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GEDNAP IV and V. The 4th and 5th Stain Blind Trials Using DNA Technology

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Abstract In the collaborative exercise GEDNAP IV one EDTA blood sample (2 ml) and 5 bloodstains (0.5 ml on cotton) were investigated and in GEDNAP V, a total of 8 bloodstains (0.5 ml on cotton), including 2 mixed bloodstains. DNA typing was carried out using the RFLP systems YNH24/*Hinf* I and MS43a/*Hinf* I and the PCR systems HLA DQα, D1S80, ApoB and YNZ22. In both exercises approximately 20 laboratories obtained results using the RFLP systems. Of the PCR systems, D1S80 was the most commonly used (14 labs in GEDNAP IV; 18 labs in GEDNAP V). The interlaboratory standard deviation for YNH24 in both exercises was approx. 0.6%, for MS43a 0.7–2.2% (GEDNAP IV) and 0.4–1.4% (GEDNAP V), depending on the fragment size. The fragment size calculation performed in each laboratory yielded a standard deviation twice that obtained when the fragment size calculation was performed centrally (IfR, Münster). In GEDNAP III, a system-specific corridor was developed to define the limits of deviation; this was modified for the present study by combining the fragment size ranges of YNH24 and MS43a. In both studies a subgroup of laboratories was involved in preliminary exercises using three PCR VNTRs and the system HLA DQα. Owing to the substantial variation in experience of the participating laboratories with PCR typing the results obtained in these two studies do not fulfil the basic quality criteria of the GEDNAP studies.

Key words GEDNAP IV · GEDNAP V · RFLP systems · PCR systems · Bloodstains

Introduction

Preceding collaborative DNA exercises organized by the stain commission of the German Society of Legal Medicine, the so-called GEDNAP exercises (German DNA profiling group), made use of the same RFLP single locus systems YNH24/*Hinf* I (Promega, USA) and MS43a/*Hinf* I (Cellmark Diagnostics, UK) as in the three previous studies (Puers et al. 1992; Bär et al. 1992; Brinkmann et

al. 1993). One of the commission's achievements has been to establish a uniform framework with standard values of cell line fragments and variation parameters. In these series the scope was extended to include PCR systems, but this was only accepted by a limited number of laboratories. Furthermore, the level of experience with PCR systems varied between these laboratories. PCR typing was performed using the sequence polymorphism HLA DQα (Saiki et al. 1989) and the PCR VNTR polymorphisms MCT118 (D1S80; Budowle et al. 1991), YNZ22 (D17S5; Horn et al. 1989; Wiegand et al. 1992) and ApoB (Boerwinkle et al. 1989).

Materials and methods

Samples

GEDNAP IV

Each participating laboratory received the following samples:
– 1 × 2 ml blood sample
– 5 × 500 µl bloodstains on cotton

GEDNAP V

The participating laboratories received:
– 3 × 500 µl "suspect" blood samples as stains on cotton
– 3 × 500 µl "pure" blood stains on cotton
– 2 × 500 µl "mixed" blood stains on cotton; both mixtures were from two individuals each and the ratios were either 1:3 or 1:9 (Tables 3, 4, 9).

RFLP methodology

The loading scheme, i.e. the sequence of samples and controls and ladders, was predefined and the restriction enzyme was *Hinf* I. Electrophoresis: 1% agarose gel, 1 × TBE buffer, 2.5 V/cm; the 2 kb fragment of the marker should have migrated 18 cm. After separation the gels were stained with ethidium bromide, DNA analysis ladder (Gibco-BRL, UK). Chemiluminescence detection.

The analysis of fragment sizes was carried out by each participating laboratory and centrally (in Münster) using a video densitometer (BioImage, Millipore, USA). The method of fragment size calculation with this machine is the local reciprocal approach (Elder and Southern 1987).

PCR methodology

PCR polymorphisms were analysed separately by each participating laboratory. PCR methods and electrophoretic separations were not standardized. For DQα and MCT118 the commercially available ladders and kits (Perkin Elmer, Germany) were predominantly used and for MCT118 there existed a ladder from the laboratory in Münster, which was made available to some laboratories. Ladders for the remaining systems were either made by the participating laboratory itself or made available by one laboratory (Münster).

RFLP comparisons

The fragment sizes obtained were compared to predefined standards. To establish these, five "reference" laboratories (defined by low intralab standard deviations) had carried out multiple tests with the same samples covering a range between 2.1 kb and 13.5 kb. From their results a standard framework was established with mean values, standard deviations, maximum variations allowed (± 3 SD range) as a common standard framework for all participating laboratories (Fig. 1a, b).

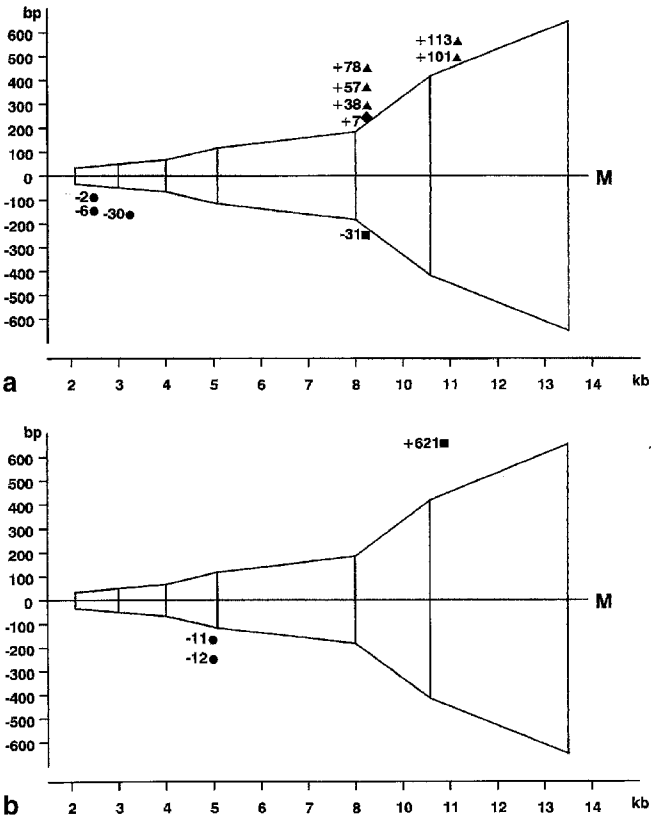

Fig. 1a, b Fragment sizes for the sample material and cell line with the corresponding variation range as a "corridor" for the fragment sizes of the "reference" laboratories from GEDNAP III (Brinkmann et al. 1993). In GEDNAP III 5 laboratories carried out more than 5 gels with the same DNA samples. The measured overall standard deviation in these 5 laboratories was used for setting the corridor boundaries (± 3 SD range) by combining the YNH24 and MS43a fragment length values of the investigated samples. The deviation values are given in bp. M = Mean value. The vertical lines indicated the ± 3 SD range of the fragment size values which were calculated from the GEDNAP III samples. a) GEDNAP IV, b) GEDNAP V

Systems

GEDNAP IV:

YNH24/*Hinf* I, MS43a/*Hinf* I; HLA DQα; D1S80; ApoB; YNZ22.

GEDNAP V:

YNH24/*Hinf* I, MS43a/*Hinf* I; HLA DQα; D1S80; ApoB; YNZ22.

Samples and laboratories were coded twice.

Table 1 Mean value (M), standard deviation (SD) and variation range (Var) of the fragment lengths from all laboratories. Fragment size calculation: video densitometer, BioImage (Millipore, USA). YNH24 – GEDNAP IV (n = 20), *n* = number of participating laboratories

YNH24	EDTA-bl		Bl-stain 1		Bl-stain 2		Bl-stain 3		Bl-stain 4		Bl-stain 5		K562	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
M (bp)	2249	2113	2247	2109	3034	2929	4280	2677	5028	4341	4031	3134	4010	2894
SD (%)	0.54	0.6	0.76	0.76	0.73	0.64	0.59	0.59	0.69	0.62	0.69	0.64	0.58	0.48
Var (%)	1.65	2.03	2.98	2.99	3.4	2.39	2.13	2.05	2.65	2.1	2.56	2.07	2.02	2.18

Table 2 MS43a – GEDNAP IV (*n* = 21); see legend to Table 1

MS43a	EDTA-bl		Bl-stain 1		Bl-stain 2		Bl-stain 3		Bl-stain 4		Bl-stain 5		K562	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
M (bp)	10542	8005	10563	8013	7725	4587	10649	9211	8363	5163	8694		13496	5255
SD (%)	1.58	1.24	1.97	1.24	0.94	0.7	1.59	1.32	1.17	0.79	1.14		2.18	0.8
Var (%)	8.31	4.66	8.39	4.99	3.26	2.35	6.09	5.14	4.28	2.98	4.72		7.94	3.44

Results and discussion

RFLPs

GEDNAP IV

YNH24 fragment sizes varied between ca. 2.1 and 5.0 kb and the interlab SD varied between ca. 0.5 and 0.8% (Table 1). – MS43a was associated with larger fragments, i.e. between ca. 4.6 and 13.5 kb and the SD varied between 0.7 and 2.2% (Table 2). A steep interlab SD increase occurred in the higher kilobase range, and this was mainly due to one lab, in which a much shorter separation had been used, thus leading to considerably higher fragment sizes than were allowed (corridor, Fig. 1a). The remaining values outside the corridor were mainly due to single labs and rather small (Fig. 1a).

GEDNAP V

YNH24 was associated with fragments varying between ca. 2.2 and 5.1 kb with SDs between 0.41% and 0.78%, while MS43a varied between 5.2 and 13.4 kb with SDs between 0.43% and 1.42%, except for one isolated SD of 5.57, which was due to one value far outside the corridor (Fig. 1b).

Mixed stains were associated with differing recognition rates; stain 1 with a ratio of 1: 3 (Tables 3, 4) was correctly typed by 15 out of 17 labs (15/17), with YNH24, the MS43a quotient was 9/17. Stain 3 (ratio 1:9) was associated with a quotient of 13/17 (YNH24) and 7/17 (MS43a) respectively. There occurred no mistyping of the weak components; they were either not detected or typed.

Comparisons of K562 results between GEDNAP III, IV, V show quite similar levels of SDs in all three series, while the variation width decreased significantly (Tables 5, 6). The extent of this decrease was between 25% and 60%. We attribute this to the decreasing number of laboratories producing one-offs which can also be derived from the "corridor approach" (Fig. 1b). These and the remaining statistical figures are also very similar to those in the EDNAP studies (Schneider et al. 1991, Gill et al. 1992).

Comparisons between decentral and centralized fragment size evaluations show: GEDNAP IV showed insignificant and significance differences in both directions while GEDNAP V was in the allelic range between 8 and 10.5 kb, associated with a doubling of the decentral SDs. We attribute this to wide variety of manual, semi-automated and automated evaluation methods used.

PCR systems

GEDNAP IV

The mistyping rate ranged from 2% (D1S80), through 3% (YNZ22) to a maximum of 10% (ApoB, HLA DQα). Reasons for mistyping were: not using a system specific allelic ladder; allelic drop out (YNZ22); incorrect interpretation of (correct) results (HLA DQα, D1S80) – owing to the different level of experience of the participating laboratory, mistypings occurred only in some laboratories (Tables 9–11).

GEDNAP V

More laboratories joined in after the start of this phase (Tables 9–11). Two approaches were taken to the mistyping rate (Tables 10, 11). If the failure to detect the weaker component in mixtures was not counted (Table 11), the level of mistakes was approximately the same as in the preceeding series (cf. Tables 9, 11). Most typing errors were in D1S80 and were due to wrong assignment of alle-

Table 3 YNH24 – GEDNAP V ($n = 17$); see legend to Table 1

YNH24	Person A		Person B		Person C		Stain1 (mixture A:C = 3:1)				Stain 2		Stain 3 (mixture B:C = 1 : 9)				Stain 4		Stain 5		K562	
	A	B	A	B	A	B	A	B	C	D	A	B	A	B	C	D	A	B	A	B	A	B
M (bp)	5050	4346	4014	2683	4678	3634	5048	4686	4348	3640	4796	2216	4678	4001	3643	2670	4007	2679	5041	4348	4015	2903
SD (%)	0.55	0.66	0.54	0.62	0.75	0.68	0.6	0.65	0.53	0.66	0.57	0.63	0.51	0.66	0.66	0.66	0.68	0.78	0.67	0.59	0.54	0.41
Var (%)	1.82	2.92	1.82	2.39	3.1	2.56	1.9	3.01	2.0	2.53	2.15	2.08	1.62	2.35	2.47	2.36	2.35	2.87	2.76	2.0	2.37	1.45

Table 4 MS43a – GEDNAP V ($n = 18$); see legend to Table 1

YNH24	Person A		Person B		Person C		Stain1 (mixture A:C = 3:1)				Stain 2		Stain 3 (mixture B:C = 1 : 9)				Stain 4		Stain 5		K562	
	A	B	A	B	A	B	A	B	C	D	A	B	A	B	C	D	A	B	A	B	A	B
M (bp)	8389	5174	10185	8736	9855	5257	9883	8414	5243	5168	10163	9823	10333	9856	8677	5254	10173	8724	8368	5170	13370	5273
SD (%)	0.63	0.58	1.11	0.78	1.08	0.67	0.93	0.98	0.51	0.43	1.09	1.2	5.57	0.78	0.98	0.49	0.86	0.69	0.59	0.55	1.42	0.45
Var (%)	2.21	2.05	4.98	2.87	4.13	2.21	3.09	3.3	1.45	1.57	4.07	5.17	13.52	2.33	3.72	1.73	3.06	2.59	2.21	1.93	5.16	1.54

Table 5 Comparison of the interlaboratory mean values, standard deviations and variation ranges of the cell line K562 fragments in GEDNAP III, IV and V. YNH24

K562		GEDNAP III	GEDNAP IV	GEDNAP V
Fragm. A	M (bp)	4016	4010	4015
	SD (%)	0.6	0.6	0.5
	Var. (%)	3.2	2.0	2.4
Fragm. B	M (bp)	2899	2894	2903
	SD (%)	0.5	0.5	0.4
	Var. (%)	2.2	2.2	1.5

Table 6 MS43a; see legend to Table 5

K562		GEDNAP III	GEDNAP IV	GEDNAP V
Fragm. A	M (bp)	13506	13496	13424
	SD (%)	1.6	2.2	2.0
	Var. (%)	7.9	7.7	5.2
Fragm. B	M (bp)	5278	5255	5278
	SD (%)	0.7	0.8	0.5
	Var. (%)	3.9	3.4	1.5

les, which was due in turn to the use of different allelic ladders and separations. The second source of error was due to allelic dropout. Also, especially with DQα we again observed misinterpretations of correct results.

Although the PCR VNTR systems showed a stronger discriminative power to mixed stains than RFLP, there occurred also band losses and weak component patterns which were not recognized. As far as we can judge this problem, insufficient quality of amplification and sometimes preferential amplification seemed to be major reasons for this artefact (see also Comey et al. 1991).

Another problem could be seen with ApoB due to the incompatibility of the allelic ladders used where six laboratories used three different allelic ladders. One laboratory typed all alleles 4 repeats too short: three laboratories typed one allele as 31, two other laboratories as 29. Sequencing of these alleles should clarify this problem.

Different electrophoretic systems were used (agarose gels, horizontal PAGE, vertical PAGE) which led to different migrations of the same fragments. An investigation by Hagerman (1990) showed that variations in migration are due to different gel systems, which especially affect AT-rich systems, such as ApoB.

There was also an overlap between laboratories with mistypings, because 8 out of 23 labs (Table 11) showed erroneous results and 4 out of 23 showed more than 1 mistyping.

Conclusions

The RFLP typing results indicated a good level of experience in most of the laboratories. In contrast the PCR typing results indicated substantial variation in experience of the participating laboratories. Reliable reproducibility in

Table 7 Allelic composition of the sample materials to be analysed in GEDNAP IV

System	K562	EDTA-bl	Stain 1	Stain 2	Stain 3	Stain 4	Stain 5
HLADQα	3, 4	1.2, 1.3	1.2, 1.3	3, 4	1.1, 1.2	1.1, 1.2	1.2, 4
D1S80	18, 29	24, 24	24, 24	24, 24	18, 24	24, 31	18, 22
ApoB	37, 37	37, 37	37, 37	33, 35	37, 37	37, 39	31, 37
YNZ22	2, 2	3, 4	3, 4	4, 9	4, 4	2, 4	3, 11

Table 8 GEDNAP V ("weak" alleles in brackets); see legend to Table 7

System	Person A	Person B	Person C	Stain 1 A : C = 3 : 1	Stain 2	Stain 3 B : C = 1 : 9	Stain 4	Stain 5	K562
HLADAα	1.1, 1.2	1.1 ,1.1	1.3, 1.3	1.1, 1.2, (1.3)	1.2, 4	(1.1), 1.3	1.1, 1.1	1.1, 1.2	3, 4
D1S80	24, 31	18, 25	18, 21	(18), (21), 24, 31	28, 33	18, 21, (25)	18, 25	24, 31	18, 29
ApoB	37, 39	35, 37	37, 49	37, 39, (49)	35, 37	(35), 37, 49	35, 37	37, 39	37, 37
YNZ22	2, 4	3, 3	4, 4	2, 4	2, 5	(3), 4	3, 3	2, 4	2, 2

Table 9 GEDNAP IV; typing errors for each system and laboratory

	HLA DQα	D1S80	ApoB	YNZ22
No mistakes	6	12	5	3
1 Mistake	2	0	0	0
2–4 Mistakes	1	2	1	1
n (Labs)	9	14	6	4

Table 10 GEDNAP V; see legend to Table 9

	HLA DQα	D1S80	ApoB	YNZ22
No mistakes	5	7	2	4
1 Mistake	7	7	2	0
2–6 Mistakes	3	4	0	0
n (Labs)	15	18	4	4

Table 11 GEDNAP V (failure to detect the "weak" component in unequal DNA mixtures was not counted as a mistake); see legend to Table 9

	HLA DQα	D1S80	ApoB	YNZ22
No mistakes	10	11	4	4
1 Mistake	3	5	0	0
2–5 Mistakes	2	2	0	0
n (Labs)	15	18	4	4

interlaboratory typing can only be reached with harmonization of the electrophoretical methods and allelic ladders. This means that the results of the PCR typing cannot be included under the quality criteria of a collaborative exercise.

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