AN EVALUATION OF SINGLE LOCUS PROBES IN CASEWORK

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INTRODUCTION

DNA profiling using Single Locus Probes has been in use in casework at the Metropolitan Police Laboratory since November 1988. The probes used are D1S7 (MS1), D7S21 (MS31), D12S11 (MS43A) AND D2S44 (YNH24). These together with the restriction enzyme Hinf1 enable us to produce results that are compatible with other members of the European DNA Profiling group (EDNAP).

Since 1988 approximately 2800 blood samples and 1000 case stains have been examined. Results from these tests have now been analysed and it has been possible to draw some useful conclusions regarding the suitability of various types of staining for DNA profiling.

TYPES OF CASES EXAMINED

DNA profiling provides extremely powerful evidence in body fluid cases but it is expensive and relatively time consuming. Hence it has been necessary to restrict its use to the more serious cases. (table 1.) There are many examples where the body fluids present are suitable for profiling but they have not been examined because of these restrictions.

Table 1. Case types examined in 1990 at the Metropolitan Police Laboratory

CASE TYPE	NUMBER OF CASES	%
MURDER	79	16
RAPE	272	55
INDECENT ASSAULT	76	15
BUGGERY	33	6
ASSAULT	18	4
MISCELLANEOUS	19	4

A wide range of body fluids are acceptable for DNA profiling assuming that intact nucleated cells are present. A high priority is placed on serious sexual assaults and semen is the most commonly encountered type of stain followed by blood. Details of the success rates with different items are given in Table 2.

SEMEN STAINING

The most numerous semen stained exhibits are vaginal swabs. The success rate with this type of item is very high.(a positive result is defined as obtaining a profile which is different from that of the donor of the swabs). The high success rate is probably due to the good quality of the DNA and the absence of contaminants that can inhibit restriction. Many swabs where there were only very low numbers of spermatozoa visible under microscopic

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Table 2. Exhibit types and success rates in 1990

EXHIBIT TYPE	SUCCESS RATE %
semen staining:	
INTERNAL VAGINAL SWABS	92
ANAL SWABS	56
KNICKERS	90
FABRIC/CLOTHING	72
SALIVA/MOUTH SWABS	43
CONDOMS	50
blood staining:	
DENIM	27
KNIVES	83
SHIRT MATERIAL	75
OTHER CLOTHING	53
LEATHER	43
saliva:	
CONTROL SALIVA SAMPLES	59
CIGARETTE ENDS	4
hairs:	
PLUCKED HAIR CONTROL SAMPLES	95
SHED HAIRS FROM CASE ITEMS	16

examination and which yielded less than 100 ng of DNA still gave good quality profiles. For this reason it is difficult to set a lower limit of semen concentration for profiling acceptability. In virtually all cases involving semen the preferential extraction method for separating spermatozoa from epithelial cells is used. Staining in the crutch of knickers also gives a high success rate and this is an option when there is no semen on vaginal swabs.

The lower rates of success with semen on anal swabs and in saliva samples probably reflects the fact that the levels of semen are often much lower. However semen profiles have been obtained from saliva samples taken up to 4 hours after an act of oral sex. Condoms are sometimes encountered in casework and they give an unusually low success rate considering the amount of semen present. This may be due to the fact that semen inside a condom will remain liquid and degrade more quickly than a stain on a dry substrate or that spermicides present in some condoms could inhibit restriction.

BLOOD STAINING

The percentage success obtained from blood staining is less than that from semen. DNA concentration in blood is much lower so a larger stain will be required and the more fragile nature of white blood cells compared with spermatozoa is likely to lead to faster degradation

of DNA in bloodstains. The most suitable substrates for bloodstaining such as knife blades can be easily swabbed and the blood concentrated. Diffuse bloodstains on clothing are difficult to extract and concentrate. The normal protocol uses direct extraction of stain material with SDS and proteinase K . This is very efficient with small concentrated stains but with diffuse stains it yields a large volume of dilute DNA which is more awkward to process.

Denim has been included as a separate category because of the difficulties encountered with restricting the DNA that has been extracted. The dye present in the denim is extracted along with the DNA and it can be difficult to remove. It appears to strongly inhibit restriction enzymes as do the dyes present in some other dark coloured fabrics. Attempts have been made to remove these contaminants with further solvent extractions, dialysis and preparative electrophoresis. These methods have not always been entirely successful. If DNA is excessively coloured after purification it is re-dissolved in 0.2 m sodium acetate and then re-precipitated with ethanol. This often makes sufficient difference to allow the sample to be restricted successfully.

The same reasons probably account for the lower number of positives in the "other clothing" and "leather" categories whereas success with blood on shirt material which is colour fast and has probably been washed many times previously is relatively good.

SALIVA STAINING

Saliva contains cells that are constantly being shed from the lining of the mouth. In cases where blood or hair samples are not available, saliva may be used as a control sample.

Attempts to prepare profiles from saliva stains on cigarette ends etc have met with low success. The small volume of saliva present in stains makes them unlikely to work and no attempts to carry out DNA profiling are made unless a reasonable number of cells can be seen using the microscope.

There have been many requests to attempt profiling of saliva present on vaginal swabs following sexual assaults. The situation here is complicated by the presence of a second body fluid (vaginal) in large excess. As it is not possible to separate the cell types by a preferential extraction a mixed profile will result. The profile of the vaginal material is likely to be very much stronger than that of any saliva and secondary bands may well appear during the long autoradiograph exposures necessary for the weak saliva profile. Therefore at present, profiling on this type of exhibit is not performed.

HAIRS

Plucked head hairs are frequently used as control samples. A sufficient quantity of good quality DNA is usually obtained from approximately 10 hairs. However the success rate with shed hairs recovered from items such as hats and masks is much lower.

CONCLUSIONS

DNA profiling has been remarkably successful at providing very strong evidence from seminal stains especially those from vaginal swabs and knickers. Blood stains have given profiles in the majority of cases although dark coloured fabrics can cause difficulties. Success with saliva stains has been considerably lower. In the absence of a blood sample control, the use of plucked hairs is more likely to give a DNA profile than a saliva sample.