

Report on the third EDNAP collaborative STR exercise

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Abstract

This report describes an inter-laboratory exercise completed on behalf of the European DNA Profiling (EDNAP) group. The exercise is one in a series designed to identify STR loci which could be used for harmonisation between participating European forensic science laboratories. Participants were asked to identify the alleles present in five bloodstains at the STR loci HUMTH01 and HUMVWFA31/A. Two of the stains were prepared from mixtures of two different blood samples. There were no special instructions and each laboratory was requested to use the methodology normally employed for crime case

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investigations. All participating laboratories achieved the same results for both loci. In addition, the laboratories were also requested to report the results obtained from any other loci which would normally be used in crime case investigations. A comparison of these results showed some inter-laboratory variation.

Keywords: European DNA Profiling group; DNA profiling; HUMTH01; HUMVWFA31/a

1. Introduction

The European DNA Profiling (EDNAP) group was formed in the latter part of 1988 in direct response to the European initiative to have an 'open-border' policy. Scientists involved with work for the various criminal justice systems realised that they could exchange and compare DNA profile results obtained during the investigation of crime cases only if their systems were compatible. The initial decisions were to settle on single locus probing in which the same enzyme (*Hinf* 1) would be used and two probes would be common to all participating laboratories. In order to ensure that all laboratories would meet the required standards for the production of accurate results, two sequential inter-laboratory quality assurance exercises were arranged. A decision was also taken which committed the group to publish the results of these exercises in the scientific literature [1,2].

EDNAP is formed of representatives from laboratories within Europe who are involved with work for the police or their criminal justice systems in general. All members have equal status and it is a measure of the understanding which exists between members that no issue has ever been decided by a vote. Each participant is responsible for representing the interests of the other laboratories doing similar work within their own countries. EDNAP is now constituted as a Working Party of the International Society for Forensic Haemogenetics.

With the introduction of PCR methodology into forensic science, the EDNAP group had to decide on which loci would be used as linking systems for further co-operation. In the first quality assurance exercise which involved PCR technology, two loci were chosen HUMTH01 and HUMACTBP2 (SE33). The former is a simple locus having six common alleles whereas the latter has greater complexity with more than 35 recorded alleles and a greater level of microheterogeneity. There was general agreement on the results obtained from HUMTH01 but rather more diversity with the results achieved in the analysis of the HUMACTBP2 locus [3].

In the second exercise a group of four relatively simple STR loci having tetrameric repeat sequences; HUMTH01 (TH01), HUMVWFA31/A (VWA), HUMF13A1 (F13) and HUMFES/FPS (FES) were tested. These were chosen as previous work had demonstrated that alleles from all four loci could be detected from a single PCR multiplex reaction [4]. The outcome of this exercise revealed that although the results obtained from the multiplex of four loci using automated detection via dye-labelled primers on ABD 373A DNA sequencers was robust, problems were

experienced with reproducibility of allele designation at two of the loci when alternative detection methods were used. The exercise did indicate, however, that results obtained from THO1 and VWA would make them candidates for harmonisation between laboratories.

As a consequence of these findings it was agreed at a meeting in Copenhagen on 26th March 1994 to perform an exercise in which alleles from these two loci would be determined, using local protocols, and the results compared for accuracy and reproducibility. The Metropolitan Police Forensic Science Laboratory volunteered to prepare samples of dried bloodstains, of known allelic designation, which would be mailed to participating laboratories for analysis. The correct designations would be known only to the organising laboratory and, following analysis, the results would be collated and the outcome presented at the following meeting in Coimbra, Portugal on 22nd October 1994. Member laboratories were asked to supply details of their amplification and detection methods and also the results from any other loci which would be used and analysed as part of the function of casework examination or development work.

In the event all alleles within the THO1 and VWA loci were correctly identified by all participating EDNAP laboratories, including those where the stains were made from a mixture of two different blood samples. There were discrepancies, however, in the nomination of alleles from other loci but as the object of the exercise was harmonisation on the two designated loci, these differences will not be discussed in depth in this communication. Although some loci have been identified as unsuitable for harmonisation without further investigation, this does not imply that they are of no value at the present time. It will be possible to use them in individual laboratories if appropriate validation exercises have been performed and good quality systems are in place.

2. Materials and methods

2.1. Materials supplied

Each laboratory was supplied with 6 bloodstains, including 5 'unknown' samples (labelled 1–5) and one control sample (labelled C), the phenotype of which, at the VWA and THO1 loci, was given. Participants were informed that two of the 'unknown' samples contained mixtures of blood. Laboratories were asked to use whichever primers and methods for amplification, electrophoresis and detection they had chosen for routine analysis or development work. The main aim of the exercise was to compare the results obtained from the VWA and THO1 loci, but participants were also invited to type the samples using any other PCR-based systems in use in their laboratory.

Allelic ladders for VWA and THO1, as used in the previous EDNAP exercise [4], were offered to participating laboratories by P. Gill (FSS, Birmingham). The allelic designation and base pair size information associated with these ladders [4] was supplied to all laboratories.

2.2. Laboratories

The exercise was sent to 18 European laboratories belonging to the EDNAP group.

2.3. Preparation of samples

Stains were prepared on washed cotton using aliquots of liquid blood samples donated by staff members. 'Mixed' stains were prepared from a 50/50 mixture of two donor blood samples of the same ABO blood group. Stains were allowed to air dry thoroughly at room temperature prior to packaging and sending to participant laboratories.

2.4. Procedure followed at originating laboratory to obtain allelic designation

The procedure detailed below was followed in the course of population databasing studies, the results of which allowed identification of potential donors for the purposes of this exercise. The same procedure was also followed for testing three randomly selected sets of stains prepared for distribution to participating laboratories.

DNA was extracted from the bloodstain samples using the Chelex® extraction method described by Walsh et al. [5] and the amount of DNA present in each extract was then determined by the use of a dot-blot hybridisation procedure with a higher primate-specific probe [6]. Template DNA (3 ng) was amplified simultaneously at the VWA, THO1, F13 and FES loci using reaction and cycling conditions described by Lygo et al. [7]. Amplified product (1.5 μ l) was added to 2.5 μ l of loading buffer (2% (w/v) dextran blue, 2.4 nM GS2500 ROX internal lane standard (ABD) in deionised formamide). Samples were heated to 90°C prior to loading on to a 6% polyacrylamide, 8M urea, 1 \times TBE sequencing gel (24 cm well-to-read). Electrophoresis was carried out on an ABD 373A automated DNA sequencer for 6 h at 2500 V, 40 mA, 30 W. Genescan 672 Analysis software was used to determine the sizes of the DNA fragments with reference to the internal size standard, by the method of Elder and Southern [8]. Allelic designation was made according to 'windows' set in the laboratory, based on the results of the electrophoresis of 360 allelic ladder samples for each locus across 10 gels [9]. Nomenclature of alleles was made according to the recommendations of the ISFH [10,11] based on the DNA sequence of each allele contained within the allelic ladders [12].

2.5. Amplification methods used by participating laboratories

A number of different amplification reaction conditions were employed by participants in the exercise to obtain results at the VWA and THO1 loci. As outlined in Table 1, the majority of participants amplified the VWA and THO1

Table 1

The range of PCR reaction types used in the exercise to obtain results at the VWA and THO1 loci

Type of PCR reaction and conditions used	No. of laboratories employing these conditions
<i>Conditions as last EDNAP exercise [4]</i>	
Single	2 ^c
Duplex ^a	2 ^c
Quadruplex ^b	3
<i>Laboratories' own protocols</i>	
Single	8 ^d
Duplex ^a	1 ^d
Quadruplex ^b	3 ^d

^aAll duplex reactions used involved the amplification of the VWA and F13 loci in one reaction and the amplification of the THO1 and FES loci in another.

^bQuadruplex reactions involved the amplification of the VWA, THO1, F13 and FES loci simultaneously in one tube.

^cOne laboratory used both single and duplex reactions as specified in the previous exercise [4].

^dOne laboratory used its own protocol for amplification in single, duplex and quadruplex reactions.

products in separate reactions. Six laboratories adhered to the reaction conditions (either single, duplex or multiplex) detailed in the previous exercise [4] and ten laboratories used their own protocols, which are summarised in Table 2 and Table 3.

Table 2

A summary of the PCR methods employed by laboratories to obtain results at the VWA and THO1 loci, other than those following the protocols given in the last EDNAP exercise [4]: PCR reaction components

Type of reaction	Amounts of components used				Final reaction volume (μ l)	Additional components added
	MgCl ₂ (mM)	dNTPs (μ M of each)	Primers (μ M)	<i>Taq</i> (units)		
Single	1.5–2.0	85–200	0.1–1.0 each	1.0–1.5	15–50	0.16 μ g/ μ l BSA
Duplex	1.5	200	VWA: 0.13 THO1: 0.15 F13: 0.18 FES: 0.05	1.0	50	None
Quadruplex	1.5	200	VWA: 0.1–0.3 THO1: 0.09–0.2 F13: 0.16–0.25 FES: 0.05–0.5	1.25	25–50	0.16 μ g/ μ l BSA

Table 3

A summary of the PCR methods employed by laboratories to obtain results at the VWA and THO1 loci, other than those following the protocols given in the last EDNAP exercise [4]: PCR cycling conditions

Type of reaction	Locus/loci	Denaturation		Annealing		Extension		No. cycles	Final extension at 72°C (min)
		°C	s	°C	s	°C	s		
Single	VWA	94–95	10–60	54–64	20–60	72	30–60	25–30	0–10
	THO1	90 ^a –95	10–60	54–64	30–60	70–72	20–60	25–30	0–10
Duplex	VWA/F13; THO1/FES	94	45	57	60	72	60	32	10
Quadruplex	VWA/ THO1/ F13/ FES	94–95	45–60	54	30–60	72	30–60	28	10

^aAfter 10 cycles employing a denaturation temperature of 94°C.

Note: Two laboratories employed “hot start” PCR, (95°C 3–5 min).

Two laboratories employed cooling “ramps” between the denaturation and annealing steps.

2.6. Electrophoretic methods and detection systems employed by participating laboratories

A number of electrophoresis and detection systems were utilised by the participants, as summarised in Table 4. The majority of laboratories employed fluorescence detection of amplified products as the method of choice, although manual systems based on the use of both agarose and polyacrylamide were also used to generate results in this exercise. The dyes used to label allelic products by the laboratories employing fluorescence detection with an ABD 373 Automated Sequencer are given in Table 5. It can be seen that the majority of these laboratories used the JOE or HEX and FAM or 6-FAM dyes as detailed in the last collaborative STR exercise [4].

Table 4

Electrophoresis and detection systems employed by participants to obtain results at the VWA and THO1 loci

Electrophoresis system	Detection system	Number of laboratories employing system
Metaphor [®] agarose	Ethidium bromide	1
Native polyacrylamide	Silver staining	2
Denaturing polyacrylamide	Silver staining	1
Denaturing polyacrylamide	Fluorescence detection	Pharmacia ALF Sequencer: 2 ABD 373A Sequencer: 12

Note: One laboratory returned results obtained from the use of both native polyacrylamide gel electrophoresis with silver staining and fluorescence detection with an automated sequencer. Another laboratory used both Metaphor[®] agarose gel electrophoresis with ethidium bromide staining and fluorescence detection with the ABD 373A Sequencer to analyse PCR products.

Table 5

Fluorescent dyes used to label allelic products in conjunction with the use of an ABD 373A sequencer

Dye/locus combination	Number of laboratories using this combination
VWA — JOE/HEX; THO1 — FAM/6-FAM	9
VWA — JOE; THO1 — TAMRA	1
VWA — FAM; THO1 — FAM	1
VWA — TAMRA; THO1 — 6-FAM	1

2.7. Additional systems used by participating laboratories

Many of the participating laboratories profiled the test DNA samples using PCR-based systems additional to the two STR loci specified in the exercise. The systems used are summarised in Table 6. The majority of laboratories typed the DNA samples additionally at the two STR loci F13 and FES. Together with VWA and THO1, these loci make up the quadruplex system described by Kimpton et al. [13] that formed the basis of the previous EDNAP exercise [4]. The other systems used were D1S80 [14] (a Variable Number Tandem Repeat locus), HLA DQ α © [18] and Polymarker© [19] (both available in kit form from Perkin Elmer), the two complex STR loci HUMACTBP2 (SE33) [15] and D21S11 [16] and AMG (amelogenin homologous gene), a PCR-based sex marker [17]. All the blood samples used to prepare stains in this exercise were typed at the F13 and FES loci by the originating laboratory. The four blood samples used to prepare the two 'mixed' stains were typed, after completion of the exercise, using D1S80 and HLA DQ α ©/Polymarker© by N. Morling (Institute of Forensic Medicine, Copenhagen) and P. Schneider (Institute of Legal Medicine, Mainz), respectively. These results were used as a reference with which to compare results returned by other laboratories.

Table 6

Systems other than VWA and THO1 used by participating laboratories to generate results in the exercise

System	Number of laboratories employing the system	Reference
FES	13	[13]
F13	12	[13]
D1S80	4	[14]
HLA DQ α ©	4	[18]
Polymarker©	3	[19]
HUMACTBP2	3	[15]
D21S11	2	[16]
AMG (sex)	2	[17]

Table 7

Allelic designations obtained for samples supplied to participating laboratories by the originating laboratory

Sample no.	VWA alleles	THO1 alleles
1	15, 18	7, 9.3
2	14, 16, 18, 20	6, 7, 9, 9.3
3	14, 19	6, 9.3
4	16, 18, 21	8, 9
5	14	5, 8
C	16, 20	6, 9

Note: Allele designations are given as observed phenotypes.

3. Results

3.1. Allelic designations obtained by the originating laboratory

The allelic designations obtained for each test sample DNA at the originating laboratory are shown in Table 7. The policy at this laboratory is to 'bin' THO1 alleles 9.3 and 10 for reporting purposes, so the 9.3 designation for samples 1, 2 and 3 would allow for the possibility of a 10 allele being present. The designation for the control sample, C, was supplied to participants. The samples containing mixtures of blood were numbers 2 and 4.

3.2. Results returned by participating laboratories

Results were returned by 16 laboratories. In every case, the allelic designations made for each sample at the VWA and THO1 loci matched those obtained by the originating laboratory. Laboratories using more than one method for amplification or detection of PCR products obtained the same results whichever method was employed.

3.3. Problems encountered by participating laboratories

Very few problems associated with typing at the VWA and THO1 loci were reported by participants. However, one laboratory reported problems with DNA extraction and another laboratory reported difficulty in distinguishing between the THO1 9.3 and 10 alleles, which have a size difference of only one base pair, when detecting PCR products on native polyacrylamide gels.

3.4. Results obtained from other PCR-based systems

The results obtained from systems other than the VWA and THO1 STR loci were not all directly compatible when compared with one another. Variations in typing were only associated with the analysis of mixed samples, as summarised in

Table 8. The anomalous profiles obtained are shown, in conjunction with the reference results obtained as detailed in the Materials and methods (Section 2). It was not possible to ascertain whether or not the results obtained from typing at the HUMACTBP2 locus were compatible, as the laboratories concerned used different allelic designation systems and the base pair sizing of the alleles contained within allelic ladders were not comparable.

4. Conclusion

All EDNAP laboratories participating in this exercise successfully typed the DNA from five stains, including two of mixed origin, at the VWA and THO1 loci. This was achieved despite variation in the amplification, electrophoresis and detection systems utilised by individual laboratories. The results demonstrate that these simple STR loci are ideal for standardisation in the forensic community, where different laboratories have varying resources.

The THO1 locus has been the subject of two previous collaborative exercises [3,4] and the VWA locus was investigated in the last exercise [4]. In both these experiments, the participating laboratories within the EDNAP group achieved consistently successful typing at these two loci. The current exercise differed from the previous two in that no amplification reagents or protocols were provided or specified and it therefore presented the final test for standardisation on the VWA and THO1 loci.

Results from analysis at the F13 and FES loci were returned by the majority of participants in this exercise. All the results obtained using these loci were in

Table 8

Summary of variations in results obtained by laboratories which returned results for systems in addition to VWA and THO1

System used	Variations ^a in results obtained		
	Sample no.	Reference resul	Anomalous resul
D1S80	2	18, 24, 28,37	18, 24, 28
	4	18, 20,22,4, 33	18, 20, 22
Polymarker ^{©b}	2	AB,AA,AB,AB,AC	AB,AA,AB,AB,ABC
FES	No variations		
F23	No variations		
HLA DQ α	No variations		
HUMACTBP2	Not possible to compare results		
D21S11	No variations		
AMG	No variations		

^aTypographical errors were found in results associated with FES and D1S80. These have not been reported as variations.

^bOn re-examination it was decided that the intensity of the B allele dot in the Gc system was below the level to satisfy reporting criteria.

Note: Polymarker[©] results are given in the following order: LDLR, GYPA, HBGG, D7S8, Gc.

agreement. Participants in the previous collaborative exercise [4] also produced consistent results at these loci. In that exercise, specific amplification methodologies were adhered to. However, the results obtained from the current exercise suggest that the F13 and FES loci may be candidate systems for standardisation without the necessity for defining the analytical procedures used.

A small number of laboratories returned results obtained at the D21S11 and amelogenin loci. These were all in agreement. There was, however, one anomalous result obtained from a mixed sample using the Polymarker© methodology. This system relies on dot-blot hybridisation as the method of detection, which might account for small differences in the interpretation of mixed results between laboratories. Typing at the D1S80 locus also produced inconsistent results from the samples of mixed origin. This is probably due to a variation in the sensitivity of detection systems used.

The system for allelic designation at the VWA and THO1 loci is simple and widely accepted [10,11]. In comparison, the results returned by the three laboratories who analysed the samples at the complex STR locus HUMACTBP2 could not be compared due to the non-compatibility of allelic designation protocols used. In a previous EDNAP exercise [3], laboratories were supplied with PCR primers and protocols for amplification at this locus, together with a reference ladder for allelic designation and all laboratories employing fluorescence detection of alleles run on denaturing polyacrylamide gels obtained the correct results. In this exercise, all laboratories using HUMACTBP2 employed fluorescence detection, but amplification and designation protocols were not specified. Work is in hand to investigate the degree to which standardisation is necessary for the designation of alleles at this complex STR locus.

In summary, the results obtained from this collaborative EDNAP exercise demonstrate that the simple STR loci VWA and THO1 are good candidates for standardisation on an international scale. It is anticipated that the use of these systems will expedite cross-border exchange of information.

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