Int J Legal Med (2012) 126:157–160
DOI 10.1007/s00414-011-0615-0

TECHNICAL NOTE

Method to predict the chance of developing a male profile out of mixtures of male and female DNA

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Received: 13 October 2010 / Accepted: 25 August 2011 / Published online: 8 September 2011
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Abstract In forensic examination it is a standard to take vaginal swabs from victims of sexual assault for further molecular genetic analysis. Laboratories then are usually confronted with mixtures of lots of female and only a small amount of male DNA. Nowadays it is possible to work with specific Y chromosomal markers after DNA extraction by differential lysis. The determined ratio of autosomal DNA and Y chromosomal DNA can be used to identify the possibility of generating a male profile in these samples.

Keywords Sexual assault · DNA quantification · Spermatozoa · Differential lysis · Plexor® technology

Introduction

Even when the victim can clearly identify the offender, it is very important to provide a forensic medical examination and asservation in all cases of sexual assault [1]. Usually swabs are taken from the rear part of the vaginal cavity of the victim and smeared onto glass slides for optical microscopy after staining [2]. In the case of the cytological detection of sperm, molecular genetic analysis is undertaken to develop a delinquent's profile to then match with a suspect or a DNA database. In cases of sexual assault today, differential lysis is used by default, to separate mixtures of cellular material and sperm. The special morphology of the spermatozoa cell wall allows a separation from other cells by mild lysis [3–6].

The amplification of short tandem repeats (STR) within a multiplex PCR is the standard technology in forensic DNA analysis [7–9]. There are many kits that offer the application with up to 16 STR systems in one reaction. All of them react very sensitively to the amount of DNA. It is, therefore, essential to quantify the template DNA in order to achieve the best results with DNA typing.

The Plexor® technology is a PCR assay, which allows the simultaneous quantification of human DNA and male DNA. During amplification the effective progress results in decreasing fluorescence. Near coamplification from the autosomal human RNU2 and the gonosomal TSPY loci, a third assignment, provides an internal PCR control (IPC). The HY Plexor® Kit is specific to human and primate DNA. Quantification, performed with the HY Plexor® Kit, provides evidence of the success of differential lysis by simultaneous detection of autosomal and Y chromosomal DNA.

The aim of this study was to use the combination of differential lysis and the HY Plexor® technology in order to detect the ratio autosomal DNA/Y chromosomal DNA (RAY) in the fraction of the hard lysis. This enabled the determination of the possibility to generate a full male profile out of a mixture of female and male DNA.

Methods

Mixture studies

In a first step, mixtures of male and female blood with a known content of DNA were prepared with ratios of 1:1,

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1:5, 1:11, 1:15, 1:20, 1:25, 1:50, and 1:100. To additionally enable conclusions of the RAY in mixtures of haploid and diploid cells, mixtures of extracted DNA from sperm and female blood were used in analog ratios. In both preparations the number of cells was calculated by using the DNA concentration derived from the quantification of the basic material and the DNA concentration of both a diploid (blood) and haploid (sperm) cell. The DNA out of the blood mixtures was purified using the QIAamp® DNA Investigator Kit and the provider's protocol for isolation of total DNA from blood.

The DNA mixtures of sperm and blood were purified using the QIAamp® DNA Investigator Kit and the provider's protocol for the hard lysis of differential isolation of total DNA from sexual assault specimens. The DNA was eluated in 50 μl of low TE buffer [10 mM Tris–HCl (ph 8.0), 0.1 mM EDTA].

Both assays were used for quantification of autosomal and Y chromosomal DNA. The ratio of these quotients could then be used as a measure for the success of differential lysis.

The standard curves were generated with the genomic DNA provided from the Plexor® HY Kit. This contained several human male DNA. The standard curves were performed by the amplification products of DNA from 16 pg/μl to 50 ng/μl.

Duplicate amplification was analyzed from each sample and standard probe, and the average results were referred. All PCR were conducted using the Qiagilty® pipett robot. The real-time PCR arrays were carried out using the RotorGene® System under following cycling parameters: 1 cycle at 95°C for 2 min and 38 cycles at 95°C for 10 s, then 60°C for 20 s.

The autosomal target sequence contains 99 bp from a part of the human RNU2 gene located at the long arm of chromosomes 17. The primers are labeled with flourescein.

The gonosomal target sequence is a component of the Y-related TSPY gene. The CAL Fluor® Orange 560 labeled primers elongate a 133-bp long fragment within the DYZ5 region.

The IPC primers bind on a template sequence included in the buffer mix. The target sequence contains 150 bp, and the product dye is CAL Fluor® Red 610. The IPC data reviewing the PCR conditions allow the detection of amplification inhibition. The buffer mix contains IC5, a fourth dye, as the passive reference for the normalization of signals. As a specification parameter, a melt curve for the human and the male product was detected.

The results were imported to the Plexor® HY Analysis Software. The raw data were analyzed using the forensic software application format. The standard curve; human, male, and IPC amplification curves; the cycle threshold (C_t) for each sample; dye; and the melt curves data were generated automatically by the software. Relations between human and male DNA were then calculated.

Results

No sample showed a noticeable value of the IPC. All melting curves showed the expected maximum temperature.

Influence of the mixture ratio concerning the RAY (Fig. 1)

Blood/blood

A RAY up to 10 could be found in mixtures with a ratio of 1:1 and 1:5. Mixtures of 1:10 to 1:25 were associated with a RAY between 10 and 45. Mixtures of 1:50 and above resulted in a RAY of over 45.

Sperm/blood

A RAY below 10 was only seen in the 1:1 ratio. From a ratio of 1:5 to 1:20, a RAY between 10 and 45 resulted. The mixture of 1:25 was the first sample associated with a RAY above 45.

Influence of the mixture ratio concerning the detectability of STR systems (Fig. 2)

A full male profile (11 STR systems + amelogenin) could only be generated in the 1:1 ratio. With a decrease of the ratio of male and female material, a decrease of the number of detected male alleles was seen. Compared to the blood/blood mixtures, the mixtures of sperm and blood showed slightly better results (1:15, 1:50, and 1:100 ratios).

Ratio autosomal DNA/Y chromosomal DNA

In a RAY of up to 10 full male profiles could be detected. Between a RAY of 10 to 45, enough alleles could be detected to create at least a partial profile. A ratio over 45 was associated with the detection of isolated alleles only.

Comparison of the detected RAY and expected RAY

The RAY found and expected in the group of mixtures of male and female blood showed a similar development of up to a ratio of 1:25. In the last two mixtures, an obvious difference between the detected and the expected RAY appeared in favor of higher values in the groups of the detected RAY. In all the ratios of the mixtures of sperm and blood, higher values of the expected RAY appeared compared to the detected RAY.

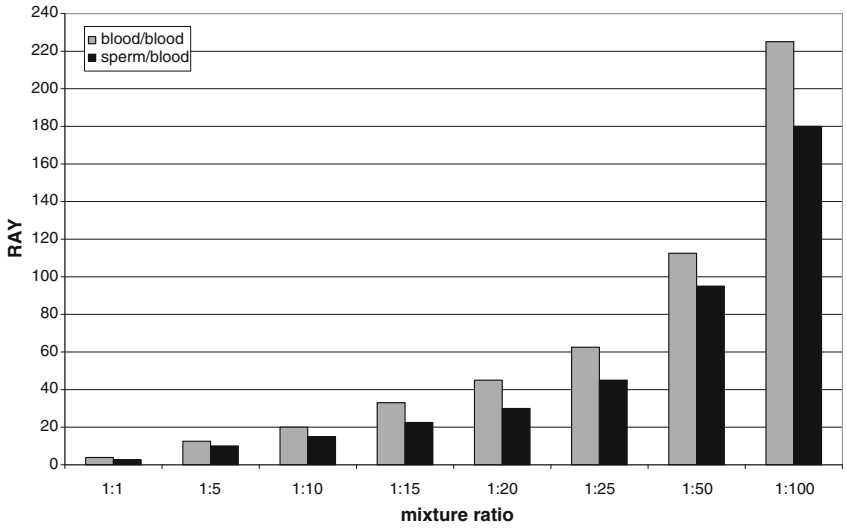

Fig. 1 Development of the RAY in different ratios of the preparation. The RAY increases with an increased ratio of blood/blood respectively sperm/blood

Discussion

In cases of mixtures of DNA, the concentrations of the involved DNA determine to what extent alleles of the minor component can be prepared [10, 11]. Due to this connection the ratio of autosomal and Y chromosomal DNA provides information on the likelihood of detecting male alleles in mixtures of male and female DNA [11].

In the performed mixture studies, it was determined that a full male profile (11 STR systems + amelogenin) could usually be generated with up to a RAY of 10, which was found in balanced mixture ratios of blood/blood and sperm/blood. With an increase of female material, a decrease of detectable male STR systems and an increase of the RAY appeared. Subsequently, only partial profiles could be generated between a RAY of 10 to 45. A RAY over 45 was associated with the detection of isolated alleles only.

The comparison of mixtures of diploid–diploid (blood/blood) and haploid–diploid (sperm/blood) cells revealed that the expected value of the RAY was similar to or below the detected RAY in the mixtures of blood cells and exceeded the detected value in the sperm/blood mixtures. A ratio of 4:1 of autosomal to Y chromosomal DNA in diploid–diploid mixtures can be expected. In contrast, in diploid–haploid mixtures, a ratio of 3:1 should be present. A reason for these discrepancies may be found in the commonly used DNA kits. Multiplex PCRs always make a compromise of all involved primers. On the one hand, the PCR conditions of the Y chromosomal probe can therefore explain the detected undulation. On the other hand, the target gene is a multilocus. As a result of the individual deviation of the number of loci, a natural variation can be expected. Additionally one could conclude that freshly ejaculated sperm not only contains sperm cells but also diploid cells (e.g., urethral epithelial cells).

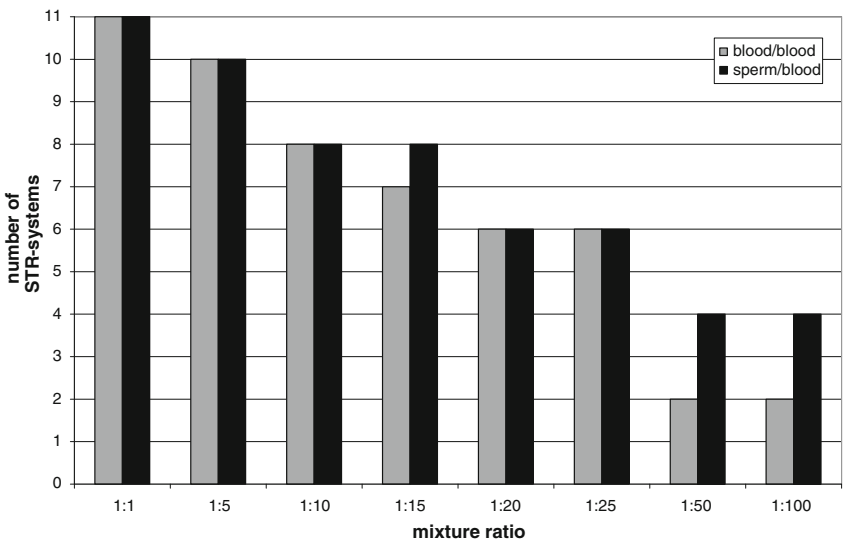

Fig. 2 Number of STR systems in different mixture ratios. The number of detectable STR systems decreases with an increase of the ratio of sperm/blood respectively blood/blood

Conclusion

Through the use of simultaneous amplification of autosomal and Y chromosomal DNA, conclusions about the existence of male, female, or mixed DNA can be made. In combination with the method of differential lysis, it is also possible to predict the likelihood of the development of a male profile. In cases, where no assignments from the authorities for further molecular genetic examinations exist and the generation of a DNA profile is therefore prohibited by law, the RAY can be used to make a recommendation about the purpose and success of additional analysis. The success of the differential lysis can be confirmed, due to the increase of male DNA and a decrease of autosomal DNA in the fraction of the hard lysis. Samples up to a RAY of 45 should be analyzed in general, because a partial profile offers at minimum the chance to exclude a possible suspect.

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