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Reverse Body Fluid Identification Workflow: A Direct to DNA Approach

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Abstract

Preliminary serological assays to detect the presence of semen in sexual assault offenses are often used by forensic laboratories as a screening method prior to DNA analysis, in order to select the best samples for extraction and determine if the victim's collected items are suitable for differential extraction. However, in many situations, samples have low amounts of semen or other male bodily fluids which can generate a semen negative result and, for that reason, some samples may not be selected for DNA profiling. To avoid this lack of information, in our laboratory until the end of 2022 and whenever possible, samples were processed in separate workflows, one to determine the presence of biological fluids and the other to generate the DNA profile. Due to the high percentage of samples detected with negative preliminary tests and without eligible DNA profiles for report purposes, we conducted a study to better quantify and understand those results. A total of 657 samples from sexual assault crimes were then analysed and it was detected that 60.4% of them had, for different reasons, no information relevant for the case under study. Given these results, a more efficient workflow (time and cost effective) was proposed to be applied to all forensic samples, reaching first a reportable DNA profile by applying a *Direct to DNA* approach followed by serological assays for body fluid identification, if necessary.

Keywords

Sexual assault, Body fluid identification, Semen detection, *Direct to DNA* approach.

1. Introduction

When forensic DNA laboratories receive evidence from a crime scene their first task is to check for the presence of biological material, namely blood, semen and/or saliva; the same principle is applied to items related to victims from sexual assault cases, which may include internal or external genitalia swabs, oral swabs, skin swabs, fingernail swabs, condoms, underwear and other clothing, towels or bedding collected in the crime scene, etc. Examination of the exhibits by naked eye or using a forensic light source is done in order to detect the presence of body fluid stains and many laboratories perform preliminary assays on items where biological material is potentially present before sending a cutting or swab for extraction and subsequent DNA typing. Nevertheless, this methodology may fail the detection of DNA profiles useful for solving the criminal case, since the recent DNA typing kits are more sensitive than many serology tests used by laboratories. Other laboratories perform a differential extraction without prior screening for the presence of semen, namely in internal swabs collected during the examination of victims of alleged sexual assaults, but male DNA can be lost during the separation between both epithelial and sperm fractions; on the other hand, some samples do not contain semen but other biological fluids from the perpetrator, which leads to a larger number of samples that need DNA profiling [1, 2, 3, 4, 5, 6].

To identify the presence of bodily fluids, our laboratory has implemented presumptive and/or confirmatory assays to detect semen, blood and saliva and, until the end of 2022, all samples selected for DNA extraction and posterior quantification/amplification were also tested to determine the type of biological evidence in question (when enough sample was available) in different workflows.

For semen identification, all presumptive positive results were then tested in order to visualize sperm cells. However, in sexual assault cases there are many samples with a semen presumptive positive result but with a negative confirmatory test, meaning that this biological fluid cannot be confirmed. It was also detected that in several situations the analysed samples did not present probative DNA results and, consequently, it would not have been necessary to test them for the presence of bodily fluids.

The aim of this study was to evaluate the methodology implemented in sexual assault samples regarding the need to perform semen preliminary tests in all samples, and propose a more efficient workflow to be applied to forensic samples received in our laboratory.

2. Materials and methods

A total of 647 samples from sexual assault crimes occurred between 2020 and 2021 were selected from female and male victims (cases already closed); the majority of the analysed samples included swabs, clothing and bed sheeting. A Crime-lite® 82S (foster + freeman) with blue light and wavelength 420-470nm was used as an alternative light source to detect suspicious stains on clothes. Whenever possible, samples that needed semen identification (stains on fabric or swabs) were subsampled by making three cuts: one to detect the presence of semen, one for non-differential extraction and one for differential extraction, if necessary. All samples were anonymized in order to not identify their donors or even the case.

Two parallel and independent workflows were applied to all selected samples: one to detect the presence of semen and the other to obtain the male genetic DNA profile of interest.

2.1. Semen detection

As a presumptive test for semen detection it was used the Seratec® Prostate-specific Antigen (PSA) Semiquant immunochromatographic rapid test (Göttingen), also used as a screening method since only positive PSA results were sent for microscopic visualization of spermatozoa using the Christmas Tree (CT) staining method [7].

2.2. DNA profiling

DNA extraction was performed with the PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems), with an elution volume of 50µL, and then quantified using the Quantifiler® Trio DNA Quantification kit (Applied Biosystems). The screening of samples that required differential extraction was done by evaluating the quantification results: in general, if the male-female DNA ratio is greater than 1:10 and the male component is above 0.5ng/µL (indicative value, which may be lower), a differential extraction was made using the Sampletype i-sep® DL-MB columns (Biotype) with subsequent purification with the PrepFiler kit followed by another quantification. Only the sperm fraction of the differential extraction was amplified.

Quantification results from both differential and non-differential extraction were then grouped into three categories according to the existing amount of male DNA: greater than or equal to 0.1 ng/µL (where DNA profiles are generally easily achieved); between 0.01 ng/µL and 0.1 ng/µL (where DNA profiles may or cannot

be obtained); lower than 0.01 ng/µL (where, in many simples, it is not possible to obtain a valuable DNA profile or any profile at all). When multiple cuts with identical importance were made from the same evidence (mainly items of clothes), only the best ones were selected for amplification (male-female ratios were taken into account and samples without male DNA were not amplified).

Eligible samples were then amplified with the GlobalFiler™ PCR Amplification Kit (Applied Biosystems) in order to obtain an useful autosomal STR profile for solving the case. When very low levels of male DNA were present (below 0.01 ng/µL) or whenever the male-female ratio did not allowed an autosomal STR profile (in most situations with ratios above 1:10), the Yfiler® Plus PCR Amplification Kit (Applied Biosystems) was used to detect a Y-STR haplotype.

All kits were performed according to the manufacturer's protocol.

3. Results

The results revealed that 391 out of 647 samples (60.4%) had no relevance for the cases analysed. This percentage includes samples without male DNA, with DNA profiles or haplotypes that could not be evaluated (namely with the majority of the alleles below stochastic level) or with DNA profiles from male victims. Redundant samples (collected, for example, from the same item of clothing) are also included in this group; only samples with the highest concentrations of male DNA and/or the best male-female ratios were selected for DNA profiling.

The remaining 256 samples (39,6%) had a reportable DNA profile or haplotype that could be considered probative for the criminal case, with 90.7% of them (232 samples) with male DNA greater than or equal to 0.01 ng/µL (Fig. 1). The majority of samples presented a complete genetic autosomal genetic profile or Y-STR haplotype, with a higher percentage of incomplete profiles in samples with male DNA below 0.01 ng/µL, as expected.

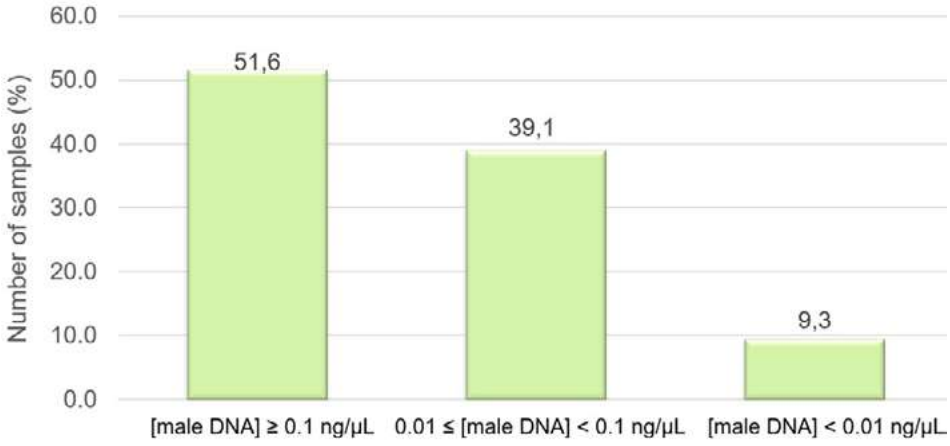
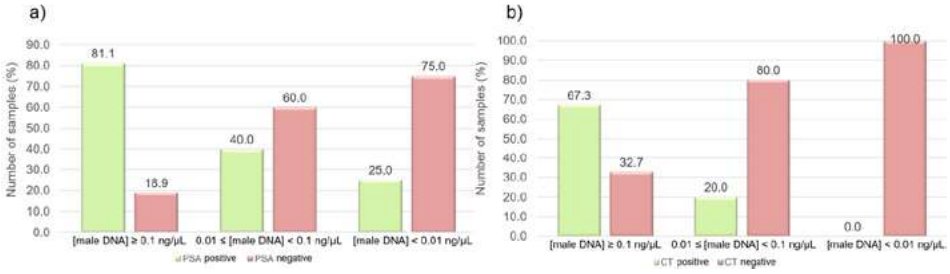


Figure 1. Number of samples (%) with reportable male DNA profiles/haplotypes distributed by male DNA concentration.

For samples with reportable DNA profiles, only 153 tested positive for PSA (107 of them with the higher concentration of male DNA) and a positive CT result was achieved in approximately half of them (80 samples), all with male DNA greater than or equal to 0.01 ng/µL. The results (in percentage) distributed according to the male DNA concentration are shown in Fig. 2.



Figre 2. Number of samples (%) with reportable male DNA profiles/haplotypes, distributed by male DNA concentration: a) PSA results and b) CT results.

It was also observed that in the group with samples without relevance for the cases under study, 83 samples (21,2%) showed a positive result for PSA even with reduced concentration (less than 0.01 ng/µL) or absent male DNA.

4. Discussion

The quantification step is essential for the selection of forensic samples for amplification. Only samples with male DNA and with the best male-female DNA ratios are selected to perform a genetic DNA profile and, as expected, higher DNA concentrations revealed a higher percentage of complete DNA profiles and positive PSA and CT results. However, in the majority of the analysed samples (60.4%), the perpetrator's male DNA was not present or could not be assessed (especially in samples with a lower concentration of male DNA). From our point of view, in these situations, carrying out presumptive or confirmatory tests to detect semen is unnecessary since the absence of a genetic DNA profile does not allow the aggressor to be identified.

It was also observed that, in many situations, a male DNA genetic profile was not associated to a positive PSA result. These results can be explained by the presence of the aggressor´s saliva or epithelial cells, which may be present in sexual assault crimes, and also because in some cases the victim is male and the genetic profile obtained is from his own DNA (unrelated to semen).

Negative CT results (for semen confirmation) are observed in almost half of positive PSA results and may be related to: lower concentration of male DNA observed in several samples, degradation of spermatozoa that have become unsuitable for microscopic visualization, or because samples originate from azoospermic or vasectomized individuals. Another explanation may be the presence of PSA in some female body fluids which can generate a positive result and, consequently a negative CT result [2, 4, 8]. On the other hand, in samples with very low concentrations of male DNA it is still possible to obtain a male reportable genetic profile/haplotype (even if incomplete) and this may be due to the high sensitivity of the current amplification kits.

5. Conclusion

If a *Direct to DNA* approach was adopted on the studied samples (where DNA analysis is performed prior to identification of bodily fluids) only 39.6% of them would have been tested for the presence of semen, saving time, costs and personnel efforts, since microscopic observation is laborious and time-consuming. This workflow (currently implemented in our laboratory) is more efficient, allows standardization of the implemented techniques and, above all, no loss of information relevant to the judicial process was detected.

6. Conflict of interest statement

The presented results are included in the master dissertation of the co-author JF.

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