STANDARDIZATION OF DNA PROFILING TECHNIQUES IN THE EUROPEAN UNION (STADNAP) MEETING

Tenerife 16 January 1999

Host: Professor Angel Carracedo

Chairman: Dr Niels Morling

A list of participants is attached at Annex 1

1. Welcome

Angel Carracedo welcomed members to Tenerife.

Niels Morling introduced Cristian Capelli (Rome), Auli Bengs (Finland), Lyn Fereday (FSS Birmingham) and Wolfgang Mayr (Vienna). Carina Schmidt and Nigel Tooke from Amersham Pharmacia Biotech were also welcomed. No representative from Perkin Elmer and Promega was present.

2. Comments on minutes from the last meeting

Although the situation regarding representation from the French Police laboratories is potentially serious, STADNAP representatives and Mr Profilis will attempt to find a solution which is acceptable to all concerned.

3. Update on STADNAP exercises

3.1 DYS385 STR Peter Schneider

Sufficient data has now been received for the production of a draft manuscript (Annex 2). Members were requested to read the draft and send comments to Peter Schneider by 31 January 1999. When complete, the manuscript will be submitted to Forens. Sci. Int. for publication.

3.2 mtDNA from head hair samples Peter Gill

The data has now been collected and analysed and is presented at Annex 3.

The samples used in the exercise were taken from a subject who was known to be heteroplasmic in at least one body tissue at position 16234. Therefore, it was not surprising to receive results which reflected this situation. Also, the fact that some differences were detected at position 16093 does not give cause for concern as this site

is highly mutable. An interesting observation was the switch from A to G at position 16129 (39.2 - 39.5) which was obtained by two laboratories (Zurich and Strasbourg).

It was suggested that the next exercise should contain hair samples from a person who is not thought to be heteroplasmic. Participants will be contacted to determine details of their methodology prior to preparation of a manuscript.

3.3 Y chromosome pentaplex Angel Carracedo

So far, nine laboratories have responded and four have also submitted population data.

In general, it was thought that the five loci could be multiplexed. Some laboratories reported the finding of DYS393 results from females. It was suggested that this can be avoided by using higher annealing temperatures or perhaps by changing the primers.

In response to the question of whether there was any benefit in the use of Y chromosome markers, it was generally agreed among laboratories using Y chromosome markers that Y chromosome typing is very useful in cases with mixtures of DNA consisting of a large proportion of DNA from a female and a very little proportion of DNA from a male as may be seen e.g. in some cases of sexual assaults.

3.4 EMD - mtDNA Angel Carracedo

Results have been received from only two laboratories at present.

Nigel Tooke (Amersham Pharmacia Biotech) gave a brief presentation on the technology of EMD in which mismatches are detected by enzyme cleavage. Information and technical support can be obtained from www.apbiotech.com/autodna which contains typical data, expected results and questions and answers. There is also an e-mail address:
mutation-detection@eu.apbiotech.com

3.5 Degraded DNA Peter Schneider

In order to produce some degraded DNA it was decided to use mechanical methods:

(i) by consecutive passes through a syringe
(ii) by ultrasonic treatment for varying times

The latter appeared much more efficient at degrading the DNA but, to date, no STR analysis has been performed. Before going ahead with large scale preparation of degraded DNA comments were invited on the method to be used.

It was suggested that there is more value in degrading the DNA in a similar way to that found in nature although it was appreciated that this could be difficult. The meeting felt that the samples could be obtained in two ways; from a putrefied corpse and from the mechanical means outlined above. Testing would be performed on both samples. There was some concern as to how the degraded DNA could be stored to provide reference samples for the future.

3.6 Pentanucleotides Ate Kloosterman

Promega are willing to provide two different triplexes for the exercise. Unfortunately, neither of the systems have been optimised, no quality control has been carried out, no ladders are ready and no population data is available. Also, no information has been received regarding chromosome location of the various loci.

There was some concern that the exercise would be seen as developing and validating systems for a commercial company. Therefore, it was considered advisable for Steve Rand and Ate Kloosterman to do an initial study to determine whether there is any value in continuing with the project in its present uncertain condition.

4. Update on ENFSI activities Dave Werrett

4.1 Current ENFSI initiatives

The ENFSI DNA Working Group currently has 27 members with 40 attendees. So far, 8 meetings have been held. The main emphasis of the group is to facilitate the implementation of methodology and to deal with operational issues. Currently, the Working Group is responding to the Sexual Trafficking of Persons (STOP) project which incorporates the exchange of DNA databases across Europe. This involves decisions on which loci to use, the compilation of population databases, QA programmes and the unification of statements for courts.

4.1.1 Common loci

The initiative has been progressed by concentrating on individual loci rather than the use of kits. The loci which are being tested include:

D3S1358, D8S1179, D18S51, D21S11, FGA, THO1, VWA and Amelogenin.

The seven STR loci will be recommended as common loci for an European (Europol) database.

Perkin Elmer and Promega are working on the development of kits contain these loci:

Perkin Elmer Profiler SGM Plus (10 loci)

Promega Powerplex 2.2 (9 loci) and Powerplex 16.2 (16 loci)

The Perkin Elmer Profiler SGM Plus kit is expected to be commercially available 1 March 1999. The Promega Powerplex kits are not expected to be commercially available this year.

4.1.2 Quality Assurance

A draft programme has been prepared with reference to TWGDAM, ISO25, EN45001 and NRC11. The draft, which has been accepted by the group, requires some additional work to accommodate GEDNAP.

The programme includes proficiency testing, competency tests and audits.

4.1.3 Statement formats

Examples of statements from the member have been collated. At the last ENFSI meeting, Taroni and Lambert reported the situation in the member states and suggested certain recommendations concerning the use of likelihood ratios for the expression of the weight of the evidence. As the great majority of the countries report frequency estimates of the DNA-profiles or match probabilities, it may take some time before agreement on this issue can be reached.

It was generally agreed that the weight of the evidence in cases with DNA mixtures could only be done by calculation of likelihood ratio(s).

Taroni and Lambert are working on a paper which includes information on expression and interpretation.

4.2 Current exercise Peter Gill

At present, 21 laboratories are involved in an exercise to determine multiplex validation and to compare results obtained. Each laboratory was asked to extract DNA from 6 reference samples and 6 casework samples and to PCR 1ng of extracted DNA.

There has been an attempt to control as many variables as possible. The study is not yet complete but the rationale has been prepared.

ENFSI is also planning studies on Y chromosome loci, extraction procedures, mtDNA minisequencing kits and affiliation with the ISFH.

4.3 Reports and minutes

EDNAP members are welcome to see the ENFSI reports. Contact Ms. Lyn Fereday, Forensic Science Service.

5. Next Meeting Niels Morling

It was suggested that the next meeting should be for 1.5 to 2 days and include presentations from eminent scientists and commercial companies on progress and developments within this specialist field.

Commercial companies suggested included:

Gene Trace Systems (Mass Spec TOF/ STRs)
Sequenon (Mass Spec/ chip technology)
Molecular Tools
Affymetrics (microchips)
Nanogen (hybridisation)
Spectrametrics
Molecular Dynamics
ABD

There was some discussion on which eminent speakers to invite but no firm decisions were taken at the meeting.

This proposal was agreed by the meeting. The date was set for 14-16 May 1999 and the venue would be Brussels.

6. Reports from Work Package Groups

6.1 WP 1 State of the Art Peter Martin

It was considered that the current technology is good and could serve the criminal justice systems for many years to come. However, it was also appreciated that developments, particularly those which were more efficient or more informative, should be embraced by the laboratories without delay.

The emerging technologies were identified as Mass Spectrometry with Time of Flight detection and microchip methodology. If laboratories could acquire the necessary microscope readers there might be a possibility for an exercise using chip technology. However, the expertise required for Mass Spec studies would probably require a collaborative exercise with scientists who posses both the equipment and the necessary skills.

At the forthcoming Brussels meeting we should invite scientists to make presentations which would include detailed information on the positive and negative aspects of these emerging technologies. It was proposed that James Robertson (FBI) should be contacted to suggest names of appropriate speakers.

6.2 WP2 Inter-laboratory exercises Peter Gill

6.2.2 mtDNA from hair shafts

The practical aspect of the first phase of the exercise is complete. The second phase involve a hair sample from a person who is not thought to be heteroplasmic.

6.2.3 Y chromosome loci

There is a need for some primer changes especially with DYS393 to avoid the interaction with X chromosomes.

6.2.4 Pentanucleotides

Ate Kloosterman and Steve Rand will determine progress after contact with Promega.

6.2.5 Degraded material

Peter Schneider will obtain some degraded material from a corpse and also artificially degrade a sample by ultrasonics. The two samples will be compared by STR analysis.

6.2.6 EMD

The exercise is on-going and a report will be made at a later date.

6.2.7 STR analysis on hair shafts

Hermann Schmitter will report back to the next meeting with suggestions for a way forward.

6.2.8 Open exercise

Peter Gill will give consideration to an open exercise.

6.3 WP3 Technology Transfer programme Bernd Brinkmann and Steve Rand

6.3.1 Phase 1

The first phase of the Technology Transfer Programme is now complete. There are no more applications other than the original seven reported at Innsbruck. All members who took advantage of the programme are reminded that, after completion of the mtDNA training, it is necessary for the guest, as well as the host, to submit a brief but concise report to WP3 for the records.

6.3.2 Phase 2

Applications can be submitted by members if they feel that their laboratory is not scientifically competent to take part in future exercises. This may be the case, for example, if projects planned by STADNAP require an extended contact with a commercial company.

No action has been taken regarding secondments of scientists from laboratories outside STADNAP as this topic requires careful and detailed planning to avoid confusion or bias. A method for the formulation and distribution of an announcement should will be considered by WP3 members. Application will probably be via the STADNAP homepage.

In connection with the European grant to STADNAP under the Thematic Network Contract N0 SMT4-CT97-7506 (DG 12-EGAA), applications are requested from laboratories who wish to extend their level of expertise in forensic DNA testing and who consider that they would benefit from a period of training at one of the

STADNAP laboratories. Priority will be given to laboratories who consider that their expertise does not meet the accepted standard for forensic DNA testing in European member states. Also urgency of training and the promotion of younger scientists will receive a priority status. Applications should be submitted in writing to:

STADNAP Secondment Programme WP3
c/o Prof B Brinkmann. Manager WP3
Institut für Rechtsmedizin der W.W.U.
Von-Esmarch-Strasse 62, D-48149 Münster, Germany

Details of the proposed secondment and a justification for sponsorship should be included in the application. The amount of funding will depend on the number of successful applicants and will be decided by a unanimous vote of the members of WP3 on behalf of STADNAP.

Further suggestions within this framework should be submitted to members of WP3. Special attention will be given to visits to commercial companies with a view to maintaining the status of STADNAP as a leader in the field of forensic DNA testing.

6.4 WP4 Databases Ernesto d'Aloja

It has been agreed that data for all commonly used STRs, except those which are Y linked, will be collected. THO1 will be used as a start to establish the collection and analysis procedure.

By 31 January 1999 the following data should be sent by members to Denise Syndercombe Court (by e-mail or floppy disc together with printed copy):

The THO1 data, either in Excel or ASCII format, organised in 3 columns:
1. A sample/lab identifier 2. The first allele 3. The second allele (homozygous alleles should be listed twice).

A list of the national (paternity and stain) labs who might be able to contribute to the survey.

A letter requesting the data will be drafted by Peter Schneider for comment by members of WP4. Bernadette Hoste will prepare a questionnaire for obtaining background data on samples and typing procedures.

It was agreed that the submitting labs will retain copyright on their data and are free to publish these in a journal (STADNAP will then reference their contribution accordingly).

WP4 plan to send out the letters, requesting samples, by the end of February and it is hoped that data will be received by the end of April.

7. Cost Statements Angel Carracedo

See reports attached at Annex 4

The importance of getting the cost statements completed accurately and on time was stressed.

8. Socio-legal Research Group Peter Martin

The sociology group at Brunel University has submitted a final report on the ESRC project which looks at DNA profiling in the UK. ESRC are still reviewing the proposal. The Brunel group is continuing to develop contacts with social scientists in Europe who are interested in pursuing a joint programme on European standardisation.

A small meeting is being arranged for the 10 May to include colleagues from Portugal, Holland, France and the USA. Peter Martin has been invited to attend.

A progress report will be made at the meeting in Brussels.

9. Communication of activities

9.1 Authorship issues Denise Syndercombe Court

See report at Annex 5

The question of multi-authorship of publications was discussed against the background of Vancouver Group deliberations and the subsequent guide lines which currently operate in a number of medical and scientific journals.

In this context, the EDNAP practice of using so many authors would be considered unethical. There are some options which could be adopted:

> STADNAP could be used as the corporate authorship and members could site that in their list of publications
>
> The acronym could be used with authors listed at the bottom of the title page.
>
> Three main authors could be used with the others listed as collaborating authors.

In the discussion, it became clear that there is no easy solution to this problem and the subject was too big for the time available at the meeting.

9.2 STADNAP Homepage Peter Schneider

Copies of the new homepage have been e-mailed to all members (Annex 6). The meeting was asked to consider whether the names of each of the STADNAP contractors should be identified or whether a list of the institutes is sufficient.

At the moment the homepage has links to 6 other sites and there was a suggestion that there should be a password covered page for drafts of papers etc. This appeared to have considerable support.

Recently, Peter Schneider attended a conference on Quality and Standardisation at which Kimmo Hindberg (Finland) gave a paper on collaborative exercises in forensic science (Annex 7). Also included in the conference were papers on reference standards. It was suggested that the Institute for Reference Materials and Measurements (IRMM) could be invited to give a talk to STADNAP on the production of reference material (Annex 8).

10 Any other business

There were no other points for discussion and the meeting was closed.

Enclosures

Annex 1.	List of participants
Annex 2.	Y chromosome STR manuscript from Peter Schneider
Annex 3.	mtDNA from hairs - data from Peter Gill
Annex 4.	STADNAP progress report
Annex 5.	Authorship discussion from Denise Syndercombe Court
Annex 6.	STADNAP Homepage from Peter Schneider
Annex 7.	Institute for Reference Materials and Measurements

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