On the effects of sampling, analysis and interpretation strategies for complex forensic DNA research with focus on sexual assault cases
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Outline

of this thesis

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Although it is only less than sixty years ago since the chemical structure of DNA was discovered by Watson and Crick, and less than thirty years since Sir Alec Jeffreys developed the DNA profiling technique, forensic DNA evidence has become a significant part of many criminal investigations. In the past thirty to sixty years, forensic DNA research has been full with developments that stimulated possibilities and success. Consequently, the demand of DNA profiling has increased, and is reflected by a four-fold increase in the DNA investigations conducted at the department of Human Biological Traces (HBS) of the Netherlands Forensic Institute (NFI) in the last decade (~7,000 in the year 2000 and ~28,900 in 2010). DNA investigations can be performed in a wide range of criminal cases and can be straightforward, but also challenging at times. This is particularly true when one or more of the following circumstances apply: (1) the amount of cellular material present on an evidentiary item is minimal and DNA profiles suffer from low template (LT) amplification artefacts, (2) an unfavourable ratio of victim’s to perpetrator’s cell material occurs due to which the perpetrator’s alleles are missed or masked by those of the victim, (3) a trace contains cell material of multiple and/or related donors which complicates donor inference and (4) the cellular origin is questioned in order to infer the activity that led to deposition of the cellular material while classical presumptive tests do not provide sufficient information.

In this thesis all of the above four issues are addressed. Chapter 1 deals with intimate (vaginal) samples taken from female victims as occurring in sexual assault cases. These specimens often contain minimal amounts of male (perpetrator) cells with a surplus of female (victim) cells. Since spermatozoa can be microscopically well distinguished from epithelial cells, laser microdissection (LMD) has great potential even when only few spermatozoa are present. When LMD is combined with XY-FISH (fluorescence in situ hybridisation) labelling, also female and male cells of similar microscopic morphology can be discriminated and selected. Evidently, LMD requires cells or cell nuclei of good quality. However, we found that the quality of the cells obtained when sampling with the standard cotton swabs is often suboptimal. Therefore, in Chapter 1, the effects of sampling with standard cotton or nylon flocked swabs were compared with the focus on the detection of the male component in post-coital vaginal samples. Comparisons included assessing the detection of proteins that are indicative for the presence of seminal fluid, the efficacy and morphology of the elution of cell material for use in advanced microscopy, the ratio of male to female cells in microscopic slides and male to female alleles in STR profiles.

The results of the experiments described in Chapter 1 led to the implementation of the nylon flocked swabs in the Netherlands Sexual Assault Forensic Examination (SAFE) kit. Around 300 to 400 SAFE kits are sent each year to the NFI, and the last version was developed in the late 90’s and was thus due for a revision. Chapter 2 describes the revision and implementation of this SAFE kit.
and revised SAFE kit are presented including a brief explanation for the adjustments made. The aim of the revision and implementation of the SAFE kit was to improve the forensic investigation of sexual offences with benefits for victims, medical examiners, police investigators and laboratory personnel.

For intimate female samplings the cellular origin of the female cells is evident. However, in some sexual assault samples it is not this clear, such as when a victim and a suspect give contradictory statements regarding the type of contact (intimate or casual) that had occurred between them. Chapter 3 focuses on questions at the activity level rather than the source level, namely what actions led to deposition of the cellular material detected? We investigated whether microbial flora analysis can assist in distinguishing a vaginal origin from other body site origins. First, the vaginal microbial flora of a large set of clinical vaginal samples was explored by a Next Generation Sequencing approach. Secondly, probes targeting families, (groups of) genera or species were selected and a microarray was designed and tested using DNA extracts from both vaginal samplings and specimens of various other body sites. The microarray results and Next Generation Sequencing dataset were then used to assess the potential for a future approach that uses microbial markers to indicate vaginal origin.

The next chapters focus on the challenging issue of LT DNA typing, an issue not limited to sexual assault cases but one that can face all DNA investigations. LT DNA typing refers to samples with minimal amounts of DNA for all or some of the contributors to the DNA profile. Various methods exist to sensitize LT analyses which were applied at the NFI in 138 (4.3%) of the 3,200 cases in the year 2010. A drawback of LT DNA typing is that these techniques are accompanied by stochastic amplification artefacts such as increased stutter, allele drop-in, allele drop-out and heterozygote peak imbalance. It is common practise to use a consensus approach to deal with these LT artefacts. The consensus method is based on replicate analysis of a sample of interest. In Chapter 4 we examined the efficacy of different consensus approaches that differ for the number of PCR amplifications and the requested level of reproducibility. Twenty-three single donor and five two-donor samples, amplified using the Identifiler STR kit were examined. The occurrence of LT artefacts in the various consensus profiles and the effect on database searches (adventitious matches and retrieving the actual donor) were assessed. The most effective consensus approach found was further assessed for more complex mixtures (n=37) amplified using the Next Generation Multiplex (NGM) kit in Chapter 5. Next to the consensus approach we tested an alternative or an additional approach that is based on blending independently amplified PCR products. This pooled sample was subjected to capillary electrophoresis analysis resulting in what we designated as a pool profile. Consensus profiles contain allele calls only, while pool profiles carry both allele calls and peak height information, which may be of use in the (statistical) analysis of the DNA profile. The effects of both approaches, and a combination of both approaches, on the percentage of detected donor alleles,
the number and position of drop-in alleles, the estimated mixture proportion and the estimated number of contributors were examined. In Chapter 6 mock cases were designed based on four of the complex LT DNA samples that were described in Chapter 5. The cases are based on four amplifications of the complex mixture, the consensus profile and include reference profiles of a hypothetical victim and suspect. Eight reporting officers (ROs) at the NFI assessed these mock cases, and classified the results of the comparisons using four categories of evidential value. The interpretations by the ROs were compared to the likelihood ratios obtained from a probabilistic model that allows the interpretation of LT DNA evidence and to the true composition of the mixtures.

The final chapter of this thesis (Chapter 7) highlights the major outcomes of the earlier chapters and gives a general overview of future challenges within forensic human biological research.