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BOOK OF ABSTRACTS

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A 1 VALIDATION OF THE USE DNA AMPLIFICATION FOR THE ANALYSIS OF FORENSIC SAMPLES BY COMPARISON WITH TESTS USING NON-AMPLIFIED DNA POLYMORPHISMS.

L. Perlee, I. Balazs*. Lifecodes Corporation, Valhalla, NY, USA.

The utility and reliability of the use of DNA amplification for forensic DNA samples was tested using material already analysed with single locus RFLP. The general procedure consisted of: 1. Determine the quality and quantity of DNA using a yield gel, followed by slot blot hybridization to chemiluminescent probe for human and bacterial DNA. 2. Amplification of sequences from the X and Y chromosomes. 3. Amplification of polymorphic sequences using 1 to 10 ng of DNA sample. 4. Gel fractionation of amplified product. 4. Confirmation of the specificity of the amplified product by hybridization with chemiluminescent probe to an internal sequence. Overall there was good agreement for the matches and non-matches obtained by DNA amplification vs. previous testing with RFLP. However, some samples from sexual assault cases showed the presence of female DNA more readily with DNA amplification. Approximately one third of the samples did not contain sufficient intact DNA for RFLP testing. DNA amplification of these samples produced interpretable results in about half of them while other samples could only be typed for the presence of male DNA. Conditions for the amplification of each sequence were optimized for maximum sensitivity and specificity. As a consequence, experimental conditions for the simultaneous amplification of multiple sequences did not have adequate sensitivity.

A 2 Investigations to improve allele definition in the Collagen 2A1 system (AMPFLP) *

The continuous band frequency distribution associated with highly polymorphic single-locus DNA systems means that it is very difficult to define alleles. A standard ladder must be used to measure fragment sizes and to group the fragments into bins for frequency calculations. This is no longer a problem if AMPFLP systems such as Collagen 2A1 are applied where the difference between the repeats is such that individual alleles can be differentiated. The Collagen system is slightly more complicated than other systems in that the repeat size varies between 31 and 34 basepairs. This means that in some cases only a 3 basepair difference will be seen between different alleles. This difference can be detected only at the lower molecular weight range so that a standard ladder consisting of a mixture of all alleles is necessary. This method has previously been used to improve the definition of the single-locus probe YNH24. The optimal conditions are different for each AMPFLP system but the principles are demonstrated here using Collagen 2A1 as an example.

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A 3 Investigations on the Forensic Application of 4 AMPFLP systems *

The use of DNA investigations in forensic science has dramatically improved the amount of information which can be obtained from mixtures of body fluids. However this is dependant on the amount and quality of the DNA present in the sample. If the DNA is highly degraded then single-locus probes, which can only be successful when high molecular weight DNA is present, will be of no avail. The amplification of small amounts of degraded DNA using PCR techniques is a highly promising method for use in these circumstances. However we know from past experience that many artefacts and problems can arise under casework conditions. Mixtures of body fluids have been investigated under laboratory and casework conditions to evaluate the 4 AMPFLP sytems, APOB, YNZ22, COL2A1 and PMCT118. The systems have been investigated to highlight problems such as artefacts and limited DNA yield under certain conditions which may prove to be critical under casework situations.

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A 4 SDS-PAGE TYPING OF HLA-DQA1 AND pMCT 118 AFTER PCR AMPLIFICATION

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Polimerase chain reaction followed by SDS-PAGE in miniaturized non-denaturing gels permits in some cases the identification of single base-pair substitutions in small DNA fragments and therefore, the study of human DNA polymorphisms. The use of automatized systems such as the PhastSystem and particularly recommended since temperature control is essential for recognizing some of the variation.
The usefulness of the system is illustrated by typing the HLA DQA1 locus, and the minisatellite recognized with the probe pMCT 118 (locus D1S80).
A simple chelating resin extraction followed by amplification, electrophoretic separation and silver staining allows the study of HLA DQA1 and pMCT 118 variation in only 5 hours. For HLA DQA1 additional variation to that provided by comercially available dot blot-ASO probes systems can be obtained. Population data from Portuguese and Spanish populations are also included.

A5

GENE FREQUENCIES OF APO B ALLELES IN A SAMPLE OF RANDOM ITALIAN INDIVIDUALS (CENTRAL AND SOUTHERN ITALY)

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A population survey on some hypervariable DNA markers detectable via enzymatic amplification (PCR) is under way in our laboratory. Here we refer on the distribution of APOB alleles in a sample of 100 unrelated individuals from Central-Southern Italy. Genomic DNA samples were amplified by a standard PCR procedure, using a couple of flanking oligonucleotide primers (Boerwinkle et al, PNAS USA,86,212-216,1989). Amplified products were separated by agarose (2.4% w/v) electrophoresis. Individual alleles apparently originated from iteration (29 to 47 repeat) of one short core sequence. A favorable distribution of alleles sizes in the APOB system resulted in safe amplification of heterozygous with minimized risks of false homozygosity. A home -made ladder formed by admixture of some very common allele products was helpful in classifying the amplified products. More than ten common phenotypes were identified and relevant gene frequencies were ascribed to each. A fair compliance with the Hardy Weinberg rule was observed.

A6

STUDY OF THE HLA DQα POLYMORPHISM IN THE POPULATION OF CATALONIA. SPAIN

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In the last years, the distribution of PCR polymorphisms has been analyzed in population groups in some areas of the world. However, in Catalonia HLA DQα studies by PCR (polymerase chain reaction) have not been reported. In this paper we show the results of a survey of this polymorphism in more than 100 samples from people living in the authonomous country of Catalonia.

The study was carried out with random blood samples obtained from the "Hospital Clinic" Blood Bank. The HLA DQα types (and subtypes of allele 1) were recognized by PCR amplification of specific DNA sequences, using allele specific oligonucleotide probes (ASO probes) and reverse dot-blot methodology.

Calculation of the phenotype distribution and gene frequencies indicates that, there is no deviation from the Hardy-Weinberg equilibrium. The six alleles define twenty-one genotypes with gene frequencies ranging from less than 0.1 to 0.2 except for allele 4 in which the frequency is around 0.3. We have observed few differences in the distribution of some variants between Catalans and other caucasians, and larger differences in relation to other ethnic groups.

We have performed the HLA DQα polymorfism in more than 50 paternity tests. All of the paternities practically proved (W ≥ 99.73 %) were confirmed. The percentage of exclusions proved the a priori chance exclusion value.

A7

Automated Analysis of Fluorescent Amplified Fragment Length Polymorphisms for DNA Typing . J. Robertson, Th. Schäfer, M. Kronick

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The polymerase chain reaction and silver staining of polyacrylamide gels has made it possible to analyze a small amount of DNA (1 to 20 ng) without Southern blotting and the use of radionucleotides, and to obtain a permanent record of the electrophoretic separation, respectively. However, lane-to-lane differences in migration rates of the DNA fragments, and the desire to obtain data for five to six VNTR loci from one sample, provides the basis for the employment of a fluorescent method. The Applied Biosystems 362 GENE SCANNER allows to utilize four fluorescent dyes per lane, one of which can be used to provide the signal for an in-lane size standard, while the other three dyes can be used to report on three different VNTR loci.

Here, we report the allele frequencies for three VNTR loci: D1S80 (pMCT118), D17S5 (pYNZ 22), and COL2A1 obtained from 100 randomly selected Caucasians. The sizes of the alleles were automatically calculated by the software. To test the accuracy of the analysis, we utilized previously published data on the same set of samples for the D1S80 locus (Budowle et al., Am. J. Hum. Genet. 48: 137-144, 1991). The allele designations obtained by the manual and automatic methods correlated with one another. Similar quality data were obtained from DNA samples isolated from either liquid blood or dried blood-stains.

A8

USE OF PCR FOR FORENSIC ANALYSIS OF DNA FROM FORMALDEHYDE FIXED AND PARAFFIN EMBEDDED HUMAN TISSUE

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The development of the polymerase chain reaction (PCR) method of specific DNA amplification has made possible the analysis of small quantities of DNA extracted from various tissues such as blood, hair roots, blood ans semen stains, bone.

In this report, we describe a method to extract DNA from tissues (heart, muscle) formaldehyde fixed and paraffin embedded.

Isolated DNA was then typed for HLA DQ alpha and we compared the genotypes obtained on this tissues with those of the blood of the corresponding victims stored at -20°C.

Using the HLA DQ alpha PCR typing, the authors show that DNA can be analysed from tissues prepared for routine histopathological examination.

A 9 SEX IDENTIFICATION OF FORENSIC SAMPLES USING PCR ANALYSIS FOR THE PRESENCE OF Y-CHROMOSOME SPECIFIC DNA SEQUENCES.

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Prompted by a case in which a child had been burnt to death, and the abdominal part charred beyond recognition of the sex, we have analysed the possibility of using in vitro amplification, by polymerase chain reaction (PCR), of Y-chromosome specific DNA sequences as a means of sex identification based on forensic samples.

In the present case DNA was isolated from intact vertebrae dissected from the body, and PCR analyses were performed with primers for repetitive as well as unique Y-specific DNA sequences, including part of the coding sequence of the gene SRY, which encodes the testis determining factor.
Primers for unique X-chromosome specific DNA sequences were included as control, as were DNA samples of known origin, both male and female.

Y-specific DNA sequences could not be demonstrated in the sample from the body in question, in agreement with the police report of a missing two year old girl.

A 10 Comparison of Population data using 3 AMPFLP systems *

Populations studies have been carried out using the 3 AMPFLP systems APOB, YMZ22 and PCMCT118 on random individuals from the Münster catchment area. Blood samples were investigated using specific primers and the PCR technique. Population frequencies obtained have been compared with other available population data to test for any significant differences. The advantage of the AMPFLP system is that definite alleles can be identified numericaly as in traditional electrophoresis systems. Family studies have been carried out to compare the results of single-locus probes with those obtained using AMPFLP systems with special reference to mutation rates and exclusion power of the systems.

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A 11 THE DEVELOPMENT AND EVALUATION OF NEW GENETIC MARKERS FOR THE APPLICATION OF PCR TO FORENSIC CASEWORK.

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Use of the polymerase chain reaction (PCR) for the analysis of biological forensic samples allows rapid typing of minute amounts of DNA. Until recently, only the AmpliType HLA DQα system was available for casework. While the HLA DQα Pd value of 0.94 can be combined with the Pd values of conventional forensic serological markers, it was desireable to increase the power of discrimination of PCR-based systems. We have developed a PolyMarker system in which 5 genetic markers (Gc, gypA, Gγ, LDL and D7S8) are co-amplified with DQα. The 5 markers are typed using the same reverse dot blot technology introduced in the AmpliType kit. The combined Pd values for DQα and these 5 markers are 0.9995-0.9997, depending on the population. Another type of PCR-based marker analysis has been termed "ampflps". Regions of DNA containing a variable number of tandem repeats (VNTR) are amplified, and the resulting PCR products are typed directly by gel electrophoresis. We have identified 29 alleles of the D1S80 locus and generated population data from over 300 individuals using a D1S80-specific allelic ladder. The Pd value for this marker ranges from 0.95 to 0.98, depending on the population. We also are developing and converting a gender identification system to the reverse dot blot format. This system may aid the interpretation of mixtures in addition to determining the gender of a sample donor. In addition to our work on the development of these markers, we are evaluating the effects of DNA degradation and mixed samples on the amplification and typing of the PolyMarker system and several ampflp loci.

A 12 AUTOMATION OF DNA PROFILING BY FLUORESCENT LABELLING OF PCR PRODUCTS

K.M. Sullivan, S. Pope and P. Gill.

The detection and characterisation of small VNTR loci has been automated by the use of fluorescently labelled PCR primers, coupled with real-time laser detection of the products during electrophoresis. This system has been successfully applied to the loci D1S80, ApoB and Collagen 2AI and is capable of resolving amplified alleles differing by a single repeat of 16bp. For example, in a population survey of locus D1S80, 10ng DNA samples from >100 British Caucasians were analysed and the fragment sizes fell in 22 distinct groups (maximum range 7bp), defining the allele sizes, approximately 16bp apart. These data indicated that the overall discrimination power of this locus was 0.935 with a heterozygosity of 80.6%.

Multiplex amplification of these small loci and of microsatellites is discussed, as is the utility of automated DNA profiling in the analysis of casework material from a variety of different sources.

A 13

AUTOMATION OF THE AMPLIFICATION AND SEQUENCING OF HUMAN MITOCHONDRIAL DNA

K.M. Sullivan, R. Hopgood and P. Gill.

Mitochondrial DNA in humans displays considerable sequence variation, much of which is clustered in two areas within the control region. Direct analysis of this DNA has been automated by a two stage PCR amplification protocol coupled with direct analysis of the products by an automated DNA sequencer. This approach has enabled sequence information to be generated from as little as 3cm of hair shaft.

Discussed is the application of automated mitochondrial sequencing in the identification of degraded human remains, and of other evidence types which are difficult to analyse.

A 14

PCR TYPING INCLUDING HIGH RESOLUTION GEL ELECTROPHORESIS REVEALS NEW ALLELES IN THE COL2A1 VNTR.

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The VNTR 3' to the collagen type II gene (col2A1) may be amplified in the polymerase chain reaction (PCR). Published PCR typing of this locus have shown a relatively poor polymorphism with three relatively common as well as two rarer alleles giving a heterozygozity of approximately 56%.

We have applied high resolution gel electrophoresis (sequencing gel) to separate the alleles in the PCR products of the col2A1 VNTR. This technique allows subtyping of the common alleles, thereby increasing the informative value of this VNTR substantially: a heterozygosity of 79% and a PIC of 0.77 in a Norwegian population material. Inheritance pattern in a small family material was in good accordance with Hardy-Weinberg expectations.

Thus the col2A1 VNTR may be included in the battery of highly informative PCR-able VNTRs.

A 15

STUDY OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM PMCT118 LOCUS IN CHINESE AND APPLICATION IN FORENSIC BIOLOGY

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The frequency distribution of amplified fragment length polymorphism (Amp-FLP)of pMCT118 locus among 98 unrelated Chinese and study on some problems about the examination of forensic biological evidences are described.With polymerase chain reaction(PCR),mini-polyacrylamide gel electrophoresis and silver stain, very smallamount of genomic DNA (1ng each sample) has been successfully amplified and detected in 12 hours.23 alleles, ranging from 340 to 780bp in size and from 0.005 to 0.3 in frequency, were detected in 98 unrelated Chinese. The heterozygosity was 79%. Genetic characteristics was consistent with Mendelian law. The preparation of micro samples and its application in frensic biological evidences such as mixed stains, single hairs and saliva are discussed. 20 cases have been examined using the method.

A 16

PARENTAGE TESTING USING DBP IN SOUTH AFRICAN POPULATIONS.

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Serum samples from 652 mother-child-alleged father trios in South African (S.A.) Caucasoids, Cape Coloureds and S.A. Negroes were tested. IEF in ultrathin polyacrylamide gels, with an ampholyte pH range of 4.5 - 5.4 was used. Visualisation of bands was by immunofixation with monospecfic antiserum. The power of exclusion (P.E.) was calculated by counting the number of cases excluded with DBP out of the total number of excluded cases, using 13 genetic systems, including HLA. The gene frequencies of the DBP alleles were estimated by direct counting and used to calculate the expected power of exclusion.

Table of gene frequencies and observed power of exclusion

	S.A. Caucasoids n = 132	Cape Coloureds n = 250	S.A.Negroes (Xhosa) n = 244
1S	0.5720	0.2820	0.0697
1F	0.1402	0.5260	0.8217
2	0.2802	0.1600	0.0574
AM	0.0	0.0200	0.0307
AB	0.0	0.0040	0.0143
variant	0.0038	0.0080	0.0062
P.E.	21.4%	32.2%	15.6%

DBP is a useful system for paternity exclusion in non-Caucasoid races of South Africa, particularly the Cape Coloureds.

A 17 DNA TYPING FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES.

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HLA alleles could be defined using polymerase chain reaction (PCR-DNA typing). As previously reported, the PCR-restriction fragment length polymorphism(PCR-RFLP) method is the most useful to define HLA-DQA1, DQB1, DRB1 and DPB1 alleles.

In this report, we demonstrated DNA typing from formalin-fixed, paraffin-embedded tissues. DNA was extracted from fixed and embedded tissues as follows: each section was extracted twice with octane to remove the paraffin and was followed by two washings with 100% ethanol to remove the solvent. After the samples were dried in a vacuum, they were suspended in a digestion buffer containing proteinase K. The samples were then incubated for 3 hours at 55°C and then again at 95°C for 8 minutes to inactivate the protease. DNA from the paraffin block was amplified by the PCR procedure with Taq DNA polymerase. After amplification, aliquots of the reaction mixture were digested with various enzymes, all of which had a single or non cleaved site, depending on the allele in the amplified DNA region. This typing from paraffin-embedded tissues using PCR is useful in forensic science practices.

A 18 HLA-DQA1 and HLA-DPB1 GENE POLYMORPHISMS IN THE JAPANESE POPULATIONS.

A. Ichinose*, M. Ota, H. Inoko, K. Sato, K. Tanaka, S. Kiyono, H. Fukushima. Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, JAPAN.

Genetic polymorphism in the HLA class II region has been identified by enzymatic amplification of specific DNA sequences using the polymerase chain reaction(PCR). In this study, we have determined HLA-DQA1 and -DPB1 alleles in 150 unrelated healthy Japanese individuals by using the modified PCR-RFLP method(1). In DQA1 alleles, 36 combinations including 8 homozygotes and 28 heterozygotes can be unequivocally determined. DQA1*0301 was the most frequent(61.3%) allele. In DPB1 alleles, 189 out of 190 combinations including 19 homozygotes and 181 heterozygotes could be determined. DPB1*0501 was the most frequent(55.3%) allele.

Reference
1. M.OTA, et al., Tissue Antiges, in press.

A 19 STUDY OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM APO B LOCUS IN CHINESE AND APPLICATION IN FORENSIC BIOLOGY

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The polymorphisms of apoliprotein B (apoB) VNTR loci were typed rapidly using polymerase chain reaction(PCR). Eleven alleles were readily detected in 100 unrelated individuals, with a heterozygosity of 70%. The allele frequencies were from 0.5% to 52. 5%. The amplified fragments were from 600 to 1000bp in size. It shows that DNA typing can be obtained from biological materials such as semen stains, mixed stains, various tissues, blood stains and single hairs.

A 20 THE USEFULNESS OF CHELATING RESINS FOR DNA EXTRACTION FROM FORENSIC MATERIAL PRIOR TO PCR AMPLIFICATION

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Different chelating resin and chelating agents were compared for DNA extraction from forensic samples (including bloodstains and root hairs) prior to PCR amplification and electrophoretic typing of HLA DQ A1 and pMCT 118.
The extraction of DNA from old and minute bloodstains and root hairs has proved to be more efficient using chelating resin when compared with other phenol-chloroform and proteinase K methods (80% positive results using the chelating resin compared with 20% using phenol-chloroform from minute 10 years old bloodstains).
The observed results are in agreement with the protective role for chelex suggested by Singer-Sam et al. [1].

REFERENCES
[1] Singer-Sam, J.; Tanguay, R.L.; Riggs, A.D. Use of Chelex to improve the PCR signal from a small number of cell. Amplifications 3:11, 1989.

A 21 APPLICATION OF HLA-CLASS II GENOTYPING BY THE MODIFIED PCR-RFLP METHOD TO THE FORENSIC SCIENCE.

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Highly polymorphic HLA systems have been used in paternity testing and to identify individuals in forensic practice. Recently HLA alleles have been determined using the polymerase chain reaction. Also, we recently reported on the modified PCR-RFLP methods(1,2,3), which were legible and easy to use in the accurate definition of HLA class II (DQA1, DQB1, DRB1 and DPB1) alleles. In this study, we report the usefulness of this method to solve forensic problems. This method allows discrimination of 36, 91, 561 and 189 combinations including homozygotes and heterozygote of the DQA1, DQB1, DRB1 and DPB1 alleles respectively. HLA-Class II genotyping could be defined by employing DNA hair samples, a small volume of whole blood , old dental pulp and bone marrow. This method is technically simple and reproducible without the need to use radioactive or nonradioactive SSO probes.

References
1. M,OTA, et al., Tissue Antigens, in press.
2. N,NOMURA et al., Tissue Antigens, in press.
3. M.OTA, et al., Tissue Antigens, in press.

A 22 SIMULTANEOUS DNA ANALYSIS OF HLA-DPB AND -DQB LOCI FROM SINGLE HAIRS: A CRIMINAL CASE REPORT.

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PCR amplification of specific DNA target sequences allows the analysis of polymorphic loci from forensic samples such as single hair. We report a case concerning five hairs with root found on the hands of a murdered man. Investigation was performed on these samples and on plucked hairs from both the victim and suspect. The unknown samples were examined one by one and DNA derived from roots was amplified for HLA-DPB locus. In addition, DNA was obtained from the shaft of the same hairs, enough to amplify a different polymorphic locus as HLA-DQB. Due to the small amount of DNA recovered from these portions 30 cycles of amplification were followed by additional 30 cycles, the latter performed on 1/10 of the first amplification product. 16 sequence-specific oligonucleotides (SSO) were employed for DPB typing and 8 SSO for DQB typing. This procedure allowed to type DPB and DQB alleles of each hair and to establish that typing was the same for all the unknown samples (DPB1*0401/*0901, DQB1*0603/*0201) , but different from both the victim (DPB1*0401/*0201, DQB1*0501/*0201) and suspect (DPB1*0402/*0201, DQB1*0301). The results demonstrate that two different polymorphic and independent loci can be analyzed simultaneously from any single hair. The high polymorphism of HLA-DPB and -DQB alleles indicates these loci as very useful markers for forensic purposes.

A 23 SEX DETERMINATION BY GENOMIC DOT BLOT HYBRIDISATION AND HLA-DQα TYPING BY PCR FROM FIXED TISSUES

Pötsch L, Penzes L, Prager-Eberle M, Schneider PM, Rittner C. Institute of Legal Medicine, University Mainz, FRG.

DNA was extracted from postmortem tissues fixed in ethanol, buffered formalin, formaldehyde and paraformaldehyde. Quality and quantity of the isolated DNA seemed to be sufficient for sex determination by dot blot hybridization using biotinylated X- and Y-chromosomal DNA probes as well as to perform PCR for HLA-DQα typing (AmpliType, Perkin Elmer-Cetus).

Sex always was correctly classified. In some cases (formalin-fixed material), difficulties during PCR procedure were observed such as complete failure of DNA amplification or incomplete determination of the DQα genotype.

A 24 HLA-DQα TYPING OF HUMAN FINGERNAILS.

Pötsch L, Penzes L, Prager-Eberle M, Rittner C. Institute of Legal Medicine, University Mainz, FRG.

In this report we describe a method to extract DNA from human fingernails. The isolated DNA was amplified by PCR. It was typed for HLA DQα with AmpliType (Perkin Elmer-Cetus) and compared with the genotype obtained from blood.

A 25 POST-MORTEM IDENTIFICATION OF FIRE VICTIMS USING DNA AMPLIFICATION

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The application of DNA typing methods after amplification by the polymerase chain reaction (PCR) of DNA derived from body tissues from charred fire victims was investigated. A total of 26 different tissue specimens from ten extensively burnt individuals were analyzed. The samples included femoral muscle, psoas muscle, bone marrow and blood. The post-mortem period varied from 38 to 183 hours. After amplifying the DNA by PCR from various tissues, the D1S80 locus was analyzed with high resolution polyacrylamide gel electrophoresis technique followed by silver staining and the alleles of the HLA-DQα locus were detected by using a reverse dot blot format. All samples could be typed for both loci and the genotypes were consistent in the various tissues from each individual. A parentage test was performed in two cases and Mendelian inheritance of the alleles for both loci was observed.

A 26 POLYMERASE CHAIN REACTION: TYPING OF DNA ISOLATED FROM VARIOUS FORMS OF BIOLOGICAL EVIDENCE.

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The polymerase chain reaction (PCR, DNA-amplification) allows the enzymatic synthesis of specific DNA-fragments in vitro and has found wide application in many different fields of research. For forensic case work this technique is of great promise, because very small stains and - sometimes - even partially degraded DNA can still be analyzed.

Several marker systems based on length or sequence polymorphisms have been described for DNA-typing by PCR. One of the most polymorphic systems is the Apo B 3' length polymorphism on chromosome 2. Up to now 16 alleles have been found with frequencies ranging from 0.002 to 0.379.

We present here our experience in typing the Apo B 3' region. Typing results could be obtained from various forms of biological evidence such as semen stains, vaginal swabs, blood stains and single hair roots. Furthermore we demonstrate the application of the PCR-technique in forensic case work.

A 27 ANALYSIS OF pMCT 118 LOCUS POLYMORPHISM IN AN ITALIAN POPULATION SAMPLE BY THE POLYMERASE CHAIN REACTION

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Non-coding human DNA has been found to contain numerous repeated sequences of short nucleotide fragments, defined as variable-number-tandem-repeat (VNTR), which display a high degree of polymorphism. For this reason VNTR systems are usefully applied in medical legal fields such as testing paternity and identifying individuals . Most of the information on VNTR so far acquired has been drawn from Southern blot analysis. These systems can also be studied by amplifying the DNA sequences with the polymerase chain reaction (PCR) technique. A limit to the extensive use of such polymorphisms is set by the lack of data on allele frequencies distribution. In the present study the PCR technique has been applied to the MCT118 system on a population sample of 125 unrelated healthy individuals living in Ancona (Central Italy). At the same time the study was extended to a family to confirm the Mendelian inheritance of the alleles.
The study was performed using polyacrylamide gel or agarose gel stained with ethidium bromide or silver stain.
Preliminary results indicate the occurrence of at least eight alleles, whose distribution over the population studied is investigated to estimate the heterozygosity rate and the agreement with Hardy-Weimberg law.

A 28 SEX DETERMINATION IN BLOODSTAINS AND SINGLE HAIRS

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The polymerase chain reaction(PCR) is an method for the primer-directed enzymatic amplification of specific DNA sequences.
In the present study, we have prepared various DNA samples(bloodstains and hairs) without a phenol/chloroform or boiling treatment procedure and tried sex determination of these samples using the PCR. After washing samples in a lysis buffer, these were suspended in a digestion buffer containing proteinase K and incubated for 3 hours at 55°C and again at 95°C for 8 minutes to inactivate the protease. The samples without phenol treatment were used for the template. PCR was carried out at 94°C, 60°C and 72°C for 30 cycles using two pairs of primers (Y1, Y2 and X1, X2). The 170-bp amplification product was detected in male DNA from bloodstains or hairs, whereas the 130-bp X specific fragment was also amplified with X1,X2 primers. The minute amount(1ul) of bloodstains and hair shafts(2-3cm) were enough for amplification by our extraction procedures.

A 29 THE USE OF THE POLYMERASE CHAIN REACTION (PCR) IN SCREENING FORENSIC SAMPLES.

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Variable number tandem repeats (VNTRs) with small allele sizes and limited size ranges can be readily amplified by PCR and both visualized and individualised with electrophoretic systems. PCR of the ApoB VNTR region was used for screening forensic samples prior to DNA profiling with single locus probes. Data are presented from case where eleven semen stains (blood group A: 1x; B: 3x; O: 7x) and nine putative donors were investigated. PCR-analysis revealed two genotypes in the blood group B stains and three genotypes in the blood group O stains. Six persons were excluded by PCR. One of the three included persons was excluded by the subsequent RFLP-analysis with probe MS 31.

A 30 ANALYSIS OF FORENSIC STAINS VIA PCR AMPLIFICATION OF POLYMORPHIC SIMPLE REPEATS FROM THE HUMAN Y CHROMOSOME

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Simple repetitive DNA stretches consist of tandemly reiterated (ca), (cac), (gata) or other short basic sequence motifs. These ubiquitous elements of eukaryotic genomes show ample lengths variabilities which can be demonstrated by single and multilocus probing as well as after PCR amplification and high resolution gel electrophoresis. We have identified several $(gata)_n$ and $(gt)_m$ simple repeats on cosmid clones originating from the human Y chromosome. The respective loci were PCR amplified. At the locus HY27LR e.g., 4 different alleles were detected due to variations in the number of $(gata)_n$ units among 30 caucasian males tested. The alleles differ from each other by distinctly 4 base pair repeat units, i.e. $(gata)_{10-13}$. In addition the alleles are transmitted regularly from fathers to sons. Meanwhile HY27LR has already been used for actual case work in order to exclude a suspect on the basis of sperm DNA from a vaginal swap after rape (and murder). Another amplification system has not been informative, probably due to mutation at the primer attachment site. Because of the limited amount and degradation of the DNA this case could not have been solved without specific amplification. Notwithstanding we favour oligonucleotide fingerprinting whenever applicable because of significantly reduced contamination and artifact problems.

A 31 THE SPECIES IDENTIFICATION BY POLYMERASE CHAIN REACTION AND DIRECT SEQUENCINGS.

Tsuchida*, F. Umenishi, S. Ikemoto. Department of Legal Medicine and Human Genetics, Jichi Medical School, Tochigi, Japan.

Polymerase chain reaction(PCR) and direct sequencing were used for the determination of the species of origin of blood and blood stains. PCR was used to amplify the region of cytochrome b in mitochondrial DNA. Primers were based on common conserved regions among human and animals in the published nucleotide sequences. DNA samples were prepared from blood and blood stains. The fragments of cytochrome b were amplified by using the DNA samples and the primers. PCR products were separated and purified by agarose gel electrophoresis. Subsequently, single-stranded DNA was generated by an asymmetric PCR and its nucleotide sequences were determined by the dideoxy-chain termination method. The amplified DNA from human and animals showed the characteristic nucleotide sequences on each species. The species of origin of blood and blood stains were able to determine by the nucleotide sequences of the amplified fragments. The present method was useful for not only making distinctions between human and animals but also the determination of origin of blood and blood stains.

A 32 The Isolation of DNA for Forensic PCR Analysis . An Evaluation of Available Methodologies.

R. Coquoz, Institut de Police Scientifique et de Criminologie, University of Lausanne, Lausanne, Switzerland.

DNA isolation is the first step of almost any DNA typing procedure. PCR is generally considered to require less stringent purifications than other analytical methods like RFLP analysis. However since it is intended to be used on particularly difficult samples (small, old, degraded,...) the success of the analysis will be highly dependent upon parameters like yield of the DNA isolation, presence of PCR inhibitors. We can expect them to be influenced by the method used for the DNA isolation. That's why an evaluation of the merits of several common or more recent methods was launched. These methods are : a) the proteinase K digestion with or without phenol/chloroform extraction, b) the Chelex extraction method from Cetus, c) the methidium - sepharose extraction method. The evaluation was made through measurement of DNA yield and efficiency of PCR amplification. Other parameters considered were the type of stain (blood stain, sperm stain, hair, saliva), their size and age. Important differences were observed between the extraction methods. The Chelex extraction method appeared to be the most convenient and efficient.

A 33

DETECTION OF THREE DIFFERENT VNTR'S BY DNA-AMPLIFICATION.

A.D. Kloosterman; R. Vossen & D. Wust. Dutch Forensic Science Laboratory; NL.

A rapid and simple procedure for the detection of three different hypervariable regions in the human genome is presented. Making use of oligonucleotide primersets from DNA flanking the minisatelite regions and thermostable Taq Polymerase reproducible and faithful amplification of the loci D1S58; ApoB and D17S30 has been demonstrated. With this PCR method it is possible to visualize the polymorphic VNTR fragments on ethidium-bromide stained 6% polyacrylamide gels. Typing these hypervariabel regions by the PCR technique is extremely sensitive. Nanogram amouts of template DNA generated good signals in all three systems.
The polyacrylamide electrophoresis could resolve the small differences in length between two successive alleles (30 bp in the ApoB system and 16 bp in the D1S58 system).
The amplification of these VNTR's show promise for the application of the PCR method in actual forensic case work.

B 1

Title: SERIAL SEXUAL CRIMES IDENTIFIED BY A DNA COMPUTERISED DATABASE

Author: J.E. Allard*. Metropolitan Police Forensic Science Laboratory, London.

The use of a computer searchable database of DNA profiles, which includes crimes where there is no suspect, has had a considerable impact on the recognition of serial sex cases in the London Metropolitan area.

A scientific application of the use of the database is described with reference to a series of rapes which was first identified by the Laboratory.

Some further crimes nominated by the police were confirmed as being part of the series and others were rejected.

Samples from approximately 300 suspects were tested using traditional blood group technology together with single locus probing. A weighted list of physical and behavioural characteristics was used by the police to prioritise their suspects.

B 2

MECHANIZED MULTI-LOCUS ANALYSIS

C.H. Brenner*, DNA·VIEW, Venice, California, USA

One criticism of calculations based on multi-locus probe "DNA Fingerprints" is that band matching decisions are subjective.

To answer this objection a computer program is used to measure and provide an objective rule for band matching. Several alternatives are offered for the computation of the likelihood ratio including

(a) number of obligatory bands shared,
(b) "+-" analysis, and
(c) Baysian analysis, according to which the graduated concept of "degree of match" replaces the need for a yes/no matching decision.

B 3 THE EFFICIENCY OF DNA FINGERPRINTS FOR INDIVIDUALISATION AND DETERMINATION OF PATERNITY

Dr Paul G. Debenham, Cellmark Diagnostics, Abingdon, England.

The properties of human DNA fingerprints detected by multi-locus minisatellite probes 33.6 and 33.15 have been investigated in 1702 caucasian paternity cases and in 36 large sibships involving the analysis of over 180,000 DNA fingerprint bands. This study establishes the effective independence of these probes and the bands they detected. Variation between the DNA fingerprints of different individuals indicates that the probability of a chance identity is very low ($<<10^{-7}$ per probe). In paternity analysis the proportion of non-maternal DNA fragments in a child which cannot be attributed to the alleged father is shown to be an effective determinant of paternity even in the presence of mutation.

B 4 TESTING THE POWER OF DNA STATISTICAL PROCEDURES

I.W. Evett.

Considerable confusion exists in the world of DNA statistics about the alleged importance of factors such as population substructuring and the representativeness of databases. Experiments have been designed within the FSS specifically for the context of forensic science and these enable the doubts which have been expressed by population geneticists to be placed in realistic perspective. The results of some of these experiments will be described together with an overview of a computer package for SLP evaluation currently under development by the FSS. Mention will be made of consultancy services which the FSS will be offering in the future.

B 5 COMPARISON OF DIFFERENT METHODS FOR THE CALCULATION OF INDICES FOR PATERNITY

R. Fimmers*, P.M. Schneider+, M.P. Baur*. *Institute for Medical Statistics, University of Bonn, Germany; +Institute of Legal Medicine, University of Mainz, Germany.

One problem for the use of the MLPs to decide about paternity (identity) is the quantification of the result in the case of a non exclusion. The genetic system (number of loci, number of alleles, linkage, ...) is usally not known. Assumptions have to be made according to most of these points. The appropriateness of the resulting methods (not of the assumptions) can be checked by applying them to real data.

Different methods for the evaluation of MLPs have been applied to family data for the probe MZ 1.3. A strong correlation can be shown for the values resulting from the different methods. The calculation on the basis of an estimated number of bandpositions and using "empty bandpositions" as evidence for paternity (Hummel) leads to a systematic bias in favor of the decision for paternity.

B 6 HOW TO DEAL WITH MUTATIONS IN DNA-TESTING

R. Fimmers, L. Henke, J. Henke, M.P. Baur. Institute for Medical Statistics, University of Bonn, Germany

In the VNTR polymorphisms de novo mutations may occur in substantial frequencies. This gives rise to the question how to deal with such observations in parentage testing.

Taking both the observed number of mutations and the exclusion rate into account we have developed a mathematical approach to incorporate an alleged mutational erant into the ESSEN-MÖLLER-formula $W = 1/(1+Y/X)$.

B 7 Title: CASEWORK EXPERIENCES WITH A COMPUTER-SEARCHABLE DATABASE OF DNA PROFILES

Authors: M.J. Greenhalgh*, J.E. Allard. Metropolitan Police Forensic Science Laboratory, London.

A data bank of DNA profiles has great potential for providing intelligence information for police officers. At the Metropolitan Police Laboratory, it has been possible to nominate suspects in unsolved cases and link cases together to show they are likely to have been committed by the same offender. Failure to use the data in this way could allow criminals to repeat their crimes when information to identify them is already available.

B 8 BIOSTATISTICAL ANALYSIS OF MULTILOCUS PROFILES: RESULTS OF A COLLABORATIVE STUDY WITH THE DNA PROBE MZ 1.3

Schneider PM[1], Fimmers R[2], Bertrams J[3], Bulnheim U[4], Feuerbach U[5], Henke L[6], Iten E[7], Osterhaus E[8], Prinz M[9], Simeoni E[10], Rittner C[1]. *Institute of Legal Medicine, University of Mainz[1], Universities of Bonn[2], Würzburg[5], Cologne[9], Kiel[10], Elisabeth Hospital, Essen[3], Institute of Blood Group Research, Düsseldorf[6], Institute of Legal Medicine, Duisburg[8], F.R.G., Swiss Red Cross, Bern, Switzerland[7].*

More than 1200 individual DNA samples mostly from families also studied with conventional blood group systems have been analysed with the multilocus DNA probe MZ 1.3 using a non-radioactive labeling and detection system (B.E.S.T. Probe MZ 1.3). On the basis of 900 meioses, the mutation rate was determined to be <1%. In all mutation cases, only a single aberrant fragment was found. By including a standard cell line DNA sample with known fragment sizes on each blot, data on the sizes of all polymorphic DNA fragments could be combined to establish frequency data bases for each laboratory as well as for all laboratories combined. The resulting frequency distributions were almost identical for all laboratories. For large fragments >10 kb, a maximal frequency of 0.1 was found, whereas for smaller fragments between 4.3 and 10 kb, frequencies increased gradually up to 0.5 - 0.7. Based on the combined analysis of all segregating maternal and paternal fragments, Mendelian inheritance could be demonstrated. Band sharing rates were found to be in agreement with previously published findings.

With these results, reliable exclusion probabilities can now be calculated in parentage testing on the basis of individual frequencies of all non-maternal DNA fragments informative for paternity. Using this approach in a typical father-mother-child case with 7 informative fragments (three bands >10 kb and four <10 kb), an exclusion probability of 8×10^{-6} is obtained.

B 9 THE RELATIONSHIP OF THE HLA PHENOTYPE FREQUENCY OF THE ALLEGED FATHER TO THE RESULTING PATERNITY INDEX IN CAUCASIAN NON-EXCLUSION PATERNITY CASES

R.H. Walker*, A.B. Eisenbrey, William Beaumont Hospital, Royal Oak, MI, USA.

Class I HLA phenotype frequencies (PFs) and HLA paternity index (PI) values in non-exclusion parentage cases have log normal distributions. HLA PF and PI values were derived using the Traver computer program. Haplotype frequencies in the data base were those published by Mickey et al. based on 5559 caucasian individuals from paternity cases and the HLA A and B specificities recognized by the 1980 Histocompatibility Workshop. The HLA PFs of alleged fathers (AFPF) were compared to their resulting HLA PI values in 500 caucasian non-exclusion cases to determine if a relationship exists between these variables. AFPF and PI were negatively correlated ($r = -0.606$; $p \leq 0.00001$). AFPFs of <1 in 100,000 have PI values which are usually >100 (median 175) while AFPF of >1 in 1,000 have PI values <70 (median 9). This correlation has little predictive value due to the broad distribution in the data about the least squares line of regression.

$y = -0.210 - 0.443x$

$Ln(PI) = -0.210 - 0.443(LnPF)$

C 1 PATERNITY INVESTIGATIONS BASED ON DNA-ANALYSIS ONLY

W. Bär* and A. Kratzer, Institute of Legal Medicine, Serology, University of Zurich, Switzerland

Despite the tremendous impact of DNA-analysis in forensic serology, the introduction of the DNA-technique in the field of paternity investigations progresses relatively slowly. Many reasons may be responsible for this fact, e.g. non-availability of reliable band frequencies, high incidence of mutations, etc.. We reasoned however that as a consequence of the growing number of methods in conventional blood group analysis, the handling of very many different elaborate techniques would increase the chance of errors. Based on our experience in providing supplementary DNA-expertise in cases where conventional blood group investigations gave non-conclusive results, we decided that the time has come to completely abandon conventional blood group techniques and to solely offer DNA-analysis as the method of choice for investigating paternity. The prerequisites for this change and the practical handling of theses cases including the information of the customers and possible pitfalls are outlined.

C 2 THE USE OF A CHEMILUMINESCENT DETECTION SYSTEM FOR PATERNITY AND FORENSIC TESTING. VERIFICATION OF THE RELIABILITY OF THE OLIGONUCLEOTIDE-PROBES USED FOR GENETIC ANALYSIS.

J. Neuweller, J. Venturini, I. Balazs*, S. Kornher, D. Hintz, J. Victor. Lifecodes Corporation, Valhalla, NY, USA.

Several VNTR loci, that had been characterized by the use of P^{32}-labeled recombinant DNA or oligonucleotides to the consensus sequence, were analyzed using oligonucleotide-Alkaline Phosphatase conjugates (AP-oligo) and a chemiluminescent detection method. The loci tested were D2S44, D4S163, D14S13, D17S79, D18S27, DXYS14 and DNF24. The size of the an AP-oligo is about 30 bases. The size of consensus sequences, for these loci, vary from 14 to 72 bp. Thus, while some of the AP-oligos include the entire consensus sequence others contain only a subset. The specificity of the AP-oligo may be affected by changes in the sequence of the variable number of tandem repeats (VNTR) at the locus. Therefore, the purpose of this study was to test whether an AP-oligo can identify the different DNA fragments (alleles) of a locus. Paternity cases, already typed using P^{32}-labeled probes, were used in this study. PstI-digested DNA from approximately 200 to 300 individuals, covering the full size range of alleles, were examined. The results obtained for these loci show that, with the exception of D2S44, all the alleles were recognized with the AP-oligos. D2S44 contained a subset of alleles that could only be detected by hybridization to recombinant DNA fragment. In forensic application, results obtained using DNA recovered from evidentiary material show that most of the probes detect 10 ng of DNA after an overnight exposure. Therefore, the sensitivity of this detection system is equal or better to that obtained with radioactively labeled probes.

C 3 ANALYSIS OF AUSTRALIAN, BLACK, CAUCASIAN, CHINESE AND AMERINDIAN POPULATIONS WITH HYPERVARIABLE DNA LOCI.

I. Balazs*, J. Neuweiler, R.C. Williams, C. Lantz. Lifecodes Corporation, Valhalla, NY.

Population genetic studies, in australian aborigine, american black, chinese, caucasian and amerindian populations, were performed with several highly polymorphic DNA loci. PstI-digested samples from random individuals were hybridized to probes recognizing 6 hypervariable loci (i.e. D2S44, D4S163, D14S1, D14S13, D17S79, D18S27). Results showed that the american black population had the highest level of heterozygosity (92%) followed by caucasian (89%) and chinese (84%). In australians it was 77% while in the various amerindian populations it varied from 74 to 84%. In general, the distributions of DNA fragments show that the most common polymorphic DNA fragments of a locus were within the same size range in all populations. The distinguishing feature of each population was the relative frequency of particular group of alleles. For example, alleles >9.0 Kb in size, in D14S13, or from 4.5 to 4.7 Kb, in D18S27, were two or more times less frequent in caucasians than in the other populations. Overall, there were group of alleles, at one or more loci, whose frequencies were different between some of the ethnic groups and therefore could be used to differentiate them from each other.

C 4 The Application of DNA-Polymorphisms in Paternity Testing

M. Basler*, W. Sprecher, M. Rink. Institute of Legal Medicine, Univ. of Göttingen, Göttingen, F:R.G.

The application of the single locus probes YNH24, MS31, g3 and the oligonucleotide probe $(GTG)_5$ was investigated in a series of paternity cases. The purpose of this paper is to demonstrate the use of DNA-polymorphism analysis, if conventional blood group tests do not lead to a clear-cut dicision. Some selected cases - with probable silent allels or probable recombination at the HLA-locus - will be reported.

C 5 ALLELE FREQUENCY IN THE POPULATION OF SPAIN USING SEVERAL SINGLE LOCUS PROBES.

C. Cabrero, E. Valverde, A. Diez, A. Carracedo y T. Borrás*. Dept. of Legal Medicine of the University of Santiago de Compostela and PharmaGen S.A., Madrid, Spain.

Numerous probes detecting tandem repeated sequences associated with single locus DNA have been isolated (VNTR). From these, six of them (YNH24, TBQ7,CMM101, pH30, MS43A and MS31) appear to detect higher variations in the number of the repeats, thus resulting in an elevated polymorphism. These probes have been adopted by the majority of laboratories engaged in human identification around the world .
Studies to determine their allele frequency have been carried out for some of the world populations. To fill the gap existing in the Iberian peninsula, and to be able to conduct identification tests in Spain, we have analyzed 250 unrelated spaniards. Samples were selected from the different regions according to their population and experiments were performed following the electrophoretic conditions proposed by the European DNA Profiling Group (EDNAP). We will present the results of HaeIII digests of these DNAs hybridized to YNH24, TBQ7, CMM101, pH30 together with the HinfI digests of the same DNA hybridized to YNH24, MS43A and MS31 and the frequency of their alleles.

C 6 DNA FINGERPRINTS OF FAMILIES FROM BEJSCE /SOUTH-EAST POLAND/

T.Dobosz*, P.Kozioł, K.Sawicki, M.Szczepaniak, S.Szymaniec, C.Vogt. Institute of Forensic Medicine, Wroclaw, Poland

The paper is a preliminary report on DNA studies in population of Bejsce /Kazimierza Wielka district, Kielce province, South - East Poland/. This population is very good known, because family data from church registers are preserved from 1550, completly from 1679. The data have been used for reconstruction of family pedigrees and relationships. DNA from 17 families numbering 95 persons were prepared by salt method and tested after digestion by Hinf I and Hae III using BIOTEST B.E.S.T. Probe M.Z.1.3. Additionaly, the blots were reprobed by oligonucleotide probe /CAC/5. The study showns that almost all families from Bejsce are related, contemporary or in the past.

C 7 A MULTILOCUS PROBE FOR FINGERPRINTING BASED ON *chi*-LIKE SEQUENCES.

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The *chi*-like octamer present in the minisatellite core sequence has been implicated in genetic recombination. Similar consensus sequences have also been found at breakpoints of oncogene translocation, immunoglobin gene rearrangement etc. There are several lines of evidence suggesting that hypervariable minisatellite sequences are recombinogenic in mammalian cells. We therefore, hypothesized that genesis of VNTRs is recombinationally mediated requiring at the minimum, the presence of *chi*-like sequences at or around the sites where VNTRs eventually occur. Hence DNA fingerprinting probes based on *chi*-like sequences alone should reveal considerable polymorphic information. A 33-mer oligo nucleotide sequence was chemically synthesized, radiolabeled and used as probe for DNA fingerprinting. Biostatistical analyses gave the band sharing frequency value of 14.2 + 2.7 % for unrelated individual and 55.4 % for parent-offspring. The probability of two unrelated individual having identical band pattern was calculated to be 1.9×10^{13}. This probe has been successfully used for DNA fingerprint analysis of forensic samples.

C 8 ACHIEVEMENT OF INTERLABORATORY UNIFORMITY - A SUMMARY OF WORK CARRIED OUT BY THE EDNAP GROUP

P. Gill, S. Woodroffe, W. Bar, B. Brinkman, A. Caracedo, B. Eriksen, S. Jones, A.D. Kloosterman, B. Ludes, B. Mevag, V.L. Pascali, H. Schmitter, P.M. Schneider, J.A. Thomson

Since 1989, approximately a dozen European laboratories have been working together, in order to address problems of uniformity and to compare results between different laboratories. Preliminary results suggested that a wide match window of at least 10% was needed with current methodologies in use. A subsequent experiment show that comparability could be achieved provided laboratories utilised similar protocol. This paper examines the quality management and organisational problems associated with ensuring that different laboratories can successfully compare and interpret results, by reference to both EDNAP and Home Office experience.

C 9

CLONING AND CHARACTERISATION OF NOVEL SINGLE LOCUS PROBES FOR FORENSIC PURPOSES

C P Kimpton, S K Watson, R Hopgood, P Gill, K Sullivan

Size selected libraries enriched for larger, more variable, tandem repeat regions have been prepared in charomid 9 vectors and screened by colony blot hybridisation employing the minisatellite probe alpha-globin 3'HVR. By hybridising insert DNA from positive clones to HinfI restricted DNA from unrelated individuals it has been shown that most positive clones contain insert DNA from polymorphic loci. Characterisation of one such clone has shown it to be a highly discriminating single locus probe (SLP) detecting a novel minisatellite locus.

Probe walking has successfully been carried out by rescreening charomid libraries with this minisatellite probe, generating a further subset of polymorphic clones. Characterisation of these clones has identified other highly polymorphic SLPs suitable for forensic applications. The cloning and characterisation of polymorphic loci will be described.

C 10

Title: THE EFFECTS OF USING DIFFERENT MOLECULAR WEIGHT MARKERS IN DNA PROFILING

Authors: M.J. Greenhalgh.* Metropolitan Police Forensic Science Laboratory, London.

A comparison has been made between a series of commonly available commercial DNA molecular weight markers (Amersham, BRL and Promega). The values obtained for the molecular weights of a series of DNA control fragments were compared using each of the systems. The largest differences occurred between those markers derived from DNA of different species. It is recommended that the same molecular weight marker system or at least one derived from the same species origin is used whenever comparison of results between laboratories is required.

C 11

The Relationships Between The Voltage Gradients And The Mobilities Of The DNA Fragments During The Electrophoresis

B.L.Guo, M.Prinz, M.Staak

An ideal seperation of DNA restriction fragments is a very important condition for a precise DNA-typing. And a possiblly short time needed by the DNA-typing is hoped in forensic investigation.

Choosing an appropriate running time depends upon the voltage gradients, the agarose concentration, and the size and the size difference of the fragments to be seperated.

The relationships between the voltage gradients and the mobilities of the DNA restriction fragments during the electrophoresis were investigated in gels with different agarose concentration.

C 12

PATERNITY TESTING WITH FIVE VNTR SYSTEMS IN DANES

H.E.Hansen*, N.Morling. Institute of Forensic Genetics, University of Copenhagen, Denmark.

The polymorphisms of the VNTR-systems D7S22 (g3), D5S43 (MS8), D7S21 (MS31), D12S11 (MS43), and D2S44 (YNH24) were investigated in 148 Danish paternity cases involving 190 alleged fathers. DNA samples from mother, child, and alleged fathers were digested with *Hin*fI and electrophoresed side by side on 0.7 % agarose gels (20 cm x 20 cm). Southern blotting, hybridization with radiolabeled probes, and autoradiography was performed with standard techniques. The band positions were measured with a ruler. A match criterion of 1.25 mm was chosen. All mother-child differences were less than 1.25 mm, except in one case with a reproducible, large mother-child difference in D7S21 (MS31) which was interpreted as a mutation. The observed mutation rate for D7S21 (MS31) in 187 mother-child pairs was 0.5 %. Of the alleged fathers, 49 were excluded with conventional systems. Using these data as the reference, the exclusion rates were: D7S33 (g3): 0.90, D5S43 (MS8): 0.47, D7S21 (MS31): 0.90, D12S11 (MS43): 0.80, and D2S44 (YNH24): 0.90. The observed combined exclusion rate of the five VNTR systems was > 0.999.

C 13 SIZE CALCULATIONS OF RESTRICTION FRAGMENTS: COMPARISON BETWEEN TWO LABORATORIES

L. Henke*, P.H. van Eede[2], J. Henke[1] and G.G. de Lange[2]; [1] Inst. f. Blutgruppenforschung,W-4000 Düsseldorf 13, Germany; [2] Central Lab. of the Netherlands Red Cross BTS and Lab. for Exp. and Clin. Immunol., Univ. of Amsterdam, NL-Amsterdam

Single locus DNA polymorphism patterns were generated on 50 human DNA samples by means of VNTR probes MS1, MS31, MS43, and G3 after HinfI digestion. The same 50 DNA samples were examined in laboratories in Amsterdam (AMS) and in Düsseldorf (DÜS). 390 fragments ranging from 0.8 to 27 kb were examined. Fragment size measurements were carried out as follows:

- different external molecular weight markers were run 4-6 times on each gel,
- in Düsseldorf, 2 manual kb size calculation were done, followed by different computer aided calculations in Amsterdam and in Düsseldorf.

Inter- and intra-laboratory comparisons revealed good reproducible fragment size measurements shown by mean difference and the standard deviation of this difference. In the range from 0.8 - 12 kb, the standard deviation of the mean differences appeared to be lower than 0.2 kb.With fragments > 12 kb much larger differences were seen. Therefore, it can be concluded that the data of fragments ranged from 0.8-12 kb can be exchanged and can be used for data pooling to obtain population frequencies. We compared this survey with a previous study in which internal markers were used and - due to the probe used - a smaller kb range was investigated (For Sc Int 49, 21-31 (1991)).

C 14 COMPARISON OF POPULATION GENETICS OF THE SINGLE LOCUS PROBES pS194 AND pL427-4

W. Martin*, H. H. Hoppe, M. Muche. Institut für Blutgruppenserologie und Genetik, Hamburg and L. Henke. Institut für Blutgruppenforschung, Düsseldorf.

Comparing population genetics of different laboratories for the single locus probes pS194 (D7S107) and pL427-4 (D21S112) it became evident that considerable differences of the distributions for the calculated kb-values of the fragments were present. The forms of the distribution curves were identical for the same population, but deviations of the determined kb-values for the fragments were found according to the technique used. On the example of the two probes, these deviations can be shown to be linear corresponding to the electrophoresis technique: particularly according to the agarose concentration and -less- to the amount of DNA applied. By that, we have the possibility to combine population genetic data of different laboratories using different techniques after linear correction of the distribution tables or curves. Since this interdependence is valid for all probes, it is quickly possible to compile relatively extensive population genetic tables as a reliable basis for the statistical evaluation in cases of disputed paternity.

C 15 COMPARISON OF MINISATELLITE DNA PROBES AND BLOOD GROUP, PROTEIN, AND ENZYME MARKERS IN PATERNITY CASES.

G.Mauff*, G.Pulverer, Ellen Mühlenbrock, L.Kochhahn, Annemarie Klein, Käthe Volz, Annette Bräutigam; Institut für Medizinische Mikrobiologie und Hygiene, Universität zu Köln, Germany.

Multilocus and single locus DNA probes, considered as very useful tools in complex cases of disputed paternity, are still judged controversially for their application in the standard trio case. In the present study 48 one man and 2 two man cases were investigated with conventional blood group, protein, and enzyme markers and with minisatellite DNA probes. The conventional expertise was carried out in all cases according to German federal regulations, the additional genetic markers Hp subtypes, GC 1 subtypes, PGM1 subtypes, PLG, C3, BF, TF C subtypes, ORM1, and in some cases A2HS, PGP, C6, and HLA. The minisatellite DNA probes MS1, MS8, MS31, MS43, and g3 (ICI Diagnostics) were applied to 20 cases, MS1, MS31, and MS43 to 30 cases, using a HinfI digest of genomic DNA, a nonradioactive digoxigenin-dUTP labeled fluorescent AMPPD detection kit (Boehringer Mannheim), and a radioactive ^{32}P assay.
In the technical evaluation the fluorescent assay was seen to be superior in time, sensitivity, and reprobing as compared to the ^{32}P assay but comparabel in experimental effort to the conventional expertise without HLA. Concordance in exclusion of paternity with MS1, MS8, MS31, MS43, g3 and 2 to 7 standard markers was seen in 8 cases, in 10 cases with MS1, MS31 and M43. No exclusion was observed with minisatellites or with standard markers alone. The distribution of minisatellite fragments among the nonrelated individuals followed published frequencies for Caucasoids.
We conclude from our data that with the nonradiocative detection system minisatellites are reliable for the paternity expertise. In view of the limited experience with DNA probes as compared to conventional markers and a comparabel technological effort their application should however be confined to complex (e.g. incest) cases or unsatisfactory probability values.

C 16 MATCHING CRITERIA FOR PATERNITY TESTING WITH VNTR SYSTEMS

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The sources of variability in duplicate testings and child-parent comparisons of RFLP VNTR data for paternity testing were analysed. *Hin*fI digestion of DNA, side by side electrophoreses on 20 cm x 20 cm agrose gels, Southern blotting, hybridization with radiolabeled probes detecting the VNTR-systems D7S22 (g3), D5S43 (MS8), D7S21 (MS31), D12S11 (MS43), and D2S44 (YNH24), and autoradiography was performed with standard techniques. The band positions were measured with a ruler with 0.5 mm resolution. Initial analyzes demonstrated that the absolute difference in migration distance was the parameter with the lowest variability. Comparisons of 350 duplicate testings on the same gel of DNA from 97 individuals showed no differences ≥ 1.25 mm. This matching criterion was used for the comparison of 866 differences in 187 mother-child pairs. All mother-child differences were less than 1.25 mm except for an assumed mutation in D7S21 (MS31), and this matching criterion has been chosen for the evaluation of Danish paternity cases. Population data of the five VNTR systems in 436 unrelated Danes will be presented.

C 17 COMPUTER-AIDED FRAGMENT SIZE DETERMINATION OF SINGLE-LOCUS DNA PROBES

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The determination of a fragment of unknown size is performed by ascertaining a compensatory function. This can be done either in drawn form as a graph or calculated, resulting in a function of measured distance. A computer-aided system for fragment size determination and integrated data management is presented in order to shorten this procedure which is rather time-consuming when done manually. It also minimizes the risk of errors in measuring, drawing or taking values from the graph: Bands are entered using a digitizer and marking them with a mouse-like stylus. The information is transmitted into the computer where a compensatory function is generated using the size marker data. Individual fragment sizes are obtained using this formula and are saved for future use, e. g. population statistics or calculation of probabilities in cases of disputed paternity. Any single-locus DNA system is supported; a variety of different size markers can be defined.

C 18 ALLELE FREQUENCY DISTRIBUTION OF TWO VNTR POLYMORPHISMS (YNH24-D2S44; ALPHA GLOBIN 3'HVR-D16) IN ITALY

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The application of hypervariable DNA profiles in forensics has raised the need for detailed studies on the population genetics of these systems. Investigations of hypervariable profiles by Southern Blot analysis (SBA) mostly produce arrays of continuous data, requiring computer databases to be created for these alleles prior to generate reliable arrays of frequencies. To achieve this goal, a standardized protocol and a centralized repository of data are higly desirable. We have organized a first nucleus of an Italian repository of VNTR frequencies on the basis of a European SBA protocol (Forensic Sci Int, 49,1-15,1991) and a simple algorithm for storage and management of continuous data (Pascali et al, submitted). A collaborative study is presently being carried out by a network of Italian laboratories, to produce frequency data on several VNTRs by the European SBA protocol. Here we present a first nucleus of data generated as above described, on the distribution of two VNTRs (D2S44; alpha globin 3'HVR) in Central-Southern Italy.

C 19 CHEMILUMINESCENT DETECTION OF SINGLE LOCUS AND MULTILOCUS HYBRIDIZATION PATTERNS

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Using nonradioactive-labeled DNA-probes the chemiluminescent detection of alkaline phosphatase has several advantages over the colorimetric detection, e.g. a less complicated process of rehybridisation of the membranes. Applying digoxigenin labeled probes and adamantyl dioxetane phosphate (AMPPD) we compared different hybridization and detection methods (ALLEFS, J.J.H.M. et al. NAR 18:3099-3100, 1990, manufacturers instructions: Tropix, Bedford, MA and Boehringer Mannheim) in order to optimize the signal to noise ratio of the results. The combination of the Boehringer blocking procedures and the Tropix chemiluminescent detection (dilution of the AMPPD in carbonate buffer) gave the highest sensitivity. Adding 0.5% blocking reagent to the alkaline phosphatase digoxigenin antibody conjugate dilution resulted in less background.

C 20 DNA-PROFILING WITH PHINS310, PMUC7, PMR24/1, PYNH24 AND PMS43 FOR PARENTAGE TESTING

T. ROTHÄMEL, H.-J. KRÜGER, W. KEIL and H.D. TRÖGER

The cummulative Paternity Index (PI) and the corresponding Relative Chance of Paternity (RCP), that can be achieved by HinfI-RFLPs of pHINS310, pMUC7, pMR24/1, pYNH24 and pMS43, is compared to the Probability of Paternity according to ESSEN-MÖLLER (W), which was determined in trios (C-M-PF) of routine casework by using a set of 26 to 31 conventional red cell and serum protein markers. This comparative study is an attempt to evaluate the evidence of these RFLPs in doubtful parentage as well as their power of exclusion in respect of the results of the conventional polymorphisms.
The DNA is precipitated by ethanol after organic extraction of proteinase K digested white cell nuclei. After HinfI-restriction the fragments are separated in a 1% agarose gel at 120 mA for 24 hours and alkali blotted to positively charged nylon membranes. The probes are labeled with P32-dCTP by the primer extension reaction.
Preliminary results show a high level of accordance (+/- less than 1%) between the "RCP" after profiling with pHINS310, pMUC7 and pMR24/1 and "W" under the conditions described above. No DNA-exclusions are found in trios with "W" exceeding 99.6%.

C 21 THE USE OF NICE PROBES IN FORENSIC CASEWORK

Michael B T Webb, Douglas H Pearston, Nicola V Fletcher, Mark D Sutton, Paul G Debenham, Cellmark Diagnostics, Abingdon, England.

We have developed a series of Non-isotopic chemiluminescent Enhanced (NICE) DNA probes for relationship analysis and forensic identification using the highly informative hypervariable loci (MS1, MS8, MS31, MS43A, G3) discovered by A. J. Jeffreys. The NICE probes consist of an oligonucleotide conjugated with alkaline phosphatase which can be detected in combination with the chemiluminescent substrate Lumi-Phos 530. Simple and rapid hybridisation procedures can now be utilised with these probes.

To assess the suitability of this technology to replace the current dependence on isotopic systems we have used these probes in parallel with their homologous isotopically-labelled single locus probes on forensic casework.

We observe that the NICE probes have at least equivalent detection sensitivities to the isotopic probes. Further, the NICE probes identify the same DNA results but do so much more rapidly. Illustrative examples of casework will be presented.

C 22 Title: FREQUENCY DATABASES FOR THE DNA PROBES MS1, MS31, MS43A AND YNH24 DERIVED FROM CAUCASIANS AND AFRO-CARIBBEANS IN THE LONDON AREA

Authors: C. Buffery*, M.J. Greenhalgh, S. Jones, G.M. Willott. Metropolitan Police Forensic Science Laboratory, London.

A frequency database for the probes MS1, MS31, MS43A and YNH24 using Hinf 1 as the restriction enzyme has been prepared from blood samples submitted to the Metropolitan Police Forensic Science laboratory in the course of forensic casework. To date there are more than 1000 Caucasian samples and over 500 samples from Afro Caribbeans. Significant differences can be observed between the distributions of the two racial groups examined with some of the probes and the relationship of this to published mutation rates is discussed.

C 23 Title: AN EVALUATION OF SINGLE LOCUS PROBES IN CASEWORK

Authors: F.J. Burridge, M.J. Greenhalgh*, G.M. Willott. Metropolitan Police Forensic Science Laboratory, London.

Since December, 1988, DNA profiling has been performed in over 1000 cases at the Metropolitan Police Forensic Science Laboratory. Stains of semen, saliva, blood and vaginal material as well as mixtures have provided evidence of great value to the police and in courts of law. Analysis of the results indicates the types of staining most likely to give a DNA profile. Some recent examples illustrate how individuals have been eliminated from the enquiries or linked to a case by DNA results.

C 24 USE OF AN OLIGONUCLEOTIDE PROBE FOR DISTINGUISHING HUMAN MALE DNA FROM FEMALE DNA.

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We have synthesized synthetic oligonucleotides that are complementary to the human DYZ1 locus. One set of oligonucleotides exhibits a high degree of specificity for male DNA. In a slot blot format it generates more than 100-fold higher signal when hybridized to human male DNA than it generates when hybridized to human female DNA. Similar results were obtained when the probe was hybridized to DNA from an agarose yield-gel. Thus, this oligonucleotide probe should be useful for sex-typing of DNA samples. Hybridization to Southern blots of Hae III-digested male DNA generated a 3.5-kb band, as well as a fainter 7.0-kb band. The 3.5-kb band has been observed in the DNA of all males tested. No hybridization was detected to Hae III-digested female DNA in Southern blots. The male-specific bands can be used as high molecular weight monomorphic markers. This can provide an in-lane control for electrophoretic band-shifting, as well as provide additional evidence that samples were correctly identified.

C 25

ACCURACY AND PRECISION IN THE ANALYSIS OF VNTR DNA POLYMORPHISMS FOR PATERNITY TESTING.

S. Reavis, T. Schlaphoff, R. Martell, E.D. du Toit*.
Provincial Laboratory for Tissue Immunology, Cape Town, South Africa.

Due primarily to inconsistencies in the electrophoretic mobility of DNA in agarose, and measurement error in determining band position on autoradiographs, a certain degree of inter-assay and intra-assay variability exists when using single-locus VNTR DNA profiling. The level of variability must be determined when excluding alleged fathers, and in calculating the probability of inclusion of non-excluded fathers. This level must also be known in order to establish accurate band (allele) frequency data bases in local populations. To ensure that the techniques in our laboratory demonstrated reproducibility similar to that reported by others, inter and intra-assay *precision* were determined by repetitive analysis of DNA from random individuals. An estimation of assay *accuracy* was determined by repetitive analysis of a Pvu II digest of the HeLa cell line, with known fragment sizes. A number of commercially available VNTR DNA probes were tested. The inter-assay coefficient of variation (CV) for all fragment sizes and all probes ranged from 0.4% to 1.0%. The intra-assay CV ranged from 0.5% to 0.9%. The overall CV was estimated at 0.74%. Precision for all probes ranged from 100 to 300 base pairs of the reported fragment size.

C 26

ON DNA TYPING OF HARD TISSUES

U. Hammer *, U. Bulnheim, R. Wegener. Institute of Legal Medicine, University of Rostock, Germany

DNA typing of tissues with a high content of fibers and minerals such as skin, tendons, cartilages, bones and teeth (roots) can be an important factor in forensic case work (f.e. identification) because of their high resistance to environmental influences.
The method of DNA preparation depends on tissue quality as the amount and type of fibers, intercellular substances, embedded horn an keratine and minerals.
Fresh cutted tissue pieces (from autopsy material) were frozen (- 80° C), than rasped and aliquoted for further investigation. Bones and teeth had to be decalcified with different proteases. Yield and quality of DNA from hard tissue were investigated in dependence on storage time and conditions. Results are compared with those of other tissues as muscles and lymph nodes.

C 27

SINGLE LOCUS DNA POLYMORPHISMS IN THE BELGIAN POPULATION AND IN PATERNITY CASES.

B. HOSTE*, M.R. MATHY. Legal Medicine, University of Liège, Belgium.

Single locus DNA hypervariable polymorphisms D2S44, D4S139, D14S13, D16S85, D18S27, were searched after HAE III restriction, in 40 cases of paternity testing. At least three probes were used in each case.
For comparison, the samples were analysed in parallel by the classical methods. These were limitted to 19 genetic markers (red cell antigens, HLA-A,B,C and protein polymorphisms), the mean exclusion probability being close to 99.9 %.
In our series, the paternity of 30 men was included and of 17 men was excluded by both methods. Only one discrepant conclusion was obtained, for which classical methods yielded an unusually low probability of paternity (98.0 %), whereas paternity was excluded by two out of three DNA probes. Two cases where the blood of the mother could not be tested and one case involving relatives were solved by both methods.
Some problems encountered with the DNA systems in paternity testing will be commented upon : non detection of very small fragments, HAEIII restriction site within the D4S139 array, ...

C 28

USE OF THE MINISATELLITE PROBE MZ 1.3 FOR IDENTIFICATION AND RELATION OF DISMEMBERED CORPSES.

W. Huckenbeck*, H. Müller. Institute of Legal Medicine, Heinrich-Heine-University, Düsseldorf, Germany.

The inquest of dismembered corpses confronts the investigator with several problems. One of them is the identification and the relation of parts of corpses to one or more persons. Putrefaction severely alters the integrity of macromolecules and receptors. We tested the usefulness of the hypervariable minisatellite probe MZ 1.3 for such sorts of examinations. The probe proved to be successful in demonstrating individual bands. The results of in-vitro experiments are discussed.

C 29 RFLP IN CONJUNCTION WITH ANATOMICAL TRAITS IN INDIVIDUALISATION OF BONE

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Highly polymorphic D4S 139 and D14S1 loci were analysed using locus specific probes to individualise a bone when sufficient quantity of high molecular weight DNA could not be recovered to prepare profiles with multilocus probes. Sex of the source was determined by hybridising bone marrow DNA with Y-specific probe 102(d)2. As the father of the deceased was not alive paternal profiles were deduced by the DNA profiles of two siblings and mother. Another seized incomplete bone, tibia depicted a callous formation in lower region revealing an old healed fracture which must have caused limping to the deceased. Age of the deceased was estimated with the odontological traits of the recovered piece of maxilla. Our results indicate that if a bone to be individualised yields insufficient quantity of high mole. wt. DNA, essential for multi-locs or a battery of locus specific profilling, the identity of source can be established with the help of alleles at two highly polymorphic loci, a Y-specific probe and a thorough anatomical examination of the bone seized.

C 30 APPLICATION OF DNA FINGERPRINTING TO PROBLEMATICAL PATERNITY CASES.

T. Kishida*, M. Fukuda, Y. Tamaki. Department of Forensic Medicine, Medical College of Oita, Oita, Japan.

Case 1 Blood samples from a paternity trio were tested for 26 genetic systems. The overall probability of paternity exclusion was 0.9563, yet none of these systems indicated nonpaternity. The overall probability of paternity was 0.4175, which seemed too low for establishing paternity. Hae III digests of their DNA samples were subjected to DNA fingerprinting with a digoxigenin-labeled MZ 1.3. probe (B.E.S.T.-Probe, Biotest). The number of shared child/alleged father bands was 4, and the band sharing frequency was calculated at 0.571 according to the formula of Schacker et al. This value was interpreted as establishing paternity.

Case 2 Typing for 27 genetic systems revealed isolated nonmaternity in the GM system and nonpaternity in the C7 system. Contrary homozygosity was observed in both systems. Based on the 25 systems except GM and C7, the overall probability of paternity exclusion was calculated at 0.9990 and the overall probability of paternity at 0.9986. Thus, the presence of silent alleles in the GM and C7 systems was strongly suggested. In addition, a new GC variant suggestive of a gene duplication was found in the mother. In DNA fingerprinting, Hinf I-digested DNA samples were hybridized with an alkaline phosphatase-labeled synthetic Myo probe (SNAP Probe, Molecular Biosystems). The child shared 4 bands with the mother and the alleged father. The band sharing frequencies were 0.500 and 0.615, which established parent-child relation.

The present study demonstrates that conventional genetic systems cannot compete with highly polymorphic DNA loci.

C 31 APPLICATION OF CONVENTIONAL POLYMORPHISMS AND SINGLE LOCUS DNA PROBES IN CASES OF DISPUTED PATERNITY.

J.A.Thomson*, P.J.Lincoln, C.P.Phillips, D.Syndercombe Court, P.H.Watts. Department of Haematology, London Hospital Medical College, London, England.

A series of disputed paternity cases have been investigated using both single locus DNA probes and a wide range of conventional polymorphisms. Using large allowable variation for determining matches (+/- 2.5%) and a wide sliding window (+/- 5%) for determination of band frequencies from databases, the PI values obtained would not be sufficient to resolve all cases if these DNA probes were used in isolation.

Combined use of these DNA probes and conventional polymorphisms gave consistently high PI and RCP values where no exclusion was found and where four probes were successfully applied a combined RCP of 99.9% was obtained in virtually every case.

The construction of false trios and subsequent analysis shows that under these conditions the DNA probes had a combined power of exclusion of non-fathers of at least 99.98%; when combined with the expected figure for conventional polymorphisms used in this investigation, the figure increased to at least 99.999%.

C 32 BIOLOGICAL PATERNITY TESTING. COMPARISON BETWEEN DNA FINGERPRINTING AND USUAL GENETICS MARKERS

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Usually, Genetic Markers such as blood groups (A1, A2, B, O, MNS, Rh), serum proteins (Hp, Gc, Tf, α–1 AT) , red cell enzymes (PGM, AcP, Glo, EsD, GPT, AKA) and the HLA system, among others, have been used for establishing a biological paternity. With these markers as a whole it's posible to obtain a 99.9 % a priori exclusion of falsely accused individuals.

The joint power of minisatellite probes 33.15 and 33.6 can detect simultaneosly many highly variable genetic loci. This joined with the stable Mendelian inheritance of the individual band patterns, allow the exclusion of all the falsely accused individals (except in the case that 2 alleged fathers were identical twins) for a particular paternity case. So, the use of the polimorphism detected by these minisatellites, allow us to solve, with a nearly absolute probability, all the filiation cases.

In order to test the exclusion power of DNA fingerprinting in our Biological Paternity Testing Service, we have applied this technique to 5 cases of disputed paternity. In 2 of the cases in which the usual marker, have not been able to detect an exclusion (without using HLA system) this has been pointed out by DNA fingerprinting analysis. In 3 of the cases, where according to the usual markers a high paternity likehood was expected, this was confirmed by DNA analysis.

C 33 DNA FINGERPRINTING PROFILES IN RESIDENT POPULATION FROM THE BASQUE COUNTRY

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Within the Human genome, there exist some regions called minisatellites consisting of a variable number of tandem repeat units. Minisatellites from the same family have a inner sequence called "core" which can be recognized by using probes such as Jeffreys' 33.15 and 33.6 ones. These probes allow us to scan simultaneously more than 30 independent autosomical loci mapping mainly within telomeric regions, except in X and Y chromosomes. The high variability in the number of repeats among loci from different people, determines that the probability of finding 2 no-related individuals with an identical restriction profile is minimum (10^{-20}). The obtained image is a real individual-specific genetic fingerprint (DNA fingerprinting).
The application of this genetic fingerprint in Legal Medicine,gives a high resolving power. Because of this, we have choosen multilocus probes 33.15 and 33.6 to start up a study of 50 people residing in the Basque Country, so that to apply the obtained results to the different areas in Legal Medicine.
4 µg of DNA from each people were cut with Hinf I and electrophoresed in 0.7% agarose gels in TAE buffer for 24 h. Transference to nylon filters and later hybridization to 33.15 and 33.6 probes, allowed us to obtain the corresponding DNA fingerprints. An estimate, according to their size in Kb (fragments of 4-6 Kb 6-10 Kb 10-20 Kb), of the mean number of DNA fragments in a individual, as well as of the probability that these fragments be simultaneously in two no-related individuals, is similar to that obtaned by Gill et al.(Electrophoresis, 1987,8:38-44) y Jeffreys et al.(Nature, 1985, 314: 76-79).

C 34

CASUISTIC: THE USE OF DNA-FINGERPRINTING IN CASES OF AFFILIATION WITHOUT THE MOTHER.

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We report a case of affiliation which can be assigned to the problem area of the so called deficiency cases. The mother and the putative father both were dead. For the examinations the putative father was exhumed after a period of four weeks. The mother had died years earlier, exhumation seemed to be pointless. The following samples were taken from the putative father's corpse: heart blood, muscle, brain and bone marrow. The conventional serological typing resulted in an isolated exclusion (AcP system). The use of the hypervariable minisatellite probe MZ 1.3. gave no satisfying result (bandsharing: about 30 %). Only the use of single locus probes confirmed the exclusion from paternity.

C 35 RESULTS OF DNA ANALYSIS FROM SIX FORENSIC SCIENCE LABORATORIES IN GERMANY

Landeskriminalamt Baden-Württemberg, Stuttgart*
Landeskriminalamt Bayern, München
Landeskriminalamt Berlin
Landeskriminalamt Niedersachsen, Hannover
Landeskriminalamt Rheinland-Pfalz, Mainz
Bundeskriminalamt, Wiesbaden

A compilation of DNA analysis in forensic science case work based on the results of six forensic science labs in Germany is given. From october 1989 (three labs) till march 1991 a total number of 215 cases, 166 of them rape cases, was analyzed by DNA profiling using single locus probes. A match of suspect samples with crime stains was observed in 57 % and exclusion of suspect samples in 20 % of the cases. No results were obtained in 20 % and the suspect was unknown in 3 % of the cases. In order to emphasize the importance of the DNA method eigthy cases from the lab of Stuttgart were chosen for a comparison between conventional stain analysis and DNA results: within the group of suspects which could be excluded by DNA profiling in rape cases, more than 50 % could not be excluded by conventional systems alone.

C 36 ALLELE FREQUENCIES FOR FIVE DIFFERENT SINGLE LOCUS PROBES IN A POPULATION OF SOUTH-WEST GERMANY

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Landeskriminalamt Baden-Württemberg, Stuttgart, Germany

Using HinfI restricted DNA we have studied the allele frequencies of minisatellite loci D1S7, D7S21, D7S22, D12S11 and D2S44. About 300 unrelated german individuals were tested after hybridization with probes MS 1, MS 31, MS 43, g 3 and YNH 24. The frequency distribution is compared with other population studies in Germany and England. The data do not differ significantly.

C 37 A SIMPLE METHOD TO PREVENT INHIBITION OF TAQ POLYMERASE AND HINF I RESTRICTION ENZYME IN DNA ANALYSIS OF STAIN MATERIAL

W.Pflug*, S.Aab, B.Eberspächer, G.Mai, U.Keller, G.Wahl, Landeskriminalamt Baden-Württemberg, Stuttgart, Germany

In forensic science case work DNA analysis of restriction fragment length polymorphisms (RFLP's) and amplified fragment length polymorphisms (AmpFLP's) have become the most powerful method. But the restriction with Hinf I and the amplification with Taq polymerase of DNA extracted from various stain material (i.e. bloodstains and mixtures of semen and vaginal secretions) and especially from cigarette tips may be inhibited. We demonstrate that the addition of bovine serum albumin (BSA) to the reaction mixture can decrease or overcome these inhibition effects.

C 38 DISTRIBUTION OF VARIABLE NUMBER OF TANDEM REPEAT (VNTR) DNA POLYMORPHISM AT D2S44 LOCUS IN TUSCANY (ITALY).

R. Domenici*, I. Spinetti, M. Nardone, M. Pistello, L. Ceccherini-Nelli. Department of Biomedicine, University of Pisa, Italy.

VNTR DNA polymorphism for YNH24/HinfI (D2S44) was examined by Southern blot in a sample of 100 healthy unrelated blood donors native of Tuscany, Italy.
We determined the restriction fragments size and frequencies by grouping them in 0.1Kb intervals. Fragment size appeared to be experimentally continous in the 5.5-2Kb range with a bimodal distribution of the frequency: at 4.4 and 3-2.7Kb. Heterozygosity was found in 95% .
Our results confirm the utility of this marker for individual identification purposes.

C 39 Optimal Size-Calling Methods for Electrophoretic Analysis Utilizing Internal Size Standars.

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Applied Biosystems, Foster City, USA

Multi-color fluorescent labeling and detection technology has been used to make possible the automated sizing of fluorescently labeled DNA fragments. The samples to be analyzed, labeled with up to three different fluorescent dyes, are combined with a fluorescently labeled size standard. The size standard is labeled with a tag different in color from that of any of the samples to be analyzed. The mixture is then applied to a gel which resides in an Applied Biosystems 362 GENE SCANNER instrument. The fluorescently labeled fragments are detected as they electrophorese past a scanning laser beam. The system software recognize the standard by its unique color of fluorescence and creates a size calibration curve for each gel lane, thus compensating for gel artifacts that can complicate lane to lane comparisons of data.
Using 2.0% agarose gels appropriate for the analysis of amplified length polymorphisms less than 1000 bp in length, a second-order least squares polynomial gives an excellent fit to the data. Sizes called are accurate to within 2% compared to lengths known from sequence data. The coefficient of variation of length measurement is within 1%, thus providing precision for DNA casework. Analysis of 100 blind samples from the FBI Research and Training Center for the D1S80 locus resulted in allelic frequencies in agreement with published data for these samples.

C 40 EXTRACTION OF DNA FROM COAGULATED BLOOD SAMPLES

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In forensic medicine, samples of coagulated blood are available mainly. Following routine manuals, yield of DNA is irregular and sometimes very low. In this paper, the two possible reasons for this phenomena, sedimentation and enclosure of nucleated cells within coagula, were investigated. Usefulnes of different preparation procedures was compared.

- Fresh blood was allowed to coagulate in vertically standing narrow tubes and sections of the columns were won by biopsy forceps. DNA was extracted
  a) from sections of the coagula without pretreatment and
  b) from sections of the coagula after the attempt of biochemical lysis
- Coagula were treated with fluid nitrogen just like tissue, and DNA was extracted from different layers of the samples.
- The ratio of DNA in the liquid phase of coagulated blood samples was determined.
- Quantity and quality of DNA extracted from whole blood samples was determined
  a) after freeze and thaw cyles and
  b) from dried and grinded whole coagulated blood samples

C 41 EXPERIENCES WITH SIX SINGLE LOCUS PROBES IN PATERNITY TESTING.

Birgit Brüggemann, Dietlinde Teixidor, Maria Kilp, S. Seidl, Inst.of Immunhaematology Univ. Frankfurt and Red Cross Donor Service Hessen, Frankfurt,Germany.

Investigation of DNA polymorphism has been found useful in cases of disputed paternity. Multilocus probes (MLP) and single locus probes (SLP) are in use, both have advantages and disadvantages. Because of problems in identifying the various bands of MLP's, most investigators prefer SLP's. Furthermore, Int. Societies (AABB, ISFH) strongly recommend the use of SLP's.
We performed DNA analysis by the use of the following biotin labeled SLP's: pS 194 (5.0-11.0 Kb), pL 336 (2.0-6.0 Kb), pL 159-1 (4.0-5.5 Kb), pL 355-8 (6.0-8.0 Kb), pL 427-4 (1.4-3.4 Kb) and pR 365-1 (1.3-3.5 Kb). The data were compared with the results obtained by conventional serological techniques utilizing 6 red cell membrane systems, 8 serum groups and 8 enzyme polymorphisms. DNA digest was performed by adding 25 units of Pst I to 10 µg of genomic DNA, followed by gelelectrophoresis (42-66 hours), hybridization for 16 hours, 3x washings, 10 min. incubation with streptavidin, again 3x washing, thereafter alkaline phosphatase followed by 4x washings, finally NBT and BCIP was added. The two bands could be easily identified. A total of 20 families of disputed paternity (mother-child-putative father) were investigated. In case of exclusion by serological procedures 4 to 6 of the used SLP's resulted in an exclusion pattern. In one case the putative father was exclusively excluded in the Tf-systems, only one of the six SLP's (pL 427-4) demonstrated also exclusion. In all cases of non-exclusion by serological technique the DNA alalysis confirmed these results.

C 42 COMPARATIVE STUDY ON DETERMINING PATERNITY BY USING CONVENTIONAL AND HYPERVARIABLE DNA MARKERS.

K. Suzuki*, S. Ito, T. Miyazaki, H. Matsumoto, Y. Mizoi. Department of Legal Medicine, Osaka Medical College, Takatsuki, Japan.

The introduction of DNA technology has provided much more information to forensic analysis. This study examined efficiency and reliability of DNA probing for paternity samples already determined by using conventional markers. DNA extraction and subsequent typing followed usual procedures with some modifications. Single-locus VNTR probes were applied to 55 cases and multi-locus fingerprinting was done for some of them in Teijin-Bio Laboratory. No discrepancy was observed between the results of DNA probing and conventional typing. All of the 25 exclusion cases were definitely determined only by a single locus pYNH24 probe. In a two-man case where the two are brothers, paternity has not been determined yet by using conventional markers and two single-locus markers. Single-locus VNTR markers were found to be quite useful for determining paternity in difficult cases with putative father deceased. Influence on mobility of sample quantity and contamination with salt or protein was examined. Protein contamination was found to decrease mobility whereas salt with concentration as high as saline gave no effect on mobility.

C 43 INVESTIGATION OF VARIATION IN BAND SIZE DETERMINATIONS FOUND WHEN USING SINGLE LOCUS DNA PROBES.

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Assessments of variability of estimated band sizes have been made by comparing bands from mother child pairs run in adjacent tracks on the same gel and by repeated testing of the same sample on different gels in the same laboratory.

A series of blood samples have also been used to prepare autoradiographs in two separate laboratories. Manual and image analysis methods have been used to size the bands from both sets of autoradiographs.

Comparison of band sizes from mother/child pairs (within-gel variation) indicates that two bands cannot be considered to be different in size (with 99.999% confidence) if they fall within +/- 2.5% of each other. Repeat testing of standard samples on separate gels shows a greater variation of around +/- 3% to +/- 5% (99.9% confidence) depending on the band size detected.

Results from the two separate laboratories indicate that the method of measurement (ruler or Biotrac image analysis) has little effect (+/- 1-2%) on band size but, as expected, the same bands, measured on gels produced in different laboratories, show a greater variation in size (around +/- 5%). These results indicate the criteria necessary to allow the exchange of data between these particular laboratories and such exercises should be undertaken whenever such exchanges are considered.

C 44 DETECTION OF DNA POLYMORPHISMS BY USING α SATELLITE PROBES: APPLICATION TO THE FORENSIC IDENTIFICATION.

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The α satellite is a complex family of tandemly repeated DNA based on a 171-bp monomer repeat and is located at the centromeric regions of human chromosomes. Here we describe that RFLPs involving α satellite can be a tool of the forensic identification. DNAs were prepared from peripheral lymphocytes from unrelated individuals and digested with BglII, EcoRI, HindIII, MspI, PvuII or XbaI. The probes, D11Z1, D17Z1 and DXZ1 (α satellite DNAs on 11, 17 and X chromosomes, respectively), were labled with digoxigenin-dUTP by random primed. After Southern blotting-hybridization procedures, the hybrids were detected by enzyme immunoassay. Mendelian inheritance of the α satellite RFLPs was also investigated. EcoRI- and PvuII-D17Z1 and XbaI-DXZ1 polymorphisms were likely to be applicable to individual identification or parentage testing. Both the pattern of bands and the relative intensity of each band were reproducible. In addition, the detection method used was practically enough sensitive.

C 45 Family Studies Using Conventional and Minisatellite DNA Probes to Establish Paternity of Deceased Alleged Father.

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An unusual Paternity case was referred to our laboratory at the Medical College of Virginia involving an alleged father who had died a few years earlier in an automobile accident. The parents of the deceased wanted evidence for the courts that the child is their biological grandchild so that he becomes entitled to social security benefits. Tested were the parents of the deceased, nine of his siblings, the boy, and his mother. Because of the large number of siblings involved, the results of RFLP analysis were used to investigate linkage between DNA fragments and the degree of heterozygosity among the bands. The paternity testing using red blood cell groups, HLA, red cell enzymes, serum protein, and immunoglobulin allotypes gave a cumulative PI of 43,000 and a combined PP of 99.998%. RFLP analysis using Hinf I and Sau 3A single digests and the minisatellite DNA probes 15.1.11.4 and 6.3 of Alec Jeffry showed no exclusion of paternity and gave nearly conclusive evidence that the alleged father was the biological father of the child.

C 46 POPULATION GENETIC STUDIES OF FOUR HYPERVARIABLE DNA-LOCI.

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Population genetic studies were performed using DNA probes (Collaborative Research/Immucor) that recognize four hypervariable loci (D7S107, D18S17, D11S129 and D20S16) in the human genome. DNA from unrelated individuals from the German Ruhr area was extracted from EDTA blood samples using the non-toxic non-organic extraction method (Miller et al. 1988). Probes were labelled with biotin. DNA was restricted with PstI and electrophorezed 42 hours at 1.4V/cm in 0.8 % agarose. DNA was blotted to ONCOR Sureblot and detected using non-isotopic alkaline phosphatase-streptavidin detection system of ONCOR. Size markers were a mixture of lambda restricted with HindIII, PstEII and SphI.

Provisional results are given in the table.

Locus	Probe	Individuals (n)	Size range (kb)	Size range of common alleles	No. of alleles
D7S107	pS194	32	5.6-8.6	5.7-7.5	21
D18S17	pL159-1	136	3.9-6.0	4.1-4.9	20
D11L129	pR365-1	58	1.5-3.3	1.5-2.0	12
D20S15	pL355-8	119	5.7-7.9	5.9-6.4	17

Size range and frequency of alleles are simular to those presented by Dykes et al. 1990. The high number of different alleles with low frequencies requires DNA analysis of a large panel of unrelated individuals ($\geq$ 500) in order to obtain reliable frequencies for calculation of likelihood of paternity.

C 47 MINISATELLITE DNA PROBE MZ1.3 : BAND SHARING RATES AMONG SIBLINGS AND PART OF INFORMATIVE BANDS AMONG CHILDREN.

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DNA fingerprint analysis with the minisatellite DNA probe MZ1.3 (BIOTEST, Art. No.: 825020) was carried out in healthy Caucasian families with three or more children as part of a joint study of 11 European laboratories in order to explore the reproducibility in different laboratories and to extend the population genetic data.
In complement to the results of this study (Schneider et al., in preparation) we analyzed the portion of informative bands among 186 children and calculated the band sharing rate among siblings according to the formula: 1/2(n/a + n/b) (n = number of common alleles; a,b= total number of bands per individual).

Among a total of 186 parent-child combinations the average portion of informative bands was 13.4 with an average of 20.5 total bands (= 65.4%).

The band sharing rate for all possible sibling combinations (n=222) was 63.0±11.2%, which is in the same order as other comparisons among first degree relatives (e.g. father/child : 61.5±5.4%, mother/child : 64.4±6.9%, Rittner et al. 1991). The parent-child comparison (n=389) yielded a band sharing rate of 59.5±11.4% (average no. of bands : 21.1), which is in the same order and nearly identical to the rate given by Schacker et al. 1990

C 48 DNA FINGERPRINTING IN PATERNITY TESTING IN LITHUANIA.

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On the basis of the observations by Vassart, G. et al. (1987) and Dzhincharadze, A. et al. (1987), proving that wild-type M13 bacteriophage DNA could serve as a molecular probe for detection of hypervariable minisatellite regions in human genome, the DNA fingerprinting technique for disputable paternity testing in forensic medicine was applied. The method used included the DNA extraction from blood samples from the mother, father in dispute and child, digestion with Hinf I (preferentially) or Bsu RI restriction endonuclease, electrophoresis through agarose gel, and Southern blot-hybridization to ^{32}P-labelled M13 probe. The latter one is prepared from single-stranded M13 DNA by partial synthesizing of the second strand from the forward sequencing primer in the DNA polymerase I Klenow fragment catalyzed reaction. Since 1990, the DNA fingerprinting method above was approved by Lithuanian authorities for the use in paternity testing. Since the fall of 1990, 28 cases of disputed paternity using M13 probe were studied. The following regularities were observed in the fingerprints obtained: in average, 20 well-identifiable DNA fragments were scored for every individual, while 6 fragments were shared by all individuals in the case. For nearly 20% of the cases studied, the number of co-migrating identifiable fragments made up to 10-13. The ambiguity obtained was eliminated by parallel use of restriction endonuclease Bsu RI. Two cases of paternity exclusion were obtained, while in one case the exclusion was obtained by DNA fingerprinting only, where no conventional biochemical or blood group marker system was effective.

C 49 DNA TYPING IN FORENSIC CASEWORK IN NORWAY. STRATEGIES AND EXPERIENCES.

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During the last four years, VNTR typing has been offered in forensic casework as a supplement to traditional diagnostics. Probes presently used are MS1 (D1S8), YNH24 (D2S44), MS31 (D7S21), and MS43A (D12S11).

The number of cases chosen for DNA typing has ranged from about 60 to about 80 per year. We have experienced a wide range of case types. Rape/sexual assault are the most common, but cases under investigation for murder, incest or body identification are also relatively frequently encountered. A selected series of cases will be demonstrated.

The typing success rate has improved greatly, reflecting a combination of more careful selection of cases and improved typing procedures. Statistical data pertinent to the usefulness of DNA typing in casework will be presented.

C 50 DETERMINATION OF INCEST IN FORENSIC CASEWORK USING MULTI-LOCUS DNA PROFILING.

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Multi-locus DNA profiling is a highly useful tool in resolving paternity disputes. However, the power of exclusion can be not high enough in instances where there is blood relationship between the mother and the alleged father. In such class of cases the absence of unassigned bands impacts heavily on the probabilities calculated for inclusion estimate.

The present study was designed to produce the model to assess the probabilities of multi-locus DNA fingerprints as means of revealing genuine relationships between blood relatives. Using statistical modelling approach the "phantom" DNA profiles were computer simulated with distribution of bands in the profile dictated by the degree of genetic relatedness predicted for the parents. The numerical consequence of the treatment could be explored for evaluating evidence in the context of determining incest. The statistical chance for any claimed relationship between two individuals is available from similarity index calculated for this particular pair.
The forensic application of this approach to the determination of incest for the sexual assault case will be reported.

C 51 DNA PROFILING INTO CARBONIZED CORPSE.

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The introduction of DNA profiling into operational casework, a carbonized corpse, is described. The technique was examined for its potential application in forensic science. DNA profiling was carried out from bone, different tissues and hairs of two different cases. We have developed a rapid procedure, essentially based on standard protocols with some modification, to isolate DNA. Briefly, excised tissues and bones were dropped into liquid nitrogen and powdered with a mortar and pestle; DNA was then purified.
The DNA extration from hairs was carried out from ten carbonized roots, containing a very small part of bulb, in a different way.
The procedure yields 300µg to 350µg / $1cm^3$ of bone or tissue and 10 hairs of high molecular weight DNA, in every case suitable for restrition enzyme digestion, Southern blotting analysis or PCR amplification.
We looked at DNA profiling after digestion with Hinf I and Southern blotting using YNH 24 (Nakamura et al.) and Glob. 3' HVR (Nicholls et al.) as single locus probes and we obtained the predicted hybridization patterns.

C 52 OPTIMISATION OF THE DIGOXIGENIN/CHEMILUMINESCENCE METHOD FOR THE DETECTION OF VNTR

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To improve the resolution of the bands pattern for the detection of the human VNTR with the non - isotopic digoxigenin/chemiluminescence method, we have try to optimised the different conditions of some parameters :
- transfer solutions for the Southern-blotting : NaOH, 10xSSC, NH4Cl
- blocking reagents for the hybridization : Promega, Tropix, Boehringer, SDS+PEG
- fixation : UV 245nm, UV 302 nm, backing at 80°C and 120 °C
- enhancer substances for the detection : albumin and PEG

For our assays, we size-fractionned 3 ug saline extracted genomic DNA, digested with Hinf I (5U/ug), on a 0.8 % agarose gel (Seakem Le) during 25h. at 35 V. followed by a capillary blotting tranfer to charged nylon membranes (Sigma) and detection was done with AMPPD (Tropix).

D 1 GENETIC AND MOLECULAR ASPECTS OF THE HUMAN RED CELL ACID PHOSPHATASE POLYMORPHISM.

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Human red cell acid phosphatase is one of the classical enzyme markers used in the forensic science laboratory. Recent results have provided knowledge of the genetic and biochemical basis of this polymorphic system. The three isozyme pairs (**Af/As**, **Bf/Bs** and **Cf/Cs**) encoded by the ACP1*A, ACP1*B and ACP1*C alleles have been characterized with respect to enzymatic, molecular and immunochemical properties. The **Af**, **Bf** and **Cf** isozymes have identical properties as have the corresponding three **s** isozymes, and from a functional point of view the acid phosphatase system consists of only two isozymes, **f** and **s**. The ACP1 alleles generate different proportions of **f** and **s** isozyme, probably by alternative splicing of specific **f** and **s** exons. The different relative content of **f** and **s** isozyme in the various ACP1 phenotypes is the key to their different properties. All 6 isozymes consist of a single peptide chain of 157 amino acid residues. **f** isozymes differ from **s** isozymes with respect to an internal segment of 34 amino acid residues. The **Bf** and **Bs** isozymes are identical in sequence to the **Cf** and **Cs** isozymes, whereas the **Af** and **As** isozymes differ from these with respect to a single amino acid residue. To further determine the nature of the allelic differences DNA-sequencing of the ACP1 gene is being performed.

D 2

THE RhD ANTIGEN IN ccDee AND ITS' INHERITANCE

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In 1969 we used CcDee, ccDEe red blood cells and red blood cells of those persons who had caused sensitization, for booster injections given to Rh negative women who are already sensitized. Since 1976 we use ccDee for booster red blood cells. Because we discovered a large number of people with this genotype in the vicinity of Ljubljana, we examined their relatives as well and determined inheritability. The results are given as well as the immune response in 523 inoculated persons - 1849 inoculations in all.

D 3 ISOELECTRIC FOCUSSING STUDY ON SERUM PROTEINS (GC, TF, PI AND ORM) IN FOUR CASTE GROUPS OF MAHARASTRA, WESTERN INDIA: APPLICATION IN PATERNITY TESTING

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Several serum protein systems failed to exhibit genetic variability with traditional electrophoretic techniques. The introduction of technique of isoelectric focussing (IEF) has made it possible to reveal in these an extensive genetic diversity in the gene pool of human and other species. The essential knowledge about the variation of such genetic subtypes in various populations of the third world countries is still lagging behind. The present study has analysed subtypes of four serum proteins (GC, PI, TF and ORM) in four endogamous caste groups from the state of Maharastra, Western India. These caste population showed a vast genetic differentiation at each of locus studied. These marked allele frequency differences observed between different caste groups are likely to be due to their breeding structure. Several of such populations have migrated to the West and now permanently settled in Europe, United States and Canada. The anthropogenetic use of these subtype variants have now become increasingly evident but work on their genetic implication in forensic science in immigrant populations is limited. Based on the subtype allele frequencies we report the exclusion probabilities for paternity examination in these Indian caste groups.

D 4 PGM1 SYSTEM: A RARE ALLELE AND AN INTRAGENIC RECOMBINATION IN TWO CASES OF DISPUTED PATERNITY.

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PGM1 subtyping by thin layer polyacrylamide gel isoelectric focusing (PAGIF), has become a commonly used method, because it is simple, highly reproducible and a relatively cheap method. However, the results are complicated by the existence of silent alleles, by at least 30 rare variants and by the ocorence of unexplained maternity exclusions and single paternity exclusions, according to the 1rst rule of heredity. Here we report two cases of exclusion: one is a paternity exclusion by some genetic markers, including PGM1 system, where is involved a rare allele; another case is a maternity exclusion only by PGM1 system, where the mother (1B2A) has one of the only two genotypes (1B2A and 1A2B) that can give rise to recombinant gametes during oogenesis, according to a phylogeny theory described by Carter et al and developed by Takahashi et al. This theory has been corroborated by many investigators including G. Wetterling (1990). This last study was completed by RFLP analysis.

D 5 INCOMPATIBLE MOTHER-CHILD PAIRS IN THE PGM1-SYSTEMS

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Incompatible mother-child pairs involved in cases of disputed paternity in Berlin during the last twenty years have been retested by isoelectric focusing in polyacrylamid gel for subtyping. Our findings will be discussed with genetic interpretations, obtained by other investigators.

D 6 GENETIC MARKERS (HP, TF, GC AND PI) IN TWO POLISH POPULATION SAMPLES.

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Two Polish population samples (198 Poles from Central Poland = P; 228 Kashubes from Northern Poland = K) were typed for HP types and TF, GC and PI subtypes. The allele frequencies are as follows: HP*1 = 0.3699 (P), 0.4289 (K); TF*C1 = 0.7677 (P), 0.8128 (K), TF*C2 = 0.1641 (P), 0.1233 (K), TF*C3 = 0.0631 (P), 0.0551 (K), TF*B = 0.0051 (P), 0.0088 (K); GC*1F = 0.0876 (P), 0.1674 (K), GC*1S = 0.5722 (P), 0.5000 (K), GC*2 = 0.3402 (P), 0.3326 (K); PI*M1 = 0.6897 (P), 0.6711 (K), PI*M2 = 0.1359 (P), 0.1970 (K), PI*M3 = 0.1359 (P), 0.0899 (K), PI*S = 0.0103 (P), 0.0219 (K), PI*Z = 0.0128 (P), 0.0153 (K), PI*Var = 0.0154 (P), 0.0088 (K). Comparing these data with that oberved in other East European populations (Hungarians, Matyos, Slovaks, Gypsies) a considerable locus and allele specific heterogeneity in the distribution of these allele frequencies was found. Genetic distance analysis disclosed the existence of two subclusters (Hungarians + Matyos; Poles + Slovaks), which are linked up to one cluster. Gypsies and especially Kashubes differ obviously from this cluster, which can be explained considering the ethnohistory of these two populations. The average heterozygosity is the lowest in Gypsies. This marked genetic heterogeneity within East European populations is not only of considerable interest to population genetics, but also with regard to the application of Essen-Møller values in cases of disputed paternity, when East Europeans are involved.

D 7 A DE NOVO MUTATION IN THE ALPHA1-ANTITRYPSIN GENE DETECTED IN A CASE OF DISPUTED PATERNITY BY DNA SEQUENCE ANALYSIS.

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Alpha-1-antitrypsin (α1AT), the major protease inhibitor (PI) of neutrophil elastase, is a highly polymorphic glycoprotein in human plasma. More than 75 different PI variants have been identified hitherto by either isoelectric focusing (IEF) of serum and/or sequence analysis. Reported here is a problematical case of paternity with an isolated exclusion in the PI system. Subtyping by IEF has shown the following results: mother PI M1M2, child PI M1-Var, and alleged father PI M1M2. In other 30 serological systems, including HLA, tested and evaluated biostatistically we found a strong evidence for paternity (W=99.9972%). DNA-haplotype analysis confirmed the serological data. The molecular basis of the child's PI variant was defined using automated direct sequencing of PCR-amplified genomic DNA. A point mutation was identified which causes an amino acid substitution in exon V of the α1AT-gene: Asp 341 (GAC) → Asn 341 (AAC). This mutation was excluded within both of the parental DNAs. We conclude: (1) The child's PI variant can be explained by this new mutation. (2) The serological systems and their biostatistical probability are sufficient to confirm the fatherhood in this case.

D 8 TWO-DIMENSIONAL ISOELECTRIC FOCUSING ANALYSIS OF RARE AND SILENT ESTERASE D TYPES. DESCRIPTION OF A NEW ESD VARIANT PHENOTYPE.

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We have recently described a new two-dimensional isoelectric focusing method (2D-IEF) that has permitted the identification of the ESD subunits from the homodimeric and heterodimeric forms of five common ESD phenotypes [Alonso, A. et al. Electrophoresis 1991 (in press)]. Here, we present the 2D-IEF pattern of three ESD variant phenotypes (ESD 7-1, ESD 7-2, and a new ESD 1-VAR phenotype observed in a Spanish population) as well as two heterozygous phenotypes for a silent allele (ESD 1-QO and ESD 2-QO). The ESD 7 monomer (M7) has been identified from the 1-D heterodimeric forms (HE 7-1 and HE7-2) as a band with the same isoelectric point found in the ESD 1 monomer. The 2D-IEF analysis of the ESD 1-VAR phenotype, which showed a very cathodal 1-D IEF pattern, has permitted the identification of the ESD VAR subunit, from the 1-D homodimeric (H VAR-VAR) and heterodimeric (HE 1-VAR) forms, with a mobility cathodal to the ESD 1 monomer. Finally, the 2D-IEF pattern of the ESD 1-QO and ESD 2-QO phenotypes are characterized by the presence of the ESD M1 band and the ESD M2 band, respectively . These results indicate that the possible allele product of ESD*QO is unable to form dimers. However, further investigations are necessary to clarify if there is an allele product that is unable to form dimers or the ESD*QO allele leads to a lack of polypeptide product.

D 9

Haptoglobin Subtypes in Lower Saxony (Germany)

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The distribution of Hp phenotypes and gene frequencies from 400 unrelated German adults will be presented. Determination of Hp subtypes are carried out according to the method of Patzelt and Schröder (1986) with slide modifications.Hp subtyping of 120 mother-child pairs has been investigated, too. Unexpected combinations in the material are not found. The data are in full accordance with the postulated genetic model of an autosomal locus with codominant alleles.

D 10

C4 PHENOTYPE AND GENE DISTRIBUTION IN A POPULATION OF EASTERN LOMBARDY (ITALY).

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The human complement factor 4 (C4) is a ß1 globulin that acts in the classical pathway of complement activation. C4 molecules are encoded by two different loci C4A and C4B located between HLA-B and DR in the MHC on chromosome 6p. It is an high polymorphic system but it's not commonly used and extensively investigated in forensic haemogenetics, particulary in Italy. The aim of this study is to present a contribution to a better knowledge of the distribution of C4 polymorphism in Italy through a population sample from the Brescia area (Lombardy, Northern Italy). C4 phenotypes were determined in EDTA-plasma samples from 250 unrelated and healthy blood donors; the separation of C4 fraction was carried out by agarose gel electrophoresis after treatment with carboxypeptidase and/or neuraminidase and subsequent immunofixation using monospecific C4 antiserum, according to Awdew and Alper (1980). Beside the common phenotype C4A3 and C4B1, we observed 6 different variants for C4A and 6 for C4B. The following allele frequencies were calculated: C4*A3=0.772, C4*A1=0.002, C4*A2=0.053, C4*A4=0.008, C4*A6=0.024, C4*A13=0.006, C4*AQO=0.109, C4*B1=0.781, C4*B2=0.090, C4*B3=0.008, C4*B5=0.006, C4*B6=0.004, C4*B51=0.006, C4*BQO=0.155. The allele frequencies have been compared with the few data published from other populations.

D 11

Distribution of Transferrin(Tf), Red Cell Acid Phosphatase(EAP), Esteras D(EsD) and Group Specific Component(Gc) Phenotypes in China.

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Population data on electrophoretic genetic markers is necessary for a statistical evaluation of forensic evidence . Since the high heterozygosity of Tf, EAP, EsD, and Gc polymorphism in population, They are very useful in paternity test and identification. The purpose of this work is to present the databases of Tf, EAP, EsD and Gc that are Phenotypes and gene frequencis in unrelated adult Chinese; to provide the probability of discrimination and the probability of excluding paternity in four polymorphisms mentioned above; and to compare the databases in Chinese with that in other populations.

The distribution of Tf, EAP, EsD and Gc Phenotypes in Taiyuan, China, was investigated by isoelectic focusing (Tf, EAP), Non-equilibrium isoelectric focusing (EsD) and Polyacrylamid gel disc electrophoresis (Gc). The gene frequencies are as follows: TfC1 0.7561, TfC2 0.2244, TfD 0.0171; EAPB 0.8076, EAP A 0.1923; EsD1 0.6065, EsD2 0.3934; Gc1 0.7559, Gc2 0.2441.

D 12

NULL AND RARE ALLELES IN PATERNITY TESTING.

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During 1990 and the four first months of 1991, we have analysed 290 disputed paternity cases in our laboratory. To resolve these paternity investigations, we have caracterized about 20 conventional markers and, in some cases HLA typing. We have found some null alleles and rare alleles in the studied genetic markers, namely: Duffy (null allele) and Pi, PLG, 6-PGD, PGM1, EsD and Tf (rare alleles). Here we report a pedigree of one studied family, which carried a null allele in the Duffy system and other families carrying rare alleles from some other genetic markers refered before. The allele frequencies of these markers are presented.

D 13

COMPARATIVE SUBTYPING OF ACP-1, PGM-1 AND ESD IN HUMAN PLACENTA AND CORD BLOOD BY ISOELECTRIC FOCUSING: PRACTICAL CONSIDERATIONS OF FORENSIC SIGNIFICANCE.

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Human red cell enzymes with genetic polymorphism (ACP-1, PGM-1, ESD...) were previously analyzed in placenta and other human tissues by conventional starch gel electrophoresis. However, to our knowledge, analysis of these enzymes from human placenta by isoelectric focusing (IEF) have not been performed hitherto. In this report, the genetic polymorphism of ACP-1, PGM-1, and ESD was studied in placental extracts and red cell lysates from the corresponding cord and maternal blood by IEF using miniaturized polyacrylamide gels. It has been demonstrated that the polimorphic variation that these enzymes displayed in placenta corresponded to the phenotypes of the newborn. However, due to maternal blood contamination, a mixture of maternal and child allele products could be observed when typing was performed in placental extracts from the maternal face. On the other hand, the following differences in the IEF pattern from placenta and red cells were found: In addition to the ACP-1 allele products, three monomorphic bands, which were demonstrated to correspond to the genetic products of the ACP-2 and ACP-3 loci, were detected in placenta. Furthermore, in this tissue, the ACP-1-C band of the B phenotype showed higher staining intensity than the ACP-1-B band, making difficult the differentiation between B and BC phenotypes. The staining intensity of the PGM-1 allele products was at least 20 times higher in placenta than in cord blood. Finally, it was observed no difference in the expression of ESD phenotypes in placenta as against in red cells.

D 14

DISTRIBUTION OF ADENOSINE DESAMINASE (EC 3.5.4.4) PHENOTYPES IN A SERIES OF HIV-SEROPOSITIVE PATIENTS.

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In several studies increased levels of the adenosine desaminase (ADA) have been described in blood of AIDS patients. To our knowledge the distribution of the phenotypes was never examined. Therefore we typed the ADA phenotypes in a series of 200 HIV-seropositive patients with a varying spectrum of severity of AIDS. The distribution found was very similiar to studies on healthy persons.

D 15

DETERMINATION OF C1R TYPES IN BLOODSTAINS.

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Polymorphism of C1R was investigated in bloodstains by means of isoelectric focusing and electroblotting. Bloodstains were made on filter paper and stored at 37°C, room temperature and 4°C. 5 x 5 mm pieces of bloodstains were extracted with 20 µl 1 M potassium phosphate buffer (pH 7.0) containing 10 U/ml neuraminidase and the extracts were subjected to polyacrylamide gel isoelectric focusing at pH 5-8 in the presence of 7 M urea using 10 x 5 mm filter paper strips. After run, proteins were transferred onto a sheet of nitrocellulose membrane by electroblotting. The focused proteins were reacted with goat anti-human C1R serum and then with alkaline phosphatase conjugated anti-goat IgG serum. The membrane was stained with ß-naphthyl phosphate and Fast Blue BB salt. All the bloodstains examined (C1R 1-1, 2-1, 2-2, 5-1, 5-2, 5-5) were correctly typed for C1R at 37°C for up to 2 weeks, at room temperature for up to 5 weeks and at 4°C even over 10 weeks. The bands became fainter and more indistinct with increasing age of stains. This polymorphism can be a useful genetic marker for the medicolegal grouping of bloodstains.

D 16

COAGULATION FACTOR XIIIB PHENOTYPING IN A JAPANESE POPULATION AND IN BLOODSTAINS.

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Polymorphism of FXIIIB was investigated on 555 unrelated Japanese individuals. Plasma was separated and treated with 40 U/ml neuraminidase. Isoelectric focusing was performed with polyacrylamide gel plates at pH 4-8. After electrofocusing, proteins were transferred onto a sheet of nitrocellulose membrane using electroblotting. The membrane was stained for FXIIIB by immunologic detection using rabbit anti-human FXIII-S serum and alkaline phosphatase-conjugated goat anti-rabbit IgG serum. In our sample five common phenotypes FXIIIB 1-1, 2-1, 3-1, 3-2, 3-3 and a rare variant type FXIIIB 15-3 were observed. The allele frequencies were FXIIIB*1 = 0.3063, FXIIIB*2 = 0.0162, FXIIIB*3 = 0.6766 and FXIIIB*15 = 0.0009. The above technique was transposed to the phenotyping in bloodstains that were made on filter paper. 5 x 6 mm stains were moistened with 10 µl 20 U/ml neuraminidase and directly placed on the gel. Phenotyping was possible from 20 bloodstains stored at 37°C for up to 4 months, and at room temperature and 4°C even over 5 months. No significant differences on the determination limits were recognized among the five common phenotypes. The FXIIIB system would provide a new powerful means for the grouping of bloodstains in forensic casework.

D 17 FORMAL GENETIC DATA ON ORM1 SUBTYPES

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ORM1 was determined in families from SW Germany and the Porto area (Portugal).Several individuals were detected,which exhibited heterozygous phenotypes ORM1 F1F2S and ORM1 F1F3S,respectively.Segregation analyses showed in both the samples,that alleles ORM1*F1, ORM1*F2 and ORM1*F3 were transmitted as haplotypes ORM1* F1F2 and ORM1*F1F3.These observations indicate,that the ORM1 locus seems to be duplicated,and the information ORM1*F1 can be arranged tandemly with those for ORM1*F2 and ORM1*F3,respectively.

D 18 ISOELECTRIC FOCUSING OF INTER-ALPHA-TRYPSIN-INHIBITOR (ITI)

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Segregation analyses of ITI in 239 families with 677 children from SW Germany support the formal hypothesis : Two common and one rare alleles,ITI*1,ITI*2,ITI*3, at an autosomal locus ITI. Gene frequencies were calculated as :
ITI*1 = 0.600, ITI*2 = 0.393, ITI*3 = 0.007 .
Additionally first frequencies in a population sample from Porto (Portugal) will be presented.

D 19 HUMAN ZN-ALPHA 2-GLYCOPROTEIN PHENOTYPING IN SEVERAL POPULATIONS.

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Human Zn-alpha 2-glycoprotein (ZAG) is a glycoprotein without reliable information concerning its biological function and found in various body fluids i.e. plasma, semen, sweat, saliva, urine, etc. The genetic variation of plasma ZAG has been reported by Kamboh and Ferrell (1986), Nakayashiki and Katsura (1989) and Ding et al. (1990). We tested ZAG in plasma or serum samples from Japanese, Korean, Thai, Phillipine, Brazilian Indian and Papua New Guinean populations. After desialization of the samples, ZAG phenotyping was carried out by means of immunoblotting after polyacrylamide gel isoelectric focusing in the pH-range of 4.5 - 5.4. Most of the samples showed a typical single band as ZAG 1 phenotype, however, ten different band patterns were found in some samples. In the ten patterns, a single band different from ZAG 1 was identical to the band produced by ZAG*5 allele and the other patterns were double bands by ZAG*1 and one of nine rare alleles, ZAG*2 to ZAG*10. In all of the tested populations, the frequency of ZAG*1 was over 0.95 and the other alleles were very low (< 0.01) except for ZAG*10 (0.048). Of nine rare alleles, five designated ZAG*6 to ZAG*10 will be first reported. From the distribution of ZAG alleles, ZAG*2 and ZAG*10 were characteristically detected in the population of Japanese and Papua New Guinean, respectively. Some allelic variants of ZAG might be distributed in restricted populations and become a useful marker to the anthropologic study on ancestral ethnic links.

D 20 SPECIES IDENTIFICATION FROM TISSUE PARTICLES BY USING THE LECTIN- AND IMMUNO-HISTOCHEMICAL METHODS.

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We report here the method for the species identification from tissue particles with both the lectin- and immuno-histochemical techniques that utilize Ulex europaeus agglutinin I (UEAI), Griffonia simplicifolia agglutinin I-B4 (GSAIB4) and anti-blood group ABH-antibodies. The samples used were formalin-fixed and paraffin-embedded tissue particles obtained from the various vertebrates. The red blood cells (RBC) and vascular endothelium cells (VE) were observed histochemically as markers. UEAI had no reaction with those cells in the fishes, frogs, saurians, birds, non-primate mammals, prosimians, new world monkeys and the old world monkeys. Only the human and anthropoid apes were found positive. The anti-ABH-antibodies showed the immuno-reactivities to the samples from the frogs and old world monkeys in addition to the human and anthropoid apes. The non-primate mammals, prosimians and the new world monkeys were detectable with GSAIB4. It is suggested that changes in the expressions of the ABH-antigens and the binding properties of the lectins, found in the evolutional process of the vertebrate species, are greatly available to identifying the species from tissue particles.

D 21 A SIMPLE TECHNIQUE FOR THE DETERMINATION OF GGTP TYPES.

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Polymorphism of gamma-glutamyl transpeptidase (GGTP) was investigated on semen samples from 350 male volunteers using horizontal starch gel electrophoresis. The bridge buffer was 0.3 M boric acid/0.0625 M sodium hydroxide (pH 8.6); the gel buffer was 0.0765 M tris/0.005 M citric acid (pH 8.6). Electrophoresis was carried out at a constant current of 1.33 mA/cm for 3.5 h at 4°C. After run, the gel was horizontally sliced and covered with a sheet of filter paper soaked in 10 ml 0.1 M glycylglycine (pH 9.0) at room temperature for 15 min. Then, the gel was incubated with another sheet of filter paper soaked in 10 ml 2 % L-gamma-glutamyl-*p*-diethylaminoanilide dihydrochloride/0.3 % α-naphtol at 37°C for 3 h. After incubation, the gel was stained with 0.2 % periodic acid at room temperature for 5 min. In our sample three common phenotypes GGTP 1, 2-1 and 2 were identified. The results for the distribution were 129 GGTP 1, 173 GGTP 2-1 and 48 GGTP 2. The allele frequencies were GGTP*1 = 0.616 and GGTP*2 = 0.384. The observed numbers were in good agreement with the expectation according to the Hardy-Weinberg law (χ^2 = 0.73, d.f. = 1, 0.50 > P > 0.30).

D 22 POLYMORPHISM OF PLASMINOGEN IN SARDINIA (ITALY).

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The distribution of PLG phenotypes in the population of Sardinia (Italy) has been investigated, by means of isoelectric focusing followed by immunofixation of desyalized sera, in a random sample of 495 unrelated healthy blood donors.
The allele frequencies calculated in our study were:
PLG*A = 0.6899 ± 0.015
PLG*B = 0.2980 ± 0.014
PLG*A3 = 0.0081 ± 0.003
PLG*M4 = 0.0040 ± 0.002
The theoretical exclusion rate in cases of disputed paternity is 18.12% (I class = 9.66% II class = 8.46 %).

D 23 HAPTOGLOBIN SUBTYPES IN A POPULATION FROM SOUTH WEST GERMANY

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Subtyping of haptoglobin (Hp) by isoelectric focusing (IEF) has been shown to lead to an isolated paternity exclusion chance of up to 33%. The Hp subtype polymorphism is therefore one of the most informative systems in paternity testing. Despite this high exclusion chance, Hp subtyping is rarely included in routine testing because of the time consuming steps in Hp purification by conventional ion exchange chromatographic methods. This limitation was overcome by an immunoprecipitation technique (Scherz R, et al, Advances in Forensic Haemogenetics 1989; 3:236-238).
We have determined the Hp subtype frequencies in 1 485 sera collected from a random population sample of unrelated German nationals who had participated in a 1985 health survey in the city of Stuttgart, South West Germany. Storage of samples at -20° C over 5 years did not affect the ability to determine Hp subtypes. All Hp phenotypes except heterozygous Hp 2FF-2SS and the homozygous Hp 2FF and Hp 2SS were detected in the sample. The calculated gene frequencies were: Hp*1F 0.1582, Hp*1S 0.2171, Hp*2FS 0.6, Hp*2SS 0.0202, Hp*2FF 0.004. The observed phenotype frequencies are in good agreement with the expected distribution under the Hardy-Weinberg law.
Gene frequencies in Stuttgart, South West Germany are consistent with those reported for other German and European populations.

D 24 NEW VARIATION IN LOW SULPHUR KERATINS DETECTED BY HYBRID ISOELECTRIC FOCUSING (HIEF)

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In previous papers, we have demonstrated variability in non-carboxymethylated keratins of human head hair using isoelectric focusing (IEF) in the presence of 6M urea and 1,5% Nonidet P40, and we have correlated the patterns with those obtained by other authors with SDS-PAGE.
Using the IEF technique, the variability could only be detected by silver staining methods, nevertheless with SDS-PAGE the patterns could be seen after Coomassie staining, so we thought that variability in other parts of the IEF gels with higher concentration of proteins should exist. With that aim we undertook the present study, applying HIEF in various pH ranges. 6M urea, 0,05M dithiothreitol (DTT) and 1,5% Nonidet P40 were used in the rehydrating solution. New variability was observed in the pH range 4.7-5.6 and its origin is discussed.

D 25

THE INFLUENCE OF INFUSED ERYTHROCYTES ON THE DETECTION OF INDIVIDUAL MEMBRANE AND ENZYME SYSTEMS.

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We report two cases in which after a massive infusion of erythrocytes we were ordered by the court to ascertain the serological markers. The blood samples taken only a short time after infusion. In the first case (case of identity, dwi-driver) we found general agreement between the first and a significantly later taken sample. In the second case (affiliation, dead putative father) we came to the conclusion that the infusion had not falsify the serological results. From our experience DNA fingerprinting is the 'conditio sine qua non' in such cases because this method is not influenced by infusion of erythrocytes.

D 26

PARISH OF BEJSCE AS A SOURCE OF UNIQUE MATERIAL FOR POPULATION GENETIC STUDY

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Bejsce is located in South-East Poland, between Krakow and Kielce. The parish was located in 1313. It comprises of 8 villages. Number of inhabitants has been slowly incrased: 1590 - 1000, 1740 - 2000, 1850 - 3000 and 1970 - 4000 and inside the Bejsce from 300 to 800 in the same time. The parish church registers are very good preservated. First book is from 1550, marriages have been regullary registered since 1583, births since 1607 and deaths since 1679. Data from these registers were used for reconstruction of pedigree during last 300 years. In this time were registered over 33000 inhabitants. Population of the parish has been under study by dr. Edmund Piasecki and his coworkers from Institute of Anthropology since 1966. The research work is concerned with problems of anthropology and population genetics. The preliminary DNA study of families from Bejsce has been performed and results are presented by dr. Tadeusz Dobosz and coworkers. Further studies of the population of Bejsce are planned.

D 27

PLASMA PROTEIN POLYMORPHISM IN HIV-SEROPOSITIVE PATIENTS: GC- AND TF*C-SUBTYPES AND PI-SYSTEM

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The paper reports on the distribution of three plasma protein systems in serum samples from 300 HIV-seropositive patients with a varying spectrum of AIDS [HIV-seropositive only, ARC (AIDS-related-complex), AIDS and Kaposi-sarcoma. The frequencies of the Gc- and Tf*C-system are similiar to those found in obviously healthy persons. In the PI-system this expanded study confirmes the results of our pilote study. We found unusual fearures of distribution. The problems of interpretation are discussed.

D 28

COMPARATIVE TYPING OF OROSOMUCOID (ORM) VARIANTS AND PROPOSAL FOR A NEW NOMENCLATURE

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Isoelectric focusing has revealed that human ORM polymorphism is controlled by two functional loci. Many variants with confusing designations have been reported thus far. In this study the ORM variants observed in 7 laboratories are compared and reclassified to propose a new nomenclature. The results showed 26 alleles including one silent and two duplicated alleles at the ORM1 locus and 28 alleles including one silent allele at the ORM2 locus. The ORM1 and ORM2 products were distinguishable in band intensity from each other. The three common ORM1 alleles should be called ORM1*F1, ORM1*F2 and ORM1*S and the one common ORM2 allele, ORM2*M. The variants were alphanumerically designated on the basis of their pI after isoelectric focusing in the presence of Triton X-100: ORM1*A4-ORM1*A1, ORM1*B1-ORM1*B10, ORM1*C1-ORM1*C6, ORM1*dF1S, ORM1*dB9S, ORM2*L9-ORM2*L1, and ORM2*H1-ORM2*H17.

D 29 INTRAGENIC RECOMBINATION WITHIN THE ALFA-1-ANTITRYPSIN LOCUS.

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A mother-child exclusion which could not be explained by a silent allele has been observed in the alfa-1-antitrypsin system. There is no doubt about the motherhood. The mother has the phenotype Pi M1,M2, the child Pi M3 and the father Pi M3,S.

The alfa-1-antitrypsin protein is commonly used in paternity testings. Several alleles have been described: Pi M, Z, S and F, where the Pi M allele is the most frequent.

The Pi M allele can be subdivided into two subloci determined by codon 101 and codon 376 in the Pi allele resulting in the following four alleles:
Pi*M1 101 Arg - 376 Glu
Pi*M2 101 His - 376 Asp
Pi*M3 101 Arg - 376 Asp
Pi*M4 101 His - 376 Glu

Usually the Pi*M3 and Pi*M4 alleles are not separated because of small charge differencies.

A cross-over in this region can give rise to false paternal exclusions. The most probable explanation to the observed mother-child exclusion is a recombination in the oogenesis.

D 30 DETERMINATION OF PGM, ESD, GLO (1) AND EAP POLYMORPHS FROM HUMAN DENTAL PULP.

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Isozymes of phosphoglucomutase, esterase D, glyoxalase 1, erythrocyte acid phosphatase have successfully been detected in human dental pulp. Comparative studies on human blood stains and teeth revealed that polymorphic forms in dental pulp were more stable than in blood stains, when subjected to different Indian ambient conditions prevailing in a year.

D 31 SOCIAL IMPLICATIONS IN IMMUNO GENETICS PLAZA DE MAYO GRANDMOTHERS ASOCIATION - BUENOS AIRES - ARGENTINA-

Between 1976 and 1983, a dictatorship was installed in Argentina.This caused the death and missing of about 30.000 people (including hundreds of children). The Abuelas de Plaza de Mayo began in 1977 a painful task: the search of their grandchildren,kidnapped or born in captivity. The dictatorship years* were really difficult and dangerous to achieve this work.With the return of the democracy,we began to reach to our goals for which we were fighting.Considering the possibility of knowing with securtty our grandchildren (among a lot of children) we began to search, all over the world, a scientific method to be sure of the biological relationship between the child and his true family. So,in this manner, our dream came true, the creation of the National Bank of Genetic Data, site in the "Carlos Durand" Hospital,depending on the Buenos Aires Municipality. Now, for seven years,we are leaving our blood for analysis and our family group's too. This blood will be kept till 2050,and our illusions is, someday, to find our grandchild, taken away by force from their parents,loosing their identity. This great pain took us to try to recover his history and their parents'.Today,15 years from our beginings,we've got the identification of 50 children, 930 people were analyzed in Argentina and in different countries.We keep on working,trying to complete the immuno-genetic information of many genealogical trees, receiving information about children or pregnant women who were kidnapped in the past. The methodology used in Durand Hospital includes: a)blood groups b)HLA histocompability c)seric proteins d)blood cell enzymes and,soon,Molecular Biology.Every scientific advance will help us in our objectives to establisch the true identity of the children, victims of the violence of the State terrorism.

E 1

HAPTOGLOBIN SUBTYPING BY POLYACRYLAMIDE GEL ISOELECTRIC FOCUSING OF SERUM, HEMOLYZED BLOOD AND BLOODSTAINS

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The aim of the present investigation was to compare two alternative methods for isolation of haptoglobin: ion-exchange chromatography (Connell et al. 1961, Patzelt et al. 1985) and immunoprecipitation (Scherz et al. 1990, Dimo-Simonin et al. 1990). The possibility of Hp-subtype determination with immunoprecipitation in bloodstains, hemolyzed blood and alcoholic blood (NaF stabilized blood) also was investigated. As result of our analyses we ascertain that the ion-exchange chromatography suits good to the routine analysis of sera. The immunoprecipitation is the method of choice for the haptoglobin subtyping of hemolyzed blood and bloodstains.

E 2

DETECTION OF BLOOD GROUP H ANTIGENS OF RED CELLS, BLOOD AND SALIVA STAINS, AND HAIRS BY ANTI-H REAGENTS.

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The sera and lectins which preferentially react with O and A_2 red cells and differentiate group O secretor salivas from non-secretors have been used for anti-H reagents. Reactivity with human red cells and absorbability and heat elution of the anti-H reagents from blood and saliva stains and hairs were examined. Human isoagglutinins, chicken anti-O red cell immune serum, monoclonal antibodies obtained from a mouse immunized with group O secretor saliva, eel sera from Anguilla japonica and Ulex europaeus agglutinins were tested for the serological examinations. Selected and appropriately diluted eel serum reacted only with group O red cells like anti-O can be used as standard serum for Bombay typing with anti-A and anti-B. The results of the absorption test of anti-H reagents with blood and saliva stains indicated that chicken and mouse monoclonal antibodies and Ulex europaeus I and II lectins showed powerful affinity to the stains. When the chicken serum was used for absorption and elution test of the stains and hairs strong agglutination of O red cells with the eluate was observed. IgM anti-H monoclonal antibodies and Ulex lectins were heardly eluted by the heating.

E 3

NEWER INVESTIGATIONS OF PROTEIN SYSTEMS BY ISOELECTRIC FOCUSING: METHODS, DISTRIBUTION, FORENSIC APPLICATION.

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This contribution describes the experiences with typing resp. subtyping of the protein systems A2HS, FXIIIA, FXIIIB, HP, ORM1, PLG, and TF including procedures of pretreatment, separation and detection. For routinely use we prefere the analysis by IEF, carried out in PAG, followed by immunofixation for detection of A2HS, FXIIIA and B, ORM1 and PLG. Data of population testing and paternity cases, including some rare variants, are presented. All studied markers are valuable tools in paternity testing. For typing of bloodstains some problems are to consider. The most useful marker for practical stain work will be the A2HS system.

E 4

DOT BLOT IMMUNOASSAY FOR DETECTION OF HLA ANTIGENS IN FORENSIC STAINS

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Analysis of polymorphic proteins and Variable Number of Tandem Repeats (VNTRs) is regularly practiced to individualise the stains of blood and other body fluids in a modern forensic laboratory. HLA system though a highly polymorphic is not yet optimally used in individualisation due to unsuitability of the microcytotoxicity method. In the present paper a simple and rapid dot blot assay which is highly specific and sensitive too, for the detection of HLA antigens to determine the source in body fluid stains, is described.

The stain extracts were immobilised on Nitro Cellulose Membrane (NCM). The stain loaded NCM was cut into discs of 3 mm diameter and placed one in each well of micro-titre plate and probed separately with a panel of antisera against eight most prevelant HLA antigens. After a thorough washing NCM discs were incubated with peroxidase labelled second antibody. The positive reactions were visualised by incubating second antibodies treated discs with substrate solution. HLA antigen could be detected even in a stain containing as small as 10 ug of dried blood and even as old as six months. Cross reactions were avoided by careful selection of antigen and antisera. The described method is found highly suitable for individualisation of blood and other body fluid stains on the basis of polymorphism in HLA loci.

E 5 THE USE OF MICROTITER TECHNIQUES FOR THE DETERMINATION OF RED BLOOD CELL PHENOTYPES IN PATERNITY TESTING.

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For the determination of erythrocyte phenotypes in paternity testing, 2 microtiter techniques are used in our routine investigations:
1) Capture-R™ (Immucor, Norcross, GA / USA) for Rh, Ss, K, Fy, Jk, Lu, Co and Xg^a, and
2) U-shaped microtiter plates (Greiner #650101, Nürtingen / FRG) for ABO, MNS, P_1, Le and Jk.
For Capture-R, polyclonal antisera are used; in the microtiter plates, monoclonal antibodies are utilized for ABO, MN, Jk and Le and polyclonal antisera for S and P_1.
The comparison of the results of the microtiter techniques with the conventional slide or tube tests showed no discrepancies in more than 500 complete typings. The microtiter techniques offer several advantages: higher dilution of the reagents used, possibility to store the trays for the repetition of the readings and easier handling of the tests.

E 6 Du detection by an automated direct agglutination method that equals detection by indirect antiglobulin test.

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Donor Samples (N=42 found to be non-reactive with Anti-D in the Dynatech microbank system and reactive 1+-3+ in tube indirect antiglobulin test (IAT) were tested by automated bi-directional tilt (ABT). In ABT, flat-bottomed microplates are processed along a track and are subjected to bi-directional tilts of 65° and 50°. All samples were typed D+ by ABT with reader values 154261-261164. Control samples with common D antigen expression read 207480-281899 and D-samples read 5300-6494. Commercial anti-D at a 1:50 dilution in a high molecular weight diluent was used.

ABT was at least as sensitive and usually more sensitive than IAT when judged by amount of antibody required to result in a positive reaction. The two methods were compared by testing each Du samples in each method with doubling dilutions of anti-D beginning with 1:12. The endpoint in IAT was the last dilution which gave a 1+ reaction. The endpoint in ABT was the last dilution to give a reader value >10.000. On average, the ABT endpoint occurred at an 11-fold greater dilution of anti-D than did the IAT endpoint.

E 7 Evaluation of sperm specific lactate dehydrogenase isozyme C_4/LBH C_4. Application to semen detection in stains. *

Human sperm specific LDH C_4 isozyme was investigated in semen and semen stains by electrophoretical and dot blot analysis. The purpose of the study was to evaluate the LDH C_4 as a semen marker in experimental semen stains and mixtures. Isoelectric focusing / IEF / of LDH C_4 with subsequent enzyme analysis detection or immunoblotting revealed multiple isoenzyme bands xompared with only one LDH C_4 band activity observed in conventional electrophoresis systems. LDH C_4 IEF in different pH gradients did not reveal isozyme polymorphisms in the analysed semen samples. LDH C_4 activity could be detected in seminal fluid and semen samples with a sperm count below 0.3 million/ml (IEF with subsequent immunoblotting) and in extracts from at least 5 month-old semen stains (dot blot analysis). Investigation of the possibility of LDH-C_4 detection in mixtures of semen with other body fluids and stability studies were also performed. Results obtained so far indicate that the antibody used is specific to human sperm.

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E 8 Blotting techniques in the detection of protein polymorphisms in stains *

The development of a new generation of immunochemical methods for protein investigations has opened new dimensions for forensic stain investigations. The application of immunochemophoresis leads to a higher specificity and an increased sensitivity of detection. Blotting techniques form an integral part of these methods because transfer of the protein under investigation from the gel to an easily handled membrane is necessary. The variety of blotting techniques at the disposal of the investigator is reviewed highlighting the advantages and disadvantages. Also reviewed are the applications of protein polymorphisms to forensic stain and paternity examinations.

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E 9

ABSORPTION ELUTION TEST FOR ABO TYPING OF SECRETOR AND NONSECRETOR SALIVA STAINS.

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The well known ammoniacal blood stain extraction method (Kind and Cleevely, 1969) was used for testing saliva stains. Up to now 55 saliva stains from A, AB or B donors (34 secretors, 21 nonsecretors) were tested, both with an inhibition method (using an anti-H and an anti-AB serum) and the absorption elution test (using anti-A and anti-B sera). In 20 of the 21 nonsecretor saliva stains the correct blood group could be determined; one A nonsecretor saliva stain showed an inconclusive result. Investigations of 30 cigarette ends showed, that in most cases the antigen A and B of the nonsecretors smokers were detectable, whereas the absorption test gave no results. The elution test can be recommended as a supplementary method, when the absorption test is performed without conclusive results. The elution test is not suitable to determine 0 nonsecretors, as reliable reactions with anti-H sera could not be obtained.

E 10

DETERMINATION OF ORM PHENOTYPES USING AS GELS PRECOTES 4-6 AND PHASTSYSTEM

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Phenotypes of ORM from blood samples were obtained using PRECOTES 4-6 gels and PhastSystem. The gel is cut as a minigel size and soaked in a solution composed of Servalyt 4-6 and Nonidet P40. The plasm samples are diluted in neuraminidase and distilled water. ORM phenotypes were carried out in microproccessor PhastSystem using IEF conditions. Visualization of results was accomplished by immunofixation using anti ORM and Coomassie Blue staining technique. The ORM patterns showed high resolution separation. Advantages provides by cutout gels PRECOTES 4-6 soaked in solution with 4-6 range Servalyt and Phast System include, highly resolved band pattern, cost saving, reduction in analysis time.

E 11

FAST DETERMINATION OF TF PHENOTYPES USING MINIGELS GRADIENT 4-6,5 MODIFICATED

M. de la Iglesia*, M.A. Martinez, A. Gremo. Department of Legal Medicine, pab. 7, Complutense University 28040 Madrid, Spain.

Application of minigels and the PhastSystem to obtain phenotyping results from blood samples in Tf typing system was investigated. Routine Tf phenotyping was carried out using 4-6,5 PhastGel. The minigels were pretreated with a solution containing: Servalyt 5-7 and Nonidet P40. Sera were treated with Ferrous Ammonium Sulfate solution. Tf phenotypes were carried out in microproccessor program until 795 vlothours. The Tf patterns were developed by Coomassie Blue staining technique. Tf typing with PhastSystem and PhastGel pretreated gave good and reproducible results, being an advantageaus hand very fast method over conventional normal gels. This technique has potential advantages not only for the study of paternity cases and population genetics research, but also in criminal investigation. PhastGel gradient 4-6,5 modificated and PhastSystem is clearly the method of choice for the detection of Tf phenotypes.

E 12

PGM-1 SUBTYPING BY ISOELECTRIC FOCUSING (IEF) IN PARENTAGE TESTING IN SOUTH AFRICAN POPULATIONS.

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PGM-1 subtyping by IEF was compared to typing with starch gel electrophoresis in 652 trios from the S.A.Caucasoids, Cape Coloureds and S.A.Negroes. Frequencies for the four alleles of PGM-1 were estimated by direct counting. The power of exclusion (PE) was calculated by counting the number excluded by the PGM-1 system, out of the total number excluded, using 13 genetic systems, including HLA. Using IEF, a PGM-1 exclusion rate of 17.9% in the S.A.Caucasoids, 31.3% in the Cape Coloureds and 25.4% in the S.A.Negroes was observed. In view of the increased PE using the IEF system compared to starch gel electrophoresis, this method is more useful especially in the Cape Coloureds and would be recommended as an additional system for disputed parentage testing.

E 13

OROSOMUCOID (ORM) PHENOTYPING BY ISOELECTRIC FOCUSING IN IMMOBILIZED PH-GRADIENT FOLLOWED BY IMMUNOBLOTTING

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Human alpha$_1$-acid glycoprotein or orosomucoid (ORM) is composed of two closely linked loci, ORM1 and ORM2, located in the neighbourhood of ABO and AK1 on chromosome 9q. Genetic polymorphism of ORM1 was demonstrated in various population and family studies. In our study an improved method of isoelectric focusing in immobilized pH-gradient followed by immunoblotting with a specific antibody was used. Serum or plasma samples from individuals living in Düsseldorf were investigated after neuraminidase treatment. The six common ORM1 subtypes (F1, F1F2, F2, F1S, F2S, and S) as well as some rare variant phenotypes are presented. In contrast to ORM2 the ORM1 polymorphism seems to be useful in forensic hemogenetics.

E 14

ABO BLOOD GROUPING AND SPECIES IDENTIFICATION OF BLOODSTAINS BY SANDWICH ELISA USING MONOCLONAL ANTIBODY SPECIFIC FOR HUMAN ERYTHROCYTE BAND3

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Species identification and ABO blood grouping of bloodstains are main subjects in medicolegal practices. However, either distinctions between species especially human and other primates, or ABO blood grouping of bloodstains contaminated by body fluids is not yet satisfactory. To resolve these problems, we produced monoclonal antibodies (mAbs) to human erythrocyte band3 which has suger chain carrying ABO blood group activity. The obtained anti-band3 mAb(2H9) was specific for human even in primates, and recognized cytoplasmic domain except suger moiety of band3. Therefore, mAb 2H9 can be used as a capture antibody in sandwich ELISA by which ABO blood grouping and species identification of bloodstain performed simultaneously. Consequently, it was quitly possible by sandwich ELISA using mAb 2H9 to judge the ABO blood groups from bloodstains contaminated by other body fluids without interferences of contaminants, furthermore to discriminate erythrocytes of human from those of chimpanzee.

E 15

MONOCLONAL ANTIBODIES TO BLOOD GROUP SUBSTANCES IN VAGINAL SECRETIONS

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Method for ABO blood grouping of vaginal secretions from mixed body fluids remains to be developed. We have carried out a series of study to produce monoclonal antibodies (mAbs) to ABO blood group substances (ABO-BGSs) in various body fluids, since sandwich ELISA using mAbs to ABO-BGSs in body fluids is seemed to be the most suitable method for resolving the problem. Consequently, we have already produced mAbs to ABO-BGS in saliva and semen and applied them to ABO blood grouping of saliva or semen from mixed body fluids by sandwich ELISA. At present, we attempted to produce mAbs to ABO-BGS in vaginal secretions. For production of the mAbs, vaginal ABO-BGS was purified by chromatography using sepharose 4B. Vaginal ABO-BGS consisted of three components which showed also Lewis blood activity. The highest molecular weight component carrying ABO blood activity was used as an immunogen for production of mAbs. One (V-4) of mAbs obtained was specific for vaginal secretions but not for ABO blood groups. And also ABO blood groups was detected only from vaginal secretions but not from other body fluids (e.g. semen, saliva) by sandwich ELISA using V-4. When mAb V-4 was applied for sandwich ELISA to judge the ABO blood groups of mixed body fluids, ABO blood group of only vaginal secretions was determined, although some vaginal secretions derived from blood group O donors showed weak blood group A activity.

E 16

GC IN HUMAN SALIVA STAINS

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A method for the detection of group specific component (Gc) in saliva stains by isoelectric focusing and subsequent immunoblotting is described. Stains were prepared on filter paper, air dried and stored at -24°C, +4°C and +23°C for 1, 3, 7 and 28 days. The amount of Gc protein detectable in saliva differed widely from person to person. Nevertheless, 90% of the fresh stains could be typed correctly. In 10% no Gc protein was detected.

Storage at 23°C lead to a rapid loss of Gc protein (after 3 days only 40% and after 7 days only 10% of the stains could be typed), whereas the Gc protein keept stable at -24°C up to 28 days. There was no incorrectly typed stain in the whole series.

E 17

IMMUNOBLOTTING AND IMMUNOFIXATION TECHNIQUES FOR SUBTYPING Gc IN OLD BLOODSTAINS AND SEMEN STAINS

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Comparison betweeen two techniques for Gc subtyping in bloodstains and semen stains is discussed. Bloodstains were stored at room temperature for ten months. An Urea 6M solution was used for extraction. IEF in 4-6,5 gradient with immunoblotting techniques or immunofixation followed by silver staining allowed discrimination of phenotypes for the Gc system in very old bloodstains and semen stains. Both techniques showed high reliability for Gc subtyping; albumin and other additional bands did not appear in any case. As immunofixation techniques are easier and less expensive than immunoblotting and in both cases the results in our experiments were similar, we advise the use of immunofixation techniques for Gc subtyping in bloodstains and semen stains.

E 18

OLD BLODSTAIN AND SEMEN STAIN CHARACTERIZATION IN THE TRANSFERRIN TYPING SYSTEM USING MINIGELS AND THE PHASTSYSTEM

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Application of minigels and PhastSystem to obtain phenotyping results in bloodstains and semen stains in the Tf system was investigated. Bloodstains were stored at room temperature for ten months. A neuraminidase solution was used for extraction and iron saturation was carried out with Ferrous Ammonium Sulfate. IEF with 4-6,5 PhastGel treated with a Servalyt 5-7 and Nonidet P40 solution followed by immunofixation techniques and developed by Coomassie Blue staining for bloodstains and silver staining for semen stains allowed discrimination of phenotypes for the Tf system. The little ammount of sample necessary made possible the reliable characterization in very old bloodstains (up to 10 months). The use of commercial miniaturized gels and an automated system reduced considerably the cost and working time.

E 19

USING OF MICROTITER PLATES AND THE APPARATUS DYNATECH FOR AUTOMATIZATION OF THE ROUTINE DETERMINATION OF ABO-GROUPS FROM BODY FLUIDS (OR STAINS) AND HAIR.

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Both absorption-elution (A-E) and absorption-inhibition (A-I) methods have been so far used for the basic determination of ABO-groups. This paper presents a simplified techniques using microtiter plates and modificated apparatus DYNATECH. The modificated ULTRAWASH is used for washing of the segments of cotton fibres soaked by body fluids or hair samples from the diagnostic serum surplus (A-E method). The apparatus DILUTER II connected with SRD II are used for the examination of the stubs, semen-, saliva-, sweat-stains etc. by A-I method. The evaluation of aglutination is provided in two ways. The results of A-E method are mostly evaluaed by stereomicroscope and each of the *wells* of the microtiter plate is individually gently shaked by air jet. The results of A-I metod are evaluated and typed by the automatic DYNAREADER MR 7000.

E 20

PI, C2, GC, ATIII, PLG TYPING IN BLOODSTAINS BY HYBRID ISOELECTRIC FOCUSING (HIEF)

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Protein markers (PI, C2, GC, ATIII, PLG) were analyzed in series of bloodstains to determinate the usefulness of the hybrid isoelectric focusing (HIEF) for typing electrophoretic markers for forensic material.
HIEF is clearly the method of choice for the detection of these electrophoretic polimorphisms in bloodstains (particularly minute bloddstains) since phenotypes are better distinguished, bands are straighter and sharper and much more sample (even with contaminants) can be loaded; and the sensitivity is similar to IEF with CA. We, therefore, recommend the routine use of HIEF for the detection of these proteins, and suggest further investigations of its utility for the determination of other polymorphisms.

LIST OF ABSTRACTS BY COUNTRIES AND TOPICS

Country	A	B	C	D	E	Total
Argentina			1			1
Belgium			1			1
China	2			1		3
CSFR					1	1
Denmark	1		2	1		4
Finland	1					1
France	1					1
FRG	8	3	17	10	9	49
India			2	1	1	4
Italy	3		3	2		8
Japan	5		3	6	3	17
Lithuania			1			1
Netherlands	1					1
Norway	1		1			2
Poland			1	1		2
Portugal				2		2
S. Africa	1		1		1	3
Spain	3		3	3	5	14
Sweden					1	1
Switzerland	1		2			3
U.K.	2	3	8	1		14
USA	3	2	4			9
USSR			1			1
Yugoslavia				1		1

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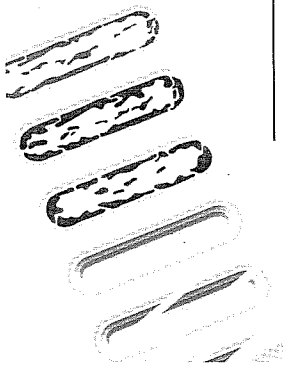
Diagnostika für die Immunhämatologie

Reagenzien für die Blutgruppen-serologie



HISTO TRAY
HLA-ABC,-B27,-DR

Mikrotestkammern mit vorgetropften Antiseren und Kontrollen für die HLA-Typisierung

Reagenzien und Zubehör für die HLA-Serologie