ABOUT A CASE OF DNA ANALYSIS FROM SOME LATENT BLOOD STAINS ON OVERALLS FOUND IN A RIVER.

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INTRODUCTION

A major problem in forensic cases concerns the identification of the nature of blood samples and the extraction of a maximum amount of DNA suitable for PCR reaction. In the most serious criminal cases it is quite frequent to find out that surfaces which were probably stained with the blood of the victims (clothes, car tapestry, etc.), underwent careful washing in order to remove traces of the crime.As we reported earlier, DNA was extracted from 5 ul experimental blood stains, hand-dried and machine washed,then amplified by PCR and finally typed by horizontal acrylamide gel for the three most common loci (HLA, D1S80 andAPO B), with a yield of 74% positive results.In this paper we report a real case linked to a murder where the evidence to be analysed was a mechanic's overalls collected from a river. Some suspected halos of probable blood were first singled out by means of catalytic tests then amplified and typed by PCR for the three VNTR's, showing a match with the victim's blood.

MATERIAL AND METHODS

The sample was a mechanic's overalls thrown away in a river by the suspect, just after the murder, hoping to eliminate the evidence of the crime. It was found by the police, in a container where all the trash collected from the river was deposited, within a river water draining plant. An accomplice suggested the police to go there, and look for the overalls. He was the author of another murder commited on that same day and had provided investigators with precise indications to prove he did not commit the first murder. The overalls were brought to the lab still damp and besides the many holes and rips, probably caused by its being thrown into the river, the front part had some small suspect areas, though nothing would make us think that it was blood. Just the same, we decided to test these suspect areas to see if there was some blood.

BLOOD IDENTIFICATION

Sangur test

The suspect areas underwent the Sangur Test strips (Boehringer Mannheim, Sangur Test Strips Cat. No.126233) which were previously dipped in a physiological solution (0.93 % NaCl).

Benzidine Test

Small cloth portions scraped from the same areas were put on a filter paper and treated with 1-2 drops of the Benzidine reagent (glacial acetic acid saturated solution of benzidine base) followed by 1-2- drops of 3% hydrogen peroxide.

Luminol Test

The stains were subsequently assayed by the Luminol Test. Three compounds were used in order to perform the test:1) 0.5 gr. of Luminol (3-Aminophtalhydrazide); 2) 25 gr. of sodium carbonate; 3) 3.5 gr. of sodium perborate in 500ml. of distilled water. The stains were sprayed with a 2% hydrochloric acid solution and then with a mixture of the previous three compounds mixed together.

DNA EXTRACTION

The stains which were positive to the presumptive tests were cut into small pieces and placed into a 2 ml microcentrifuge tube where an aliquot of a yellow resin portion (DNA SINGLE TUBE - BIOTECX Houston, Texas cat.NO.BL5500) had previously been added. The following steps of extraction proceeded according to the standard phenol-chloroform extraction protocol and the instructions given by Biotecx Bullettin NO. 8,1990.

AMPLIFICATION

Amplification and typing of the HLA-DQ alpha locus.

DNA was amplified by PCR using the Amplitype HLA-DQ alfa Forensic DNA Amplification and Typing Kit (Cetus Corporation,Emeryville, CA).

Amplification and typing of the VNTR's loci D1S80 and Apo B.

Amplification of the VNTR's loci D1S80 and Apo B was carried out by the methods previously described by Budowle et al. and Boerwinkle et al., respectively. The electrophoretical separation of the P.C.R. products was perfomed using a high resolution horizontal rehydratable polyacrylamide gel as described by Budowle et al.. The gel was exposed through silver stain.

RESULTS

The preliminary blood tests using both the Sangur strips and the Benzidine reaction produced negative results,while the treatment with Luminol was positive and it showed the areas originally stained with blood by producing a characteristic bluish-white luminescence. As to amplification and further typing of the three loci taken into account, HLA-DQ alpha provided results which could not be interpreted since they were probably due to contaminants and inhibitors connected to the substances the evidence got in contact with in the river.Very good results, on the other hand, were obtained both with D1S80 and with APO B with well-defined bands which proved to match with the victim's blood.

DISCUSSION

Besides confirming the results achieved in our previous experiments, this case stresses once more that in order to solve crime cases, it is very important to have a proper analytical strategy which enables to type more possible loci and, among them, especially those which are highly polymorphic. It also confirms that the use of Luminol is practical, effective and extremely sensitive and suited for aged and/or latent blood stains.It also indicates that choosing an appropriate method for DNA extraction is of basic importance.
Finally this case shows the high potentials of forensic biology. Therefore it is necessary and appropriate to analyse evidence that may appear traceless and could be discarded after a superficial analysis because they are considered useless for investigation purposes.

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