The GEDNAP (German DNA profiling group) blind trial system

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1. Introduction

Any laboratory which performs tests and produces results that are to be used in an evaluation, must ensure that these results are correct and that they meet the standards set for acceptance. This is true for all laboratories but is especially true for those which produce results that have consequences for the public. Forensic science and forensic medicine (in Germany this is combined in the term Rechtsmedizin or legal medicine) are both disciplines which produce results for the legal system and therefore have a great responsibility to ensure that very high standards of accuracy and precision are maintained.

1.1. Basic principles of the blind trial

The basic principles of the GEDNAP blind trial system are the same as any other system of quality control and attempts to evaluate the following problem areas:

1. The ability of an analytical method to produce results for the examination in question
2. To test the specificity of the method by examining the criteria:
    - -To test the accuracy of the results
    - -To test the precision of the results
    - -To test the limits of detection of the method

The first stage in the validation of any method for use in forensic work, is the background research performed by the laboratory which has done the development. This involves a refinement of the technical and experimental conditions followed by genetic and statistical evaluations of population studies carried out on an adequate number of related and unrelated individuals. The criteria defining what is adequate and which tests have to be performed to validate the method for DNA STR systems have been published mainly in the form of recommendations and guidelines by the DNA Commission of the International Society for Forensic Haemogenetics (recently changed to International Society of Forensic Genetics). These recommendations have been published at regular intervals over the past years to keep pace with the ever-changing repertoire of DNA systems available to the forensic community.

In all forms of recommendations, guidelines or even regulations, the next stage of any method is the peer-review system. The system must comply with the generally accepted state of-the-art which means that the system must not only be proven to be reproducible within the developing laboratory but must also be reproducible in other equally qualified laboratories.

All recommendations go further and regulate what controls must be performed by the laboratory when using the method or system for testing. The most important of these are:

1. Internal controls which must be included in every test procedure
2. External controls - Participation of the laboratory in a form of blind trial

This external form of control serves not only to test the ability of the method to come up with the correct answer under the conditions used but also, and this is imperative in this context, to test the ability of the laboratory itself to come to the correct conclusion after having performed the test.

This is a very important aspect of any testing procedure and this blind trial procedure is designed to test the following:

1. Has the laboratory tested the correct stain?

-Are the safety precautions within the laboratory sufficient to avoid confusion or contamination of samples?

2. Has the laboratory arrived at the correct result?
3. Has the laboratory come to the correct interpretation of the result obtained?

All of these aspects must be tested by a blind trial system and therefore serve as a quality control system for the laboratory doing the testing.

It is also expected that participation in a blind trial will stimulate the laboratories to be more self-critical of the standard and quality of their organisation and results. It is also expected that an increased level of awareness of problems involved will in turn lead to constructive criticism of the blind trial system itself and an improvement in the parameter testing procedure.

1.2. Development of the GEDNAP system

The basis for the GEDNAP group (German DNA Profiling Group) began in the early 1980s when a "Stain Commission" was set up by the German Society for Legal Medicine (Deutsche Gesellschaft für Rechtsmedizin) to examine and formulate ways and means of checking the quality of results obtained by laboratories performing

forensic testing for the Judicial system. The Commission consisted initially of 5 members from Institutes of legal medicine but now includes 4 from legal medicine, 4 from the governmental State laboratories (Landeskriminalamt LKA) and the Federal laboratory (Bundeskriminalamt BKA), a neutral Chairman and a non-voting secretary. With the introduction of DNA systems the institute in Münster was designated by the Stain Commission and by a unanimous decision of the participants to undertake the evaluation.

At present the state-of-the-art DNA testing is made using STRs mostly in multiplexes and commercially available kits.

Throughout the development of the GEDNAP trials, efforts have been made to treat the results with a maximum degree of fairness whilst maintaining a high level of integrity and impartiality to the evaluation. This has been upheld by using firstly an unbiased approach to the evaluation supported by anonymity of the participating laboratories as far as it is possible without being detrimental to the quality of the trial.

GEDNAP is the German-speaking working group of the EDNAP group (European DNA profiling group), which was established in 1989 by a handful of European laboratories in an attempt to harmonise the extremely rapidly developing field of DNA profiling throughout Europe.

1.3. Aims and requirements

One of the basic requirements of a blind trial is that all participants receive exactly the same material to be tested enabling a direct comparison with the known standard as well as an interlaboratory comparison to be carried out.

The aims of the blind trial procedure are fourfold:

1. Standardisation of methods and procedures
2. Standardisation of nomenclature
3. Evaluation of the competence of a laboratory to obtain the correct result
4. Elimination of errors in typing

A blind trial is one essential element of the complete quality assurance programme which a laboratory engaged in DNA typing (or any other type of laboratory) is obliged to conform to.

In the field of forensic examinations in general, and DNA typing in particular, this has two main goals:

1. To ensure that results obtained from evidential material which are to be used within the penal system in a Court of Law, reflect the true nature of this material.
2. That results from DNA investigations, which are to be submitted and stored in a National DNA data bank, are given in a standard form (nomenclature) and have been correctly typed.

2. Structure of the current GEDNAP blind trials

2.1 Participants

The number of participants in the last GEDNAP blind trials was 92 laboratories from 12 European countries taking part in two blind trials per year (GEDNAP 20 and 21 for the year 2000) plus a total of 21 laboratories taking part for the first time as ENFSI (European Network of Forensic Science Institutes) members who only examined the samples from GEDNAP 21.
In 2003 154 laboratories from 31 Ländern participated in GEDNAP 26 und 27.

Participation in the GEDNAP blind trial is basically open to any laboratory, whether private institutes, university institutes or governmental laboratories from any country in Europe. Although GEDNAP was originally a German working group laboratories from non-German-speaking countries have gradually been included and now the ENFSI group has adopted GEDNAP as the officially accepted external trial system.

2.2. Construction

The GEDNAP blind trials are organised in such a way that each participating laboratory receives 2 sets of stains to be tested during the year.
The number and the type of samples sent out for each blind trial has varied in the past depending on the number of participating laboratories, the systems to be tested and public consensus.
The DNA systems to be included in the blind trial system have varied depending on the current state-of-the-art and are decided by unanimous decision between the Stain Commission and the general consensus opinion. For the present trials the following systems can be evaluated:

- TH01	- D16S539	- Penta D	- DYS392
- VWA	- D2S1338	- Penta E	- DYS393
- FGA	- D19S433	- Amelogenin	- DYS437

- D21S11	- TPOX	- DYS19	- DYS438
- ACTBP2	- CSF1PO	- DYS385	- DYS439
- D3S1358	- D5S818	- DYS389I/II	
- D8S1179	- D13S317	- DYS390	
- D18S51	- D7S820	- DYS391	

The systems are all components of commercially available kits such as the SGM Plus and Profiler Plus kits from Applied Biosystems (Foster City, CA), PowerPlex Y, PowerPlex ES and PowerPlex 16 from Promega (Madison, Wisc.), MPX2 from Serac (Bad Homburg, Germany) or Mentype M9.1 from Biotype (Leipzig/Dresden, Germany).). These have been included because many laboratories use these kits routinely. At present laboratories receive a total of 7 samples for each trial, consisting of 3 control bloodstains from known and tested individuals and 4 stains of unknown origin with which they are to be compared.

There are also some laboratories who do not engage in stain analysis but wish to participate in the blind trial. These are mostly engaged in typing samples for the DNA data bank only and will only type the control samples.

The stains to be included in a blind trial are decided by the Stain Commission which meets at regular intervals of at least twice a year, taking into account the general opinion of the participants who are consulted on the occasion of the Workshop to present and discuss the results of the preceding trials.

In general, the type and size of the stains are designed to reflect the state-of-the-art of the DNA typing to be tested and attempts to be as near practice-oriented as possible.

2.2.1. Planning

The planning for subsequent blind trials is undertaken by the organising laboratory (Münster) in consultation with members of the Stain Commission and also with the participants.

Prior to the Workshop, the Stain Commission convenes to discuss the results and to make suggestions for the subsequent trials based in part on the outcome of the previous trials and on the latest relevant developments in the field.

On the occasion of the presentation of the result of the previous trials (February of each year) the participants will be asked for their opinions regarding these aspects. This will then be taken into consideration when the Stain Commission convenes to make the final planning for the forthcoming trials.
A time lapse of approximately 2 months then allows all possible comments to be registered before the final decision is made.

2.2.2. Registration

All previous participants and new applicants are informed of the decision and requested to register for the next set of trials and to reply within 2--3 weeks.
In the past the form has always included a question asking the laboratory to state which systems will be tested. This practice has been introduced to allow the organiser to have an overview of the extent of the procedure. This practice will be maintained in the future unless circumstances dictate otherwise.
When confirmation is received from a laboratory either by fax or by mail, the list of participating laboratories is established, whereby each incoming registration is assigned a code number (laboratory number) in chronological order of receipt. This ensures that no bias is given to any laboratory and that laboratories will probably never receive the same number as before and maintains the anonymity of the system. Once a code number has been assigned this will be used in place of the name for all future evaluations.

2.2.3. Preparation of samples

The samples are prepared in the organising laboratory according to the recommendations laid down by the ISFH and other organisations to include a maximum of integrity of the samples and a minimum of contamination.
In general, samples are obtained from members of the Institute because the DNA profiles are known and been thoroughly tested beforehand. For each blind trial different persons or combination of persons are used to avoid duplication (and ipso facto recognition).
1. New cotton cloth is used as the substrate for blood and mixed stains. This is washed 3 times before use to prevent contamination and to remove any traces of chemicals used in the manufacturing process.

2. Stains are prepared in such a way that there is sufficient blank cloth for negative blank cloth controls.
3. Disposable gloves and face-masks are worn at all times. All containers and utensils are sterile and used only once.
4. Blood is taken in sterile citrate containers and the appropriate volume is dispensed using a calibrated pipette.
5. Saliva is collected in sterile Falcon tubes by drainage and vortexed continuously to maintain homogeneity. The appropriate volume is dispensed using a calibrated pipette
6. Mixtures of body fluids are prepared in a similar way and great care is taken to maintain homogeneity of the sample by vortexing thoroughly between each sampling during the spotting procedure.

The effective relationship between components in a mixture is also checked by a comparison of the peak heights (intensity) after amplification and typing. While this does not give an absolute value, it does reflect the actual relationship as measured under experimental conditions equivalent to those encountered in the trial.

Some examples of stains prepared for the blind trials in the past are blood/blood mixtures in various proportions, blood/body fluid mixtures, semen/saliva mixtures, semen/vaginal fluid mixtures, smoked and unsmoked filter cigarettes etc. A variety of stain substrates have also been used including jeans, leather, cardboard, cotton wool swabs etc. To illustrate this for the blind trial GEDNAP 20 the following samples were prepared:

GEDNAP 20

1. Person A: 25µl blood (female) on cotton cloth
2. Person B: 25µl blood (male) on cotton cloth
3. Person C: 25µl blood (male) cotton cloth
4. Stain 1: unsmoked filter cigarette with 10µl saliva
5. Stain 2: 25µl blood mixture (Persons A:B, mixed 1:2 v/v)
6. Stain 3: 25µl blood mixture (Persons A:C, mixed 3:1 v/v)
7. Stain 4: Buccal swab from Person A

Samples are prepared in isolation in different areas of the laboratory and by spotting onto the substrate which are then air-dried overnight. The individual stains are then cut out (one stain type at a time) and placed in a parchment paper bag labelled with the corresponding number and sealed.

All stains are marked directly on the substrate to enable an identification of the stain at a later date if necessary. This will enable any possible or claimed interchange errors to be clarified. For each laboratory a set of stains is prepared and stapled together. Each package is then checked by a further assistant to ensure that the set is complete and correct. The sets are then placed in an envelope labelled with the name and address of the participant, and checked again by another assistant.
Before sealing the appropriate documentation for submitting the results is also placed in each envelope in turn, check by an observer and the envelopes are sealed.

2.2.4. Distribution of samples

Each set of documents is labelled with the laboratory code only which is entered by the organising laboratory before being sent.
The envelope containing the set of samples and the documentation necessary for returning the results are prepared for posting and sent via the university postal system.

2.2.5. Typing of samples

Laboratories must retain an adequate part of the sample for future testing in case of any disagreements over the identity or claims of contamination prior to the sample being received by the participant.

The participants are requested to use the convention for nomenclature as laid down in the ISFG guidelines. Allele numbers should not be rounded-up and should be given with a 1bp precision and do not give the fragment length in bp alone as this cannot be accepted and this will be regarded as incorrect result (group IV).

Laboratories are expected to comply with the international recommendations for DNA typing and include all the appropriate positive and negative controls as well as the various ladders (internal and external where appropriate), but this is no longer

explicitly laid down and it is up to the individual laboratories to fulfil this condition. The organising laboratory recommends that for the system ACTBP2 (SE33) an extra mixture (compound) standard which, e.g., contains the alleles 14.3, 21, 21.1, 31.3 should be included in every run, at least once at the beginning and once at the end. If separation can be achieved then the run is valid. This is also commercially available from, e.g. Serac (Bad Homburg, Germany).

All ACTBP2 alleles in samples sent out for blind trials have been previously sequenced to establish the correct number of bases as a guideline for typing.

Not all alleles in all systems are sequenced before the samples are sent but this applies to all alleles which are off-ladder or rare or show any other sort of variation.

2.2.6. Returning results

Participants are requested to return the results by the closing deadline of 3rd December of this year in question to allow the organising laboratory sufficient time to evaluate and present the results at the Workshop in February of the following year.
If results are received after this date they may be included if there is sufficient time. However, once the statistical evaluation has been made, no more results can be included.
Laboratories were previously requested to also submit the original data (print-out or original gels) when returning the results, so that possible error sources can be identified. In order to refute any possible suggestion of collaboration between participating laboratories, the submission of the orignal data is now obligatory. This can be done by e-mail or other forms of electronic data storage (e.g. discs, 100MB ZIP, CD ROM, eMail-Attachment).

When the results are received the date is entered and the results are filed under the appropriate laboratory code number before being processed.
The results for each individual laboratory are then entered by one person into an excel file.
When all the results have been received (or the deadline has passed) and entered into the appropriate file for the lab code number, a print-out is made and the results

are double checked by comparing the original data (from the lab) with the data entered in the excel file.
Any errors in the excel file are corrected.

3. Results

3.1. Evaluation of results

All data in the excel files are then checked again by the department head by comparison of the original data with the excel files.
At this stage any errors or discrepancies from the established values made by the participating laboratory are checked (if possible) by referring to the orignal data submitted.
However, due to the development of more sensitive techniques it has been decided that for mixtures, it would be reasonable to expect that alleles should be detected if they are present in more than 20% as a mixture component based on the proportion of alleles in the mixture.
Assuming an equal degree of efficiency of the amplification and equal number of cells containing DNA in the sample.
All errors are classified into categories in an attempt to identify the most common source of errors. The types of errors are classified as poor quality, over-interpretation of stutter bands and/or very weak bands, false alignment of the ladder and transcription errors.

3.2. Criteria for categorisation

After checking all results, each individual result for each system and for each stain is categorised according to the following criteria:

Group I: Correct results obtained

Group II: - An allele in a mixture has not been detected but which constitutes at least 10% of the total and has been detected by less than 90% of the participants.
- No result has been reported for isolated systems, but this has been detected by less than 90% of the participants.

Group III: This group has been removed. The option „reportable“ or „no reportable results“ will not longer be available.

Group IV: - incorrect results

- An allele in a mixture has not been detected but which constitutes at least 10% of the total and has been detected by more than 90% of the participants.
- No result has been reported for isolated systems, but this has been detected by more than 90% of the participants.

Only errors classified under **Group IV** are considered to be true errors for the final evaluation.

3.3. Presentation of results

Each laboratory receives a copy of the results which are presented beforehand at the Workshop.

In order to simplify the results not all results are given, only the code numbers are given of those who have made errors from categories 2--4.

3.4. Informing participants of results

The results are presented at the workshop held every year in February at a predefined location (usually proposed at the previous Workshop and finally decided by the Stain Commission). The results are made public in the form of a verbal presentation illustrated using overhead projection and/or slides. Over the past years it has become impractical to present all the results for all laboratories and for all systems: with over 100 laboratories this is unfeasible but all the results can be made available on request.

The complete files containing all the results submitted are taken to the Workshop to enable immediate checking if so desired. The various categories are demonstrated and the laboratory code numbers where errors have been made are given, usually together with examples, so that each affected laboratory has the chance to see the problem. After the presentation each laboratory receives a copy of the relevant tables.

Laboratories are given the chance to appeal if they feel that their results have been incorrectly classified or if they have been unfairly treated. This is made clear at the Workshop and all laboratories are given the right to appeal if they wish to do so. Laboratories also have the right to receive more of a particular sample if they wish to retype a stain in order to check the method or to convince themselves that nothing untoward has occurred.

In the event of any problem with typing or if a participant requests that the sample in question be retested, the following procedure has been invoked:

1. The portion of the sample which was retained by the laboratory will be returned to a member of the Stain Commission selected by the participant (a list of members and contact information can be supplied on demand)
2. The member of the commission selected should be from an Institute of Legal Medicine if the participant is a government laboratory and vice versa.
3. A private laboratory has a free choice.
4. The selected laboratory will then carry out the desired testing and report the findings back to the Commission and if necessary, consult with the organising laboratory before a decision is made.

4. Certification

A certificate is then issued by the organising laboratory in which it states that the laboratory in question has successfully completed the blind trial in the named systems. Certificates of participation will be issued in the name of the Institute which has actually undertaken the investigation. The investigation by a substitute or affiliated laboratory is not valid. False results (errors) are not explicitly named but are not included in the list.

The certificates are completed by an assistant in the organising laboratory based on the final evaluation of the Workshop and include all alterations which have been agreed and validated after making the results public, counter-checked by the department head and signed by the Chairman of the Stain Commission.

Laboratories also have the right to appeal at this stage if a typographical error has been made by the issuing laboratory and when the certificate is sent out, information to this effect is included in the accompanying letter.

All the documentation sent for analysis to the organising laboratory will be returned with the certificate. The participating laboratory is responsible for archiving and storage for an as yet undefined period of time.

5. State of the arts

At the present state-of-the-arts the STR systems form the backbone of the blind trial and are expected to do so for some time to come. Newly developed and/or possible candidates for inclusion in the blind trial system will be considered by the Stain

Commission and a decision will be reached after consultation with the participants in general. This system has been employed and has proved successful during the previous stages of GEDNAP and will be employed in the future as long as the participants are in agreement.

Up to now the organising laboratory has also participated in the blind trial. While this situation is not optimal, the organising laboratory has always attempted to treat these samples in an impartial way and the testing is performed by another person independent of the preparation of the trial samples. In addition another independent governmental laboratory sends other unknown samples to the organising laboratory for testing in a similar way to the official blind trial. This proposal was also suggested by the report on the GEDNAP proficiency testing programme by B. Budowle (FBI). The Stain Commission adopted this proposal and it was unanimously decided that additional samples would be provided to the organising laboratory by Dr. Schmitter (BKA Wiesbaden) which would then be tested and evaluated using the same criteria as employed in the blind trial. The organising laboratory also participates in the EDNAP trials so that the quality of results produced is also open to official scrutiny from external sources.

6. Conclusions

Since the blind trial system was first conceived in its present form, there have been many changes in the construction and implementation of the system as well as the several generations of DNA systems. The organising laboratory took over the sole responsibility of distribution, collection and evaluation of the blind trial but decisions as to which systems and which samples were to be tested were always made and will be made in the future by full consultation with the members of the Stain Commission and with the participating laboratories. This system has proved invaluable in the past for the selection of systems as well as for solving problems which may have arisen at any stage of the process. The complete feed-back regarding criticism of performance and implementation, problems and solutions together with an open discussion of all aspects at the Workshop, has proved to be a successful combination and will be maintained as long as the forum so desires.

The organising laboratory has also gained a great deal of experience over this period of time which has been put to practical use in the various aspects of management.

7. Review of the GEDNAP proficiency testing programme

In November 2000, a review of the GEDNAP procedure was carried out by Dr. B. Budowle of the FBI as part of an overall review of the databank system organised by the BKA. This included a visit to the organising laboratory where all phases of the procedure were examined for possible sources of error or inconsistencies in the system. A report was made and submitted to the head of the Forensic Science Institute of the BKA. There were no major criticisms but some recommendations were made to improve the standing and validity which have now been incorporated into this document and into the blind trial system.

8. Appendix

Addresses of Members of the Stain Commission:

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