Blood Group Typing and PCR-Analysis in Stored Blood Samples

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Checks concerning the identity of stored blood samples may be requested by the court in cases where the blood alcohol concentration (BAC) or the results of toxicological analysis are doubted by the defendant, who may subsequently maintain that blood samples were mixed up during analysis. In most cases, linkage of original and comparison sample provide no difficulties. Sufficiently high statistical probabilities to satisfy even the high-level requirements of criminal law are attained by typifying an adequate number of membrane, serum and enzyme systems. Problems concerning the identity check may arise if:

- a toxicological analysis was performed beforehand, due to the fact that quantitative analysis requires homogenisation by means of Ultraturax, which leads to a virtually complete destruction of erythrocyte membranes and membrane systems. Also, serum can no longer be separated by centrifugation. Our experiences show that, on top of this, enzyme systems are suppressed.
- only few systems can be determined due to hardly adequate sample volumes and/or long storage times. Note that no correlation exists between storage time and preservation (Osterhaus, E., Birkner, P., 1985; Sachs, V., et al. 1988).
- less than 2 ml of sample remains for analysis

The following results will show that a definite statement (exclusion/no exclusion) can be made in problematic cases by means of supplementary PCR analysis.

Materials and methods

Blood samples were stored at 4°C after BAC analysis. Systems which are utilised in routine identity checks could only be successfully typified in very few of the cases mentioned below. AB0, MNSs, Rh, Kk, P, Gm (1,2), Km (1), Hp-sub, Gc-sub, Tf-sub, Pi, a2HS, SEP, PGM, AK, ADA, GPT, 6-PGD, GLO, EsD. If phenotype frequency was too low or exclusion was not definite in this first analysis, DNA-PCR-Analysis was performed.
DNA was isolated from the remnant blood samples of serological examination using standard methods (Manniatis et al. 1982). The amount of DNA was determined by comparison of the ultraviolet fluorescence of an aliquot of each sample with known quantities of lambda DNA. Size was estimated on agarose gels and in some cases southern blotted and hybridized with single locus probes MS43a or MS31 from Zeneca. PCR reactions were carried out using 20 - 50 ng DNA. Amplification and typing of the HLA-DQα locus followed the recommended protocols (Amplitype™ User Guide). Primer sequences for the D1S80 locus were described by Budowle et al. (1991) and for the YNZ22 (D17S30) locus by Horn et al. (1989) . Reaction

mixtures (total volume 50 µl) consisted of 0,2 mM dNTPs, 10 mM Tris-HCl (pH 8,4), 1,5 mM $MgCl_2$, 50 mM KCl, 0,25 µM each primer and 2,5 U Taq DNA polymerase. No paraffin was layered over the reaction mixtures. Amplification was carried out in the GeneAmp PCR System 9600 from Perkin Elmer Cetus. Temperature cycling conditions were as follows: D1S80, denaturation 15s, 95°C, annealing 15s, 66°C, extension 30s, 72°C, 30 cycles, final extension 10 min). YNZ22, denaturation 30s, 94°C, annealing 30s, 63°C, extension 120s, 72°C, 30 cycles, final extension 10 min. PCR products were separated on 2% agarose gels in TBE puffer at 3,5 V/cm for 7 h. The bands were visualized by staining with ethidiumbromid.

Results and discussion

	storage time (months)	notes	blood group systems	frequency	frequency including PCR-analysis
1	5		ABO, Rh	1 : 5	1 : 110000
2	5		ABO, Rh	1 : 5	1 : 37 mio.
3	9	2x tox. analysis	ABO, Rh, Kk, P, PGM, AK, GPT, EsD,	1 : 107	1 : 15 mio.
4	8		ABO, Rh, Kk, P, PGM, AK	1 : 25	1 : 625000
5	14	2x tox. analysis, rem. volume 1 ml	ABO indirect, AE	1 : 2	1 : 213 *
6	9	2x tox. analysis, rem. volume 1,5 ml	ABO indirect, AE	exclusion	exclusion
7	14		ABO, Rh, Kk	diff. A_1/A_2	1 : 100000

*PCR-Systems: HLA-DQα, D1S80

As you can see from the chart, cases 1,2 and 4 provided unsatisfactory statistical results in spite of empty case history and sufficient sample volume, as only between 2 and 6 systems with insufficient phenotype frequency could be typified.

Case 7 was originally typified as A_1,while the blood group ID card, which had accompanied the blood sample, said A_2. The subsequently drawn comparison sample was typified as A_2 in the ABO system. However, PCR analysis proved beyond doubt that original and comparison sample were of the same origin. Apart from this, it could be shown again that changes in blood group properties can occur through storage, which was first reported by Thomsen, O. and Friedenreich, V. in 1926 and now confirmed by us by means of PCR.

Due to complete haemolysis and between only 1 and 1,5 ml remnant sample volume in cases 5 and 6, samples were split and a blood stain was created for ABO typification. Indirect blood group determination was carried out using absorption-elution technique (AE). In doing so, the constellation of case 6 made exclusion possible: blood stain/original sample 0, comparison sample B. The remainder of the sample was used for PCR analysis, where exclusion for all

three systems could be demonstrated. In this case, there was no real mixing-up of samples. According to documentation, the defendant's claim that he had not been at the wheel of his vehicle at the time in question was confirmed. Someone had apparently furnished the defendant's name instead of his own.

Summary

Several reports on the subjects frequency and importance of identity checks concerning stored blood samples, the difficulty of blood group determination in these samples as well as DNA analysis by SLS or MLS (DNA fingerprinting) have been made (literature survey Henke, L. et al, 1990, Kleiber, M. 1987).

As shown by our results, DNA analysis with PCR-systems HLA-DQa, D1S80 and YNZ22 provides adequate results even in cases where prior toxicological examination, putrefaction of the sample or small sample volume have occured. Cases in which classic methods of blood group determination and DNA fingerprinting on samples stored at 4°C may lead to problems.

More and more of the samples taken in the area served by our institute and analysed by us for BAC are subsequently ordered to be examined toxicologically and the results, if positive for drugs, are often doubted; an increasing number of samples which were again analysed toxicologically are then sent to our institute to have their identity checked. On the basis of experiences made by us, we suggest that PCR analysis is the method of choice in cases such as the above.

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7. PCR methodology