 | 2.7 |
| Post-coital 96h | 12 | 0.04 | PC-Female | 40/40 | 494.5 ± 181.9 | PC-Male | 14/48 | 49.3 ± 28.6 | 10.0 |

(B)

| Sample (Sperm fraction) | Total recovered sperm | Template amplified (ng) | Major contributor | Major alleles present | Major average peak height (rfu)* | Minor contributor | Minor contributor alleles present* | Minor contributor mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|
| Post-coital 12h | - | 0.49 | PC-Male | 48/48 | 2984.9 ± 1072.3 | PC-Female | - | - | - |
| Post-coital 24h | - | 0.51 | PC-Male | 48/48 | 4441.7 ± 1220.2 | PC-Female | 19/40 | 443.8 ± 214.2 | 10.0 |
| Post-coital 48h | - | 0.5 | PC-Male | 48/48 | 800.9 ± 318.1 | PC-Female | 25/40 | 738.0 ± 353.9 | 1.1 |
| Post-coital 72h | - | 0.62 | PC-Female | 40/40 | 2377.7 ± 705.9 | PC-Male | 32/48 | 868.7 ± 320.6 | 2.7 |
| Post-coital 96h | - | 1.1 | PC-Female | 40/40 | 455.0 ± 230.3 | PC-Male | 21/48 | 88.7 ± 78.4 | 5.1 |

(C)

| Sample (Epithelial cell fraction) | Total recovered sperm | Template amplified (ng) | Major contributor | Major alleles present | Major average peak height (rfu) | Minor contributor | Minor contributor alleles present* | Minor contributor mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|
| Post-coital 12h | - | 0.5 | PC-Female | 39/40 | 659.5 ± 350.5 | PC-Male | 10/48 | 83.4 ± 57.5 | 8.6 |
| Post-coital 24h | - | 0.46 | PC-Female | 40/40 | 1803.8 ± 956.9 | PC-Male | 11/48 | 77.8 ± 40.2 | 25.1 |
| Post-coital 48h | - | 0.50 | PC-Female | 40/40 | 993.6 ± 380.2 | - | - | - | - |
| Post-coital 72h | - | 0.50 | PC-Female | 40/40 | 2040.6 ± 971.5 | PC-Male | 1/48 | 70 | 99.0 |
| Post-coital 96h | - | 0.48 | PC-Female | 40/40 | 2019.9 ± 625.2 | PC-Male | 5/48 | 36.9 ± 18.9 | 54.7 |

Table 3: DEPArray™ processed epithelial cell fractions from the post-coital samples. Note the primary goal of this study was to identify and separate sperm cells from mixtures of epithelial and blood cells. This demonstrates that epithelial cells can be recovered when needed.

| Sample – DEPArray™ E-cell | Total recovered E-cell | Template amplified (ng) | Major contributor | Observed Major Contrib alleles / total expected alleles | Major contributor average peak height (rfu) | Minor contributor |
|---|---|---|---|---|---|---|
| Post-coital 12h | 5 | 0.33 | PC-Female | 35/40 | 102.9 ± 69.5 | n/a |
| Post-coital 24h | 10 | 0.66 | PC-Female | 39/40 | 209.9 ± 161.8 | n/a |
| Post-coital 48h | 10 | 0.66 | PC-Female | 40/40 | 322.4 ± 159.0 | n/a |
| Post-coital 72h | 8 | 0.528 | PC-Female | 40/40 | 719.3 ± 354.5 | n/a |
| Post-coital 96h | 3 | 0.198 | PC-Female | 39/40 | 213.8 ± 101.1 | n/a |

*Mock Samples*

Mock samples were generated to further test the sensitivity and resolution of the DEPArray™. A pair of sample sets were generated each comprised of female-1 and either sperm donor T3806 or T4333 on a Dacron fiber swab or a cotton cutting from underwear. Another sample set was produced in the same manner as the previously described however this set included a third, blood donor. Each replicate sample was processed using the DEPArray™ and the DEPArray™ LysePrep Kit or using a differential extraction. Our aim was to collect only single sperm or pure sperm event. This is in contrast to the post-coital samples where we collected sperm despite the possible co-recovery of an epithelial cell in the same cage.

Sperm cells were identified in 27 of 32 DEPArray™ processed samples, with 26 of 27 (96.2%) yielding single source profiles. The only mixture was obtained from the T3806 cutting with a 1:1000 dilution of semen to epithelial cells sample. This sample had sperm cells collocated with epithelial cells or epithelial cell debris (Figure 5). These events were recovered expecting that we would have Female-1 activity present. In contrast, single source profiles were obtained from only nine of 28 (32.1%) differentially extracted samples (Tables 4 and 6).

We observed that the deposition substrate had a potentially significant effect on the ability to recover sperm and develop a profile. Single source make profiles were obtained from 73% (22/30) of the samples deposited on Dacron swabs (DEPArray™-12/16 and differential-10/14) and 97% from the underwear cuttings (29/30) (DEPArray™ - 15/16 and differential - 14/14).

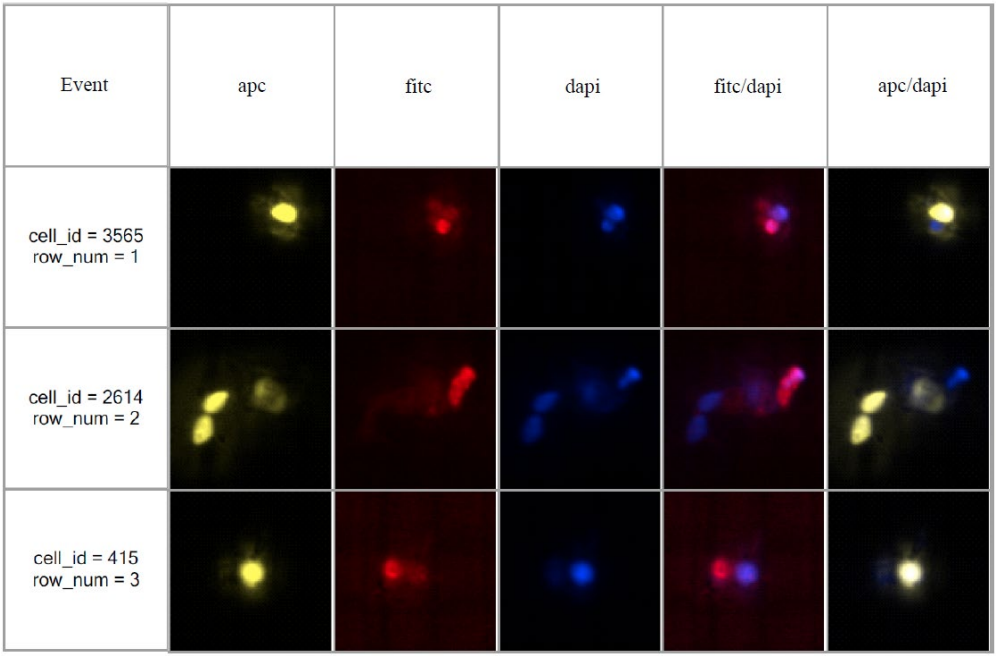


Figure 4: Epithelial cell debris recovered with T3806 sperm cells from a 1:1000 dilution of T3806 male semen with Female-1 epithelial cells on a cloth cutting. This sample represented the only mixture profile obtained from the DEPArray™ processed samples. (apc - human spermatozoa specific, fitc - epithelial cell specific, dapi – nucleic acid/nucleus)

*Semen-epithelial cell dilution series*

Single source male profiles were obtained from DEPArray™-recovered sperm cells from all cloth and swab samples down to a 1 to 100 dilution of semen to epithelial cells. The male contributor was identified in both of the 1 to 1000 dilutions on cloth, with 46/48 alleles present and an average peak height of 154.6 ± 98.9 rfu in the T3806 sample and 30/47 alleles present and an average peak height of 43.8 ± 25.3 rfu in the T4333 sample. In contrast, in-cage sperm cells were not identified in the swab based 1:1000 counterparts. A single out-of-cage (non-recoverable) sperm cell was identified in the 1:1000 T4333 swab and 10 in the T3806 swabbing, however they were all collocated with epithelial cells. A single sperm cell was identified in one of four 1:10,000 dilutions (T3806 cutting) with 14 of 48 alleles detected and an average peak height of 27.7 ± 18 rfu. These results are consistent with the results obtained from the microscopic identification of sperm, in which one sperm was identified in each of three of the four samples (data not shown). These results indicate that a 1:10,000 dilution is the limit of detection for the DEPArray™.

The differentially extracted samples yielded single source profiles in only two of the 16 samples (12.5%) and 2/12 (16.6%) in the samples in which the male donor was the major contributor. This is in stark contrast to the DEPArray™-processed counterparts in which single source male profiles were obtained in 14/15 (93.3%) in the samples where sperm were identified. The differentially extracted samples, as expected had higher peak heights due to the increased number of cells present in the samples. It is possible to achieve higher peaks heights with the DEPArray™ recovered samples through the inclusion of sperm cells that are collocated with epithelial cell components however this would lead to mixtures thus may be unnecessary to obtain interpretable results.

*Semen-blood-epithelial cell dilution series*

Single source male profiles were obtained from all DEPArray™-processed semen/blood/epithelial cell dilutions (1:1:1, 1:1:10 and 1:1:100); swab (6/6) and cloth (6/6) (Table 4). Sperm cells were recovered across all dilutions, ranging from a minimum of seven to a maximum of 43 leading to average peak heights between 159.4 ± 141.3 and 2359.5 ± 1037.1 rfu. Single source samples were obtained in 7/12 differentially extracted samples. The T4333 1:1:100 sample was the only sample with alleles from the semen, epithelial cell and blood donors, with 3 alleles present from the minor epithelial (1 allele) and blood (2 alleles) donors. The remaining four samples had a maximum of two minor alleles present from either the epithelial or blood donor. The ratio of major to minor contributors was greater than 23 to 1 across all differential samples, permitting a simple deconvolution of the major contributor.

73

74 Table 2: Dilution sets of sperm and epithelial cell samples processed using the DEPArray™ workflow. Note, only spermatozoa recoveries
75 displayed. * The observed minor alleles and mean peak heights were calculated using unshared alleles to more accurately represent the level of
76 contribution. Image galleries of the cell recoveries can be found in supplementary information Table 3S.

| **Substrate** | Sperm Donor | Dilution | Total recovered sperm | Estimated template amplified (ng) | Major contributor | Major contributor alleles present | Major contributor mean peak heights (rfu) | Minor contributor | Minor contributor alleles present* | Minor contributor mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Swab | T3806 | 1:1 | 135 | 0.4455 | T3806 | 48 / 48 | 4380.3 ± 2456.9 | - | - | - | - |
| | | 1:10 | 73 | 0.2409 | T3806 | 48 / 48 | 3411 ± 1681.5 | - | - | - | - |
| | | 1:100 | 3 | 0.0099 | T3806 | 39 / 48 | 172.7 ± 83.5 | - | - | - | - |
| | | 1:1000 | 0 | 0 | - | - | - | - | - | - | - |
| | | $1:10^4$ | 0 | 0 | - | - | - | - | - | - | - |
| | T4333 | 1:1* | 20 | 0.066 | T4333 | 48/47 | 388.3 ± 173.9 | - | - | - | - |
| | | 1:10 | 209 | 0.6897 | T4333 | 47 / 47 | 6627.2 ± 2662.9 | - | - | - | - |
| | | 1:100 | 1 | 0.0033 | T4333 | 15/47 | 53.2 ± 21.6 | - | - | - | - |
| | | 1:1000 | 0 | 0 | - | - | - | - | - | - | - |
| | | $1:10^4$ | 0 | 0 | - | - | - | - | - | - | - |
| Cutting | T3806 | 1:1 | 83 | 0.2739 | T3806 | 48 / 48 | 1578.6 ± 800 | - | - | - | - |
| | | 1:10 | 51 | 0.1683 | T3806 | 48 / 48 | 1328.3 ± 828 | - | - | - | - |
| | | 1:100 | 23 | 0.0759 | T3806 | 48/ 48 | 530.8 ± 329.2 | - | - | - | - |
| | | 1: 1000 | 6 | 0.0198 | T3806 | 46/48 | 154.6 ± 98.9 | Female-1 | 8/42 | 41.3 ± 24 | 3.7 |
| | | $1:10^4$ | 1 | 0.0033 | T3806 | 14 / 48 | 27.7 ± 18 | - | - | - | - |
| | T4333 | 1:1 | 33 | 0.1089 | T4333 | 48/47 | 768.3 ± 362.7 | - | - | - | - |
| | | 1:10 | 15 | 0.0495 | T4333 | 48/47 | 350.1 ± 162.1 | - | - | - | - |
| | | 1:100 | 13 | 0.0429 | T4333 | 48 / 47 | 329.8 ± 194.7 | - | - | - | - |
| | | 1:1000 | 2 | 0.0066 | T4333 | 30 / 47 | 43.8 ± 25.3 | - | - | - | - |
| | | $1:10^4$ | 0 | 0 | - | - | - | - | - | - | - |

Table 3: Dilution sets of sperm and epithelial cell samples processed using the *differential extraction* workflow. Note, only sperm fractions are displayed. * The observed minor alleles and mean peak heights were calculated using unshared alleles to more accurately represent the level of contribution.

| **Substrate** | Sperm donor | Dilution (Sperm:Ecell) | Amplified template (ng) | Major contributor | Major contributor alleles present | Major contributor mean peak heights (rfu) | Minor contributor | Minor contributor alleles present* | Minor contributor mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|---|
| Swab | T3806 | 1:1 | 0.95 | T3806 | 48/48 | 18702.5 ± 6125.5 | - | | | |
| | | 1:100 | 0.49 | T3806 | 48/48 | 1688.9 ± 812 | Female-1 | 24/42 | 231.2 ± 110.4 | 7.3 |
| | | 1:1000 | 1.092 | Female-1 | 42/42 | 905 ± 366.4 | T3806 | 33/48 | 737.3 ± 220.4 | 1.23 |
| | | $1:10^4$ | 1.04 | Female-1 | 42/42 | 1155.5 ± 334.9 | T3806 | 7/48 | 56.8 ± 19.5 | 20.33 |
| | T4333 | 1:1 | 0.56 | T4333 | 48/47 | 2149.6 ± 517.2 | Female-1 | 2/48 | 44.3 ± 13.8 | 48.58 |
| | | 1:100 | 0.5 | T4333 | 48/47 (34/47) | 1914 ± 748.2 | Female-1 | 42/42 (28/42) | 1175.4 ± 330.9 | 1.63 |
| | | 1:1000 | 0.51 | Female-1 | 42/42 | 6183.4 ± 1636.9 | T4333 | 18/47 | 234.8 ± 128.1 | 26.34 |
| | | $1:10^4$ | 0.5 | Female-1 | 42/42 | 3387.6 ± 500 | T4333 | 6/47 | 60.1 ± 20.1 | 56.38 |
| Cutting | T3806 | 1:1 | 1.0 | T3806 | 48/48 | 6583.7 ± 1377.7 | - | - | - | 0 |
| | | 1:100 | 0.98 | T3806 | 48/48 | 13066.8 ± 3590.1 | Female-1 | 1/42 | 231 | 56.57 |
| | | 1:1000 | 1.0175 | T3806 | 48/48 | 2661.7 ± 659.6 | Female-1 | 5/42 | 73.5 ± 43.8 | 36.23 |
| | | $1:10^4$ | 0.225 | T3806 | 48/48 | 814.6 ± 393.2 | Female-1 | 23/47 | 150.3 ± 108.1 | 5.42 |
| | T4333 | 1:1 | 1.0 | T4333 | 48/47 | 5653.3 ± 1477.8 | Female-1 | 1/42 | 106 | 53.33 |
| | | 1:100 | 0.96 | T4333 | 48/47 | 2994.5 ± 926.8 | Female-1 | 2/42 | 90.3 ± 87.3 | 33.18 |
| | | 1:1000 | 0.525 | T4333 | 48/47 | 4271.6 ± 1624.6 | Female-1 | 24/47 | 276 ± 148 | 15.48 |
| | | $1:10^4$ | 0.37 | T4333 | 40/42 (25/42) | 471.9 ± 222.3 | Female-1 | 48/47 (33/37) | 424.5 ± 198 | 1.11 |

Table 4: Semen:Blood:E-cell (S:B:E) dilutions processed using the DEPArray™ workflow. Note, only sperm fractions are displayed. Image galleries of the cell recoveries can be found in supplementary information Table 3S. * The observed minor alleles and mean peak heights were calculated using unshared alleles to more accurately represent the level of contribution. Note, no minor epithelial cell or white blood cell donor profiles were detected.

| **Substrate** | Sperm Donor | Dilution (S:B:E) | Total recovered sperm | Estimated template amplified (ng) | Major contributor | Major contributor alleles present | Major contributor mean peak heights (rfu) | Minor contributor 1 (E-cell) or 2 (blood) |
|---|---|---|---|---|---|---|---|---|
| Swab | T3806 | 1:1:1 | 12 | 0.0396 | T3806 | 47/48 | 432.1 ± 219.8 | - |
| | | 1:1:10 | 23 | 0.0759 | T3806 | 47/48 | 366.9 ± 211.8 | - |
| | | 1:1:100 | 26 | 0.0858 | T3806 | 48 / 48 | 496.4 ± 238.7 | - |
| | T4333 | 1:1:1 | 10 | 0.033 | T4333 | 43 / 47 | 224.3 ± 128.7 | - |
| | | 1:1:10 | 13 | 0.0429 | T4333 | 42 / 47 | 358.4 ± 190.4 | - |
| | | 1:1:100 | 12 | 0.0396 | T4333 | 46 / 47 | 292.8 ± 135.1 | - |
| Cutting | T3806 | 1:1:1 | 52 | 0.1716 | T3806 | 48 / 48 | 1131.8 ± 660.3 | - |
| | | 1:1:10 | 43 | 0.1419 | T3806 | 48 / 48 | 871.7 ± 420.3 | - |
| | | 1:1:100 | 22 | 0.0726 | T3806 | 48 / 48 | 394.3 ± 198.4 | - |
| | T4333 | 1:1:1 | 40 | 0.132 | T4333 | 47 / 47 | 2359.5 ± 1037.1 | - |
| | | 1:1:10 | 20 | 0.066 | T4333 | 37 / 47 | 159.4 ± 141.3 | - |
| | | 1:1:100 | 7 | 0.0231 | T4333 | 42 / 47 | 179.4 ± 109.7 | - |

Table 5: Semen:Blood:E-cell (S:B:E) dilutions processed using the *differential extraction* workflow. * The observed minor alleles and mean peak heights were calculated using unshared alleles to more accurately represent the level of contribution.

| **Substrate** | Sperm Donor | Sample | Amplified Template (ng) | Major contributor | Major contributor alleles present | Major contributor mean peak heights (rfu) | Minor 1 alleles present | Minor 1 (E-cell) mean peak height (rfu)* | Minor 2 (blood) alleles present | Minor 2 (blood) mean peak height (rfu)* | Ratio of contributors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Swab | T3806 | 1:1:1 | 0.97 | T3806 | 48/48 | 19625.5 ± 5740.1 | - | - | - | - | - |
| | | 1:1:10 | 0.5 | T3806 | 48/48 | 4805.6 ± 1086.4 | - | - | 2/47 | 107.5 ± 89.8 | 44.7 |
| | | 1:1:100 | 0.49 | T3806 | 48/48 | 1707.4 ± 528.7 | - | - | - | - | - |
| | T4333 | 1:1:1 | 1.0 | T4333 | 48/47 | 5119 ± 1052.4 | - | - | - | - | - |
| | | 1:1:10 | 0.5 | T4333 | 48/47 | 3734.5 ± 792.4 | 1/42 | 39.5 | - | - | >94.5 |
| | | 1:1:100 | 0.94 | T4333 | 48/47 | 2788.3 ± 805.9 | 1/42 | 61 | 2/47 | 120.2 ± 21.4 | 45.7:23.2: 1 |
| Cutting | T3806 | 1:1:1 | 1 | T3806 | 48/48 | 6599.5 ± 2780.8 | 1/42 | 53 | - | - | 124.5 |
| | | 1:1:10 | 0.5 | T3806 | 48/48 | 7780 ± 2028.5 | - | - | 1/47 | 56 | 138.93 |
| | | 1:1:100 | 0.5 | T3806 | 48/48 | 6377.6 ± 1565.7 | - | - | - | - | - |
| | T4333 | 1:1:1 | 1.0 | T4333 | 48/47 | 3947.1 ± 1268.8 | - | - | - | - | - |
| | | 1:1:10 | 0.5 | T4333 | 48/47 | 6173 ± 1776.9 | - | - | - | - | - |
| | | 1:1:100 | 0.94 | T4333 | 48/47 | 3326.4 ± 927.3 | - | - | - | - | - |

*Reproducibility*

Between 175 and 238 spermatozoa were recovered from the four proficiency test samples, yielding DNA concentrations between 0.58 and 0.79 ng/µL. The PT samples yielded the expected profiles peak heights greater than 5000 rfu (Table 5).

Slides were made from portions of the PT samples that were stained using the standard Christmas tree staining procedure and analyzed using light microscopy at 40x. All samples yielded greater than 75 spermatozoa per field, 1500 total over 20 fields. This is greater than the total number of sperm positive events identified using the DEPArray™. This difference is immaterial due to the presence of ample quantities of DNA to yield high quality profiles without concern for stochastic effects.

Table 5: Proficiency test samples processed using the DEPArray™, extracted using the MSB cell lysis kit and amplified using PowerPlex Fusion 6c (Promega).

| Sample | Sperm positive events | Recovered sperm | Estimated DNA quantity (ng) | Alleles observed / Alleles expected | Average peak height (rfu) |
|---|---|---|---|---|---|
| PT2303-2332 | 492 | 188 | 0.6204 | 45 / 45 | 5272.3 ± 3006.5 |
| PT2301-2312 | 537 | 238 | 0.7854 | 49 / 49 | 8280.7 ± 3881.1 |
| PT2404-2422 | 818 | 215 | 0.7095 | 45 / 45 | 10560.5 ± 5438.9 |
| PT2202-2222 | 339 | 175 | 0.5775 | 46 / 46 | 7819.5 ± 3861.5 |

## Discussion

Protocol optimization was conducted to enrich the sample for the presence of sperm (and not blood or epithelial cells). Reduction of the cell-substrate release volume was to avoid cell loss by avoiding removal and transfer of large volumes. The incubation and centrifugation speed and time were also optimized because these changes demonstrated the release of more sperm from the substrate. The DEPArray™ cartridge has limited cell capacity, therefore, to avoid epithelial cell saturation; a size-exclusion filter was used to enrich the presence of spermatozoa. This reduced the total number of epithelial cells and led to an improvement in the efficiency of each DEPArray™ run by allowing for an uninhibited cell routing/recovering phase. Simply, by reducing obstructions and obstacles in the routing path of the target cell we were able to route an increased number of cells leading to improved peak heights. The filtration also promotes more single celled events leading to single source profiles.

Areas for improvement for DEPArray™ focus on cell loss during routing and recovery phases. There are instances, where sperm cells cannot be recovered because they are out of cage. As cells move to the main chamber, they have the potential to become un-routable due to their positioning (out of cage). This is due to the quality of the cell and inability of the cell to properly respond to the dielectrohporesis. The user is able avoid losing cells by adjusting the speed in which the cages alternate the activity to move the cell electrophoretically. Adjusting this speed can reduce the cellular loss and maximize recovery. Non-target cells can also become entrapped

in cages with the targeted cellular event. This can result in the recovery of non target cells, however all trials we were able to avoid recovering non-target cells with the expectation of the one mixture where the only sperm cells that were identified were collocated with epithelial cells. Most of these challenges have been addressed with Menarini Silicon Biosystems' latest model the DEPArray™ NxT.

We believe that the most critical factor leading to successful operation of the DEPArray™ is the training and experience of the user. This applies to both the sample preparation and data analyses. A trained forensic DNA analyst should have sufficient laboratory skills to master the sample preparation after a week of onsite training and a second week of independent sample preparations. Data analysis, i.e. the identification of the target cells, is similar to the identification of spermatozoa on slides using light microscopy. Our ability to positively identify sperm improved over time; this improvement was most significant when focusing on more challenging identifications such as sperm cells that were in clumps of other cellular material or in unique 3-dimensional positions. We believe the training time required for DEPArray™ data analyses would be similar to that of sperm identification.

Although the DEPArray™ is ready for routine use, we noted several areas of improvement for the DEPArray v2.0 system. However, the primary disadvantages of the DEPArray v2.0 system have been or are being addressed by Menarini Silicon Biosystems through the release of the new benchtop DEPArray NxT has been released that address many of the shortcomings of the v2.0.

The Menarini Silicon Biosystems developed DNA an extraction method (DEPArray™ LysePrepKit -REF DALYS), which has significant advantages in both DEPArray-based and low template sample preparation procedures. The procedure has been optimized to lyse and purify DNA samples in a total volume of 4-5µL. This low volume reaction avoids the use of DNA extraction methods that may lead to inherent loss of the sample such as bead or column based methods. In addition, it avoids the use of centrifugal concentration procedures, which can lead to further decreases in DNA yield and allows for a cost savings. The procedure has minimal hands on time and sample manipulation, it is more similar to an amplification procedure than standard DNA extraction protocols. In addition, the amplification reagents are added directly to the extraction tube and amplified. This avoids additional sample manipulation and tube transfers, thus minimizes the probability of contamination and loss.

DEPArray™-mediated cell separation allows the user to recover specific cell types and eliminates the primary disadvantage of differential extractions, leading to higher confidence allele calls knowing that the only profile present is a result of the selected cells. We were able to obtain approximately 30% of the expected alleles from haploid single cells, demonstrating that it is possible to obtain valuable data from this quantity of genetic material. Interpretational issues are commonly present when signal is low, in the sub 100rfu range, however it is noteworthy, the baseline noise in samples purified using the DEPArray™ have a minimal amount of noise compared to the differentially extracted counterparts. This is likely due to the purification of only select components from the sample, leading to an increasingly interpretable allelic signal.

The signal obtained from three sperm cells, approximately 9.9pg of DNA, is interpretable with, on average, under 10% locus dropout and average peak heights of approximately 150rfu.

We believe that aneuploidy or partial aneuploidy was identified in an unexpectedly large number of sperm cells, however consistent with levels observed in recent publications [5]. The identification of this was only possible because we knew the number of analyzed sperm cells. The result of aneuploidy or partial aneuploidy within the construct of this study is either the appearance of an extra allele peak or the potential dropout of an allele. In this study, we were only readily able to identify the presence of an additional peak. The detection of these extra chromosomal events were unexpected, however recent studies have indicated that partial aneuploidy and smaller chromosomal aberrations can occur in between 11 and 14% of sperm cells from a normal individual [5]. We strongly believe that aneuploidy was present in three single sperm recoveries from PT2301-2312 had 4 instances at D22S1045, D1S1656, Penta E and FGA, PT2404-2422 at D1S1656 and T3806 at D13S317. These instances had heterozygous peaks that matched the expected donor and not shared with the analyst performing the wet bench work or samples that were processed simultaneously. In addition, these peaks were not in stutter position of one another. We observed five additional instances of possible heterozygous peaks from single sperm recoveries. These peaks were in stutter position of one another and we believe these could be high stutter given single copy amplification. The instances of potential high stutter were three standard deviations over the average stutter [4], thus outside of the 99.7% confidence interval. This meiotic abnormality has the potential of causing interpretational challenges when analyzing low levels of DNA and without knowing the number of cells. We expect however that when analyzing larger volumes of DNA, the occurrence of this phenomenon would exert less influence on the interpretation of the sample because the extra copy of the allele will not be as prominent when more than one cell is being analyzed.

The post-coital samples were of critical importance to this study owing to how well they simulated sexual assault evidence. We chose to utilize the DEPArray™ in a non-intended manner. Our approach was to maximize genetic material (sperm cells) rather than isolate single or groups of sperm. This approach resulted in mixtures of the male and female contributors, a common occurrence when using differential extractions. The most impactful result is that the DEPArray™-mediated cell separation technique was able to resolve mixtures at increased durations post-coitus, 72h, where the differential extraction resulted in a female major component. In addition, the results indicate that mixture interpretation is easier using DEPArray™-generated data, primarily based on more favorable mixture ratios.

A significant area of need in forensic DNA analyses is the separation of mixtures of semen. This has been addressed through Y-STR analyses, however the DEPArray™ offers a new and more powerful method to deconvolute the autosomal STR-based alleles. The ability to recover several groups of sperm in separate tubes allows the user to potentially vary the ratio of the two sperm donors, therefore increasing the likelihood of obtaining an interpretable profile that can be deconvoluted. This is similar to consensus profiling, however with higher template DNA levels.

The mock samples were dilutions of semen, saliva and blood, generated to further evaluate the sensitivity and specificity of the DEPArray™ and to compare to samples processed using a standard differential extraction method. Dilutions were added to Dacron swabs and cotton cloth cuttings. The primary goal of this sub-study was to obtain single source male profiles through the recovery of sperm only events. These events could consist of either single sperm cells or clusters of sperm. A clear advantage of this approach is obtaining single source male profiles; however, we observed stochastic effects beginning at recoveries of 23 sperm cells or 0.0759 ng of DNA. Although these samples yielded single source profiles, more allelic information would benefit the case by collecting more events. The potential of epithelial cell "contamination" may be of higher value than a single source profile. The value of single source versus a mixture would need to be weighed by the circumstances surrounding the case.

In 26 of 27 dilution samples the DEPArray™ produced single source male profiles. The one instance of a mixture can be easily deconvoluted, with a male to female ratio of 3.7 to 1. As stated previously, there was a lack of cages with exclusively sperm cells therefore the only way a male profile could be generated was to recover sperm collocated with an epithelial cell. We believe that this colocation can be expected routinely however not commonly. In contrast, the differential extraction method is not an absolute separation of cell specific DNA, there is an expectation for carryover in all fractions. Prior to analysis, the source contributing to the DNA concentration determined during quantitation is arbitrary, subsequently, rendering uncertainty with the profiles generated from each extraction. The DEPArray™ reduces this uncertainty and arbitrariness by recovering and subsequently, amplifying specific cell types, rather than the amplification of total DNA obtained from differential extractions.

An effective limit of detection of 1 to 10,000 was established through the reduction of the proportion of spermatozoa to epithelial cells in the mock samples. We were able to obtain a profile from a single sperm recovered from a 1 to 10,000 sample however we were twice as successful using 1 to 1000 dilutions. Therefore, a more practical limit of detection is 1 to 1000. These limits allow for the identification, isolation, recovery, and subsequent amplification and interpretation of the spermatozoa at low template levels. This limit of detection holds true for the differentially extracted counterparts however all the 1 to 10000 counterparts were mixtures where either the female contributor was the major contributor or the ratio of male to female approached a 1:1 ratio. In addition, we observed that sperm cells were more readily released from the cloth (cotton) substrates than Dacron swabs, thus the profiles obtained from cloth were higher quality than the Dacron counterparts (using both the DEPArray™ and differential methods). We hypothesize that the less complex matrix of the cotton cuttings increased the release of cells and led to higher quality results.

With the mock semen, blood and saliva dilution samples, the DEPArray™ produced all single source sperm profiles with no contribution from either the blood or epithelial cell donors. This demonstrates the specificity of DEPArray™-processed samples using three commonly encountered cell types. In contrast, the differential extraction method contained mixtures of all three contributors. Differential extractions lyse cells to obtain cell specific DNA, however, as seen in this study, there is potential carryover of cell specific DNA within each fraction.

Although some of these differential extractions profiles are simple and likely simple to deconvolute, the DEPArray™ processed samples do not require deconvolution.

This study demonstrates the readiness of the DEPArray™ for incorporation into casework analyses in forensic laboratories. The clear niche of the DEPArray™ is sexual assault evidence processing however, the use can be extended to other types of evidence such as trace or 'touch' evidence. The system provides several advantages over standard differential extraction protocols including higher specificity and sensitivity. The most significant advantage is the ability to provide highly interpretable data resulting from scenarios with multiple sperm donors. Although this scenario was not the focus of this initial study, the data supports this conclusion and this will be the focus of follow-up studies. All scientific instruments have inherent limitations; we strongly believe that the limitations of the DEPArray™ are outweighed by the advantages. The system permits a powerful and reliable method to analyze sexual assault evidence, permitting unprecedented sensitivity and specificity while also eliminating the need for traditional confirmatory tests for human sperm and qPCR based DNA quantification.

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