International Congress Series 1288 (2006) 622–624

www.ics-elsevier.com

Use of fluorescence in situ hybridisation and laser microdissection to isolate male non-sperm cells in cases of sexual assault

C. Murray *, C. McAlister, K. Elliott

The Forensic Science Service®, 2960 Trident Court, Birmingham Business Park, Solihull, B37 7YN, UK

Abstract. To assist in cases of sexual assault involving an azoospermic assailant, we have developed a method to identify male (non-sperm) cells present on post-coital vaginal swabs using fluorescence in situ hybridisation, and subsequently isolate them using laser microdissection. We have tested this method on 26 samples taken at a range of times since intercourse (TSI), and have obtained a full male profile from a sample taken when TSI was 24 h.

Keywords: Fluorescence in situ hybridisation; Laser microdissection; DNA; Male epithelial cell; Post-coital

1. Introduction

In cases of rape where vaginal swabs taken from the victim test positive for the presence of semen, but no sperm can be found (i.e. the semen is azoospermic), other cells originating from the perpetrator may still be present. These may include epithelial cells from the ejaculatory duct and urethra, and white blood cells [1]. However, lysis of the harvested cells is likely to yield only the victim's profile due to the large number of vaginal epithelial cells collected on the swab. Analysis of Y chromosome markers could be carried out, however, an autosomal STR profile with which to search The National DNA Database® may be preferable, particularly in no-suspect cases.

Here we describe a method to identify male (non-sperm) cells present in the cell harvest by labelling the X and Y chromosomes using fluorescence in situ hybridisation (FISH), and subsequently isolate them using PALM® laser microdissection.

* Corresponding author. Tel.: +44 121 329 5440; fax: +44 121 622 2051.
E-mail address: caroline.murray@fss.pnn.police.uk (C. Murray).

0531-5131/
doi:10.1016/j.ics.2005.09.109

2. Materials and methods

2.1. Sample preparation

A total of 26 post-coital vaginal swabs from 7 donors were used in this study; the time since intercourse (TSI) of these ranged from 1 h to 24 h. Presumptive testing for semen was carried out on each of the samples using the acid phosphatase (AP) test. Cells were harvested from each swab and resuspended in Carnoy's fixative (3 : 1 methanol/acetic acid). 20 μl aliquots of the cell suspension were added to PEN membrane microscope slides (PALM); these were allowed to age at room temperature for 1–3 days.

2.2. FISH

Prior to hybridisation, slides were treated with protease solution (Vysis) at 37 °C for 12 min, followed by 1% formaldehyde, PBS and 70% ethanol washes. When dry, 10 μl CEP® X SpectrumOrange™ Y SpectrumGreen™ DNA Probe solution (Vysis) were added to the sample area on each slide. A glass cover slip was applied and sealed with rubber solution.

Table 1
Results obtained from post-coital vaginal swabs taken at various times since intercourse, and processed using FISH and laser microdissection to capture male cells

Donor	TSI (h)	Percent male profile obtained (%)	Cells catapulted	Approximate mixture ratio (male : female)	AP result at 2 min
A	1	100	40 good	70 : 1	Weak
A	8	87	32 good + 14 possible	4 : 1	Weak
A	16	80	26 good + 26 possible	3 : 1	Weak
A	20	20	12 good + 14 possible	1 : 2 (major female)	Negative
A	24	0	5 possible + 11 poor	n/a	Negative
B	5	100	74 good	3 : 1	Strong
B	8	100	70 good	1 : 1	Strong
B	16	71	62 good	2 : 1	Strong
B	20	100	80 good	3 : 1	Medium
B	24	100	34 good, 35 possible, 22 single green	1 : 2 (major female)	Medium
C	1	100	68 good + 9 possible	3 : 1	Medium
C	5	94	25 good + 25 possible	7 : 1	Weak
C	8	19	5 good + 16 possible	1 : 2 (major female)	Weak
D	2	93	42 good + 30 possible	6 : 1	Strong
D	5	Search abandoned			Negative
D	8	Search abandoned			Negative
D	16	Search abandoned			Negative
1	8	83	49 good	6 : 1	Strong
1	16	100	77 good	1 : 2 (major female)	Medium
2	8	92	70 good	19 : 1	Strong
2	12	85	61 good + 33 single green	2 : 1	Strong
2	16	23	42 good + 25 possible	2 : 1	Medium
3	8	74	27 good + 20 possible	4 : 1	Strong
3	16	95	62 good	1 : 1	Negative
3	20	100	47 good + 45 possible	1 : 1	Weak
3	24	Search abandoned			Negative

In donor couples A–D, the male was known to have been azoospermic as a result of a vasectomy. Sperm may have been present on swabs from donor couples 1–3; however, only non-sperm cells were used for this study.

Samples were denatured on a heat block at 73 °C for 5 min, then incubated in a humidified chamber for approximately 6 h at 39 °C. Following hybridisation, excess probe was washed off in 0.4 × SSC at 73 °C for 2 min, and 2 × SSC/0.1% NP-40 at room temperature for 1 min. The slides were allowed to air dry in the dark, before 10 μl DAPI II counterstain and a cover slip were applied.

2.3. Laser microdissection and cell lysis

Slides were examined at 400× magnification using a PALM MicroBeam System, which was equipped with appropriate DAPI/Green/Orange filters for detection of fluorescent signals. Cells containing both an X and a Y chromosome were identified by the presence of an orange and a green signal, respectively. The locations of these male cells were recorded and the cover slip and DAPI were removed. The UV laser was then used to cut through the membrane around each selected cell, and catapult it upwards into 55 μl 1 × ABD TE buffer held in the lid of a 0.2-ml bubble top PCR tube positioned above the slide. Tubes were then centrifuged and each had 10 μl proteinase K (125 U/ml) added to it. Samples were incubated at 56 °C for 2 h to lyse the cells, then boiled for 10 min to denature the proteinase K.

2.4. Amplification and analysis

AMPF*l*STR® SGM Plus® PCR was carried out in triplicate at 34 cycles as described by Gill et al. [2], using the maximum volume of DNA extract. Electrophoresis and analysis using Genescan and Genotyper software were carried out as described by Cotton et al. [3]. Consensus profiles were generated from duplicated alleles. Results were based on the proportion of the male donor's profile obtained, excluding any alleles shared by the male and female. Mixture ratios were calculated using peak areas of unshared alleles.

3. Results and discussion

A summary of the results obtained is shown in Table 1.

In general, a good DNA result (> 70% of the male donor's profile) was obtained from all samples which tested positive for AP.

With respect to time since intercourse, sufficient male cells were, or were likely to be, identified in all samples taken when TSI was low (1–2 h). From one donor, 100% of the male DNA profile was obtained when TSI was 24 h. Variation in success rates from different donors is likely to be a result of several factors, including the number of cells present in the semen, drainage of semen from the vagina, and sampling methods.

Mixed profiles were often obtained. The collection of female cells along with the male cells of interest is unavoidable in many cases, due to their relative quantity and their close proximity on the slides. However, in casework, the victim's reference profile will usually be available to aid interpretation of mixed results.

This technique is currently being validated for use in forensic casework and will be of significant benefit in a number of cases where current methods fail to provide a result.

References

[1] O.J. Pollak, Semen and seminal stains, Arch. Pathol. 35 (1943) 140–184.
[2] P. Gill, et al., An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, Forensic Sci. Int. 112 (2000) 17–40.
[3] E.A. Cotton, et al., Validation of the AMPF/STR® SGM Plus™ system for use in forensic casework, Forensic Sci. Int. 112 (2000) 151–161.