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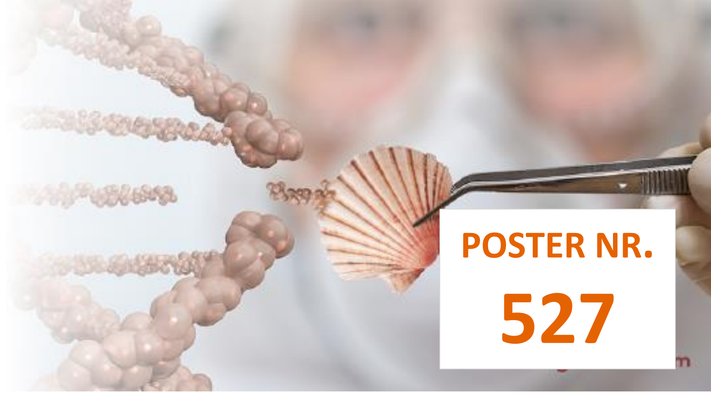

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Reverse Body Fluid Identification Workflow: A Direct to DNA Approach

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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 or saliva; the same principle is applied to clothing or swabs related to victims from sexual assault cases. 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. Many laboratories perform preliminary tests on items where biological material is potentially present before sending a cutting or swab for extraction and subsequent DNA typing (1, 2, 3, 4).

To identify the presence of body 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 amplification were also tested to determine the type of biological evidence in question (whenever enough sample was available) in two independent 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.

On the other hand, it was 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: 1) Evaluation of the implemented workflow in sexual assault samples and 2) Propose a more efficient workflow to be applied to all forensic samples of our laboratory.

Materials and Methods

SAMPLING

- **149 sexual assault** cases occurred in 2020 and 2021
- **647 samples** (female/male victims)

WORKFLOW 1
Semen

Presumptive test:
- Seratec® PSA Semiquant

Confirmatory test:
- Christmas Tree (CT) (for spermatozoa visualization in positive PSA samples)

WORKFLOW 2
Extraction + Quantification

Extraction:
- PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems)
- AutoMate Express™ Forensic DNA Extraction System (Applied Biosystems) - 50 µl elution volume

Quantification*:
- Quantifiler® Trio DNA Quantification Kit (Applied Biosystems)
- ABI™ 7500 Real-Time PCR System (Applied Biosystems)

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If human/male ratio > 10:1 and male component is > 0.5 ng/µl:

Differential Extraction:
- Sampletype I-Sep® DL columns (Biotype) followed by another extraction and quantification with the same reagents/equipments already mentioned

WORKFLOW 2
Amplification

- GlobalFiler™ PCR Amplification Kit (Applied Biosystems)
- Yfiler Plus™ PCR Amplification Kit (Applied Biosystems) whenever the human/male ratio did not allow an autosomal STR profile

In both kits:
- ➢ DNA input according to the quantification data
- ➢ Amplification according to manufacturer's instructions

- Verity® 96-Well Thermal Cycler (Applied Biosystems)
- GenAmp® PCR System 9700 (Applied Biosystems)

* When multiple cuts with identical relevance were made from the same evidence, human/male ratios were taken into account and only the best ones were selected for amplification.

WORKFLOW 2
Electrophoretic separation

- ABI 3500 Genetic Analyser (Applied Biosystems)
- Analysis in GeneMapper ID-X v.1.4

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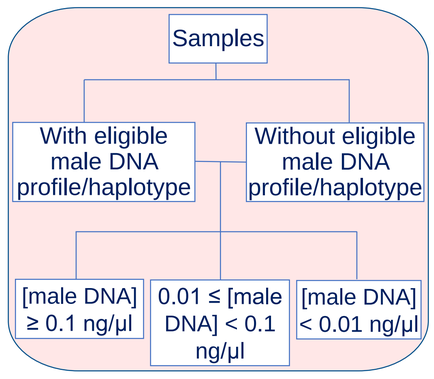
Analytical | Stochastic threshold:
70 RFU | 200 RFU

WORKFLOW 2
Data Analysis



Results

The results revealed that 391 samples (60,4%) presented a genetic profile with no relevance for the analysed cases. This percentage includes samples without male DNA, with DNA profiles that could not be evaluated (namely with the majority of alleles below the stochastic level) or with DNA profiles from male victims (Fig. 1).

The remaining 256 samples had results that can be considered probative for the criminal case, with 90,7% of them (232 samples) with male DNA equal to or greater than 0,01 ng/µl (Fig. 2). The majority of the samples showed a complete genetic autosomal genetic profile or Y-STR haplotype (Fig. 3).

However, for samples with a probative result, only 153 tested positive for PSA (107 of them with the higher concentration of male DNA) (Fig. 4) and a positive CT result was achieved in approximately half of them (80 samples), all with male DNA equal to or greater than 0,01 ng/µl (Fig. 5).

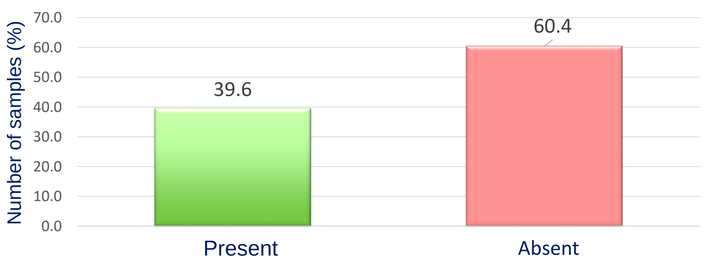


Fig. 1 – Number of samples (%) with the presence or absence of an eligible male DNA profile/haplotype in samples from sexual assault cases (2020 and 2021)

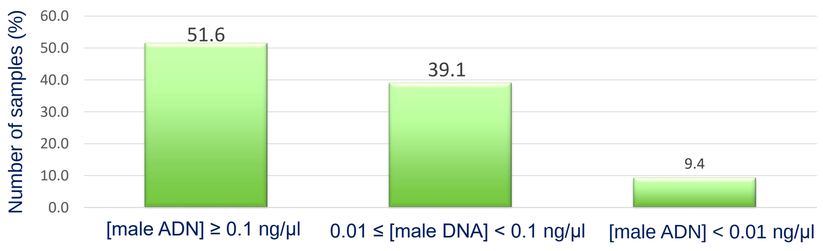


Fig. 2 – Number of samples (%) with eligible male DNA profile/haplotype distributed by male DNA concentration

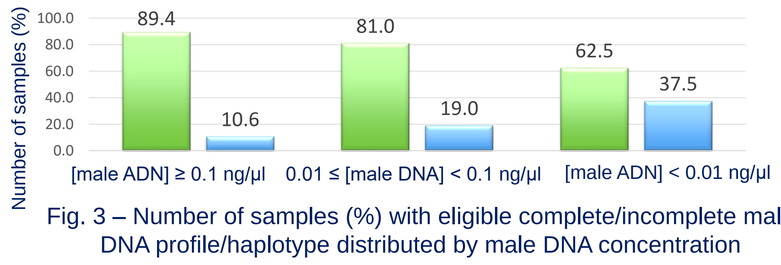


Fig. 3 – Number of samples (%) with eligible complete/incomplete male DNA profile/haplotype distributed by male DNA concentration

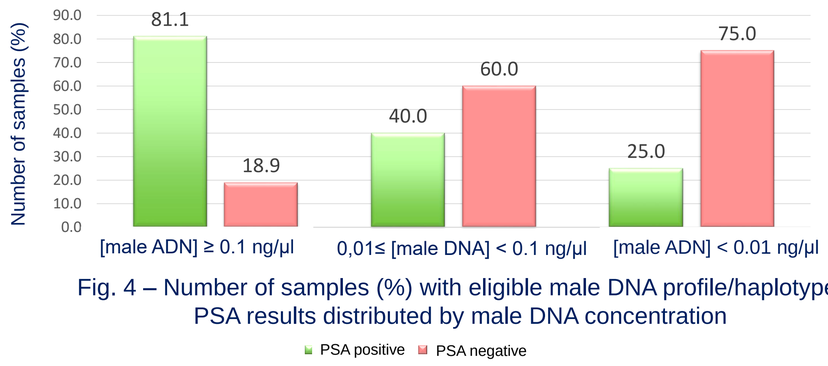


Fig. 4 – Number of samples (%) with eligible male DNA profile/haplotype: PSA results distributed by male DNA concentration

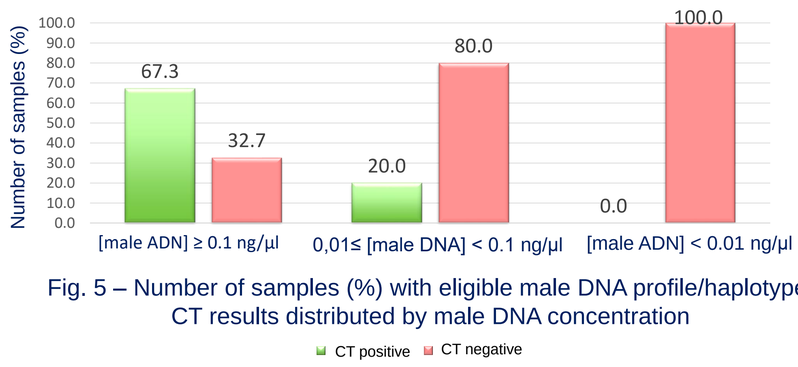


Fig. 5 – Number of samples (%) with eligible male DNA profile/haplotype: CT results distributed by male DNA concentration

Discussion and Conclusion

The quantification of forensic samples is essential to select the best ones for the amplification step. Only samples with male DNA and the best human/male 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/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 agressor to be identified.

It was 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 epithellial 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 oligospermic 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, 5). On the other hand, in samples with very low concentrations of male DNA it is still possible to obtain a male genetic profile/haplptype (even if incomplete) and this may be due to the high sensitivity of the amplification kits.

Therefore, if a ***Direct to DNA*** approach was adopted on these samples (where DNA analysis is performed prior to identification of bodily fluid) (6), 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 implemented techniques and, above all, no loss of information relevant to the judicial process was detected.

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