



The GEDNAP (German DNA profiling group) proficiency testing system

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1. Introduction

1.1 Basic principles

Any testing laboratory must ensure that their analysis results are correct and that they meet accepted quality standards. This is especially true for laboratories which produce results that have consequences for the public. Forensic genetic practitioners produce results for the legal system and therefore have a responsibility to ensure that very high standards of accuracy and precision are maintained.

Another aspect is the introduction of any new method in forensic practice, where an extensive validation has to take place. The criteria defining what is adequate and which tests have to be performed to validate the method for a genetic typing system have been published since 1992 in the form of recommendations and guidelines by the DNA Commission of the International Society for Forensic Genetics (ISFG). 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 [1-4].

The GEDNAP proficiency tests serve as an external quality control and are in accordance with the basic principles of quality control. Thus they validate new laboratories seeking accreditation, as well as support accredited laboratories wishing to introduce new DNA profiling methods. Since the European Union has declared accreditation according to the international standard DIN EN ISO/IEC 17025 as binding for all forensic-genetics testing laboratories from November 1st, 2013¹, participation in proficiency tests has become the central element in the field of quality control for any laboratory.

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

1. The ability of an analytical method to produce results for the examination in question,
2. The specificity of the method according to the following criteria:
 - accuracy of the results,
 - precision of the results,
 - limits of detection of the method.

The external assessment by GEDNAP does not only serve to evaluate the ability of the method to provide the correct answer under the conditions used, but also, and this is imperative in this context, to assess the ability of the laboratory itself to come to the correct conclusion after having performed the analytical method. To this end, the GEDNAP organizers send defined stain samples to participating laboratories as a blind trial.

¹ COUNCIL FRAMEWORK DECISION 2009/905/JHA of 30 November 2009 on „Accreditation of forensic service providers carrying out laboratory activities“

This is a very important aspect of any quality control, and this blind trial procedure is designed to assess the following questions:

1. Has the laboratory examined the correct stain?
2. Are the safety precautions within the laboratory sufficient to avoid mix-up or contamination of samples?
3. Has the laboratory arrived at the correct experimental result?
4. Has the laboratory correctly interpreted the experimental result obtained?

The results of the proficiency testing are an important element of the laboratory quality management system. It is also expected that participation in a blind trial will stimulate the laboratories to be more self-critical of the standard procedures and analytical results, as well as regarding the quality of their organizational structure.

One of the fundamental requirements of a proficiency test is that all participants receive exactly the same material to be tested, enabling a direct inter-laboratory comparison.

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. Detection of error sources and consequently, elimination of typing errors

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

1. To ensure that results obtained from evidential material which are to be used in a Court of Law reflect the true nature of this material.
2. To ensure that results from DNA investigations, which are to be submitted to and stored in a national DNA database, are given in a mandatory standard format (nomenclature) and have been correctly typed.

1.2 Development of the GEDNAP system

The origin of the GEDNAP group (German DNA Profiling Group) can be traced back to the early 1980s when a “Stain Commission” was set up by the German Society for Legal Medicine (Deutsche Gesellschaft für Rechtsmedizin) to examine ways and develop recommendations for checking the quality of results obtained by laboratories performing forensic testing for the judicial system. The ‘Joint Commission of Institutes of Legal Medicine and Forensic Science’ in Germany now consists of 4 delegates of the German Society for Legal Medicine, 4 delegates of the local State criminal laboratories (Landeskriminalamt, LKA) and the Federal criminal laboratory (Bundeskriminalamt, BKA), and a chairman (see Appendix).

Initially, serological systems were applied in the proficiency test, and this was organized by the Institute of Legal Medicine Hanover. With the introduction of DNA systems the Institute of Legal Medicine Münster was designated by the Stain Commission and by a unanimous decision of the participants to undertake the evaluation.

Throughout the development of the GEDNAP proficiency test, 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.

GEDNAP (German DNA Profiling Group) is the German-speaking correlate 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 Europe².

² www.isfg.org/EDNAP

2. Structure

2.1 Participants

In 2012, 235 different laboratories from 37 different, mainly European countries participated in the proficiency test GEDNAP 44. For the members of the 'ENFSI DNA Working Group' (*European Network of Forensic Science Institutes*³) participation in the GEDNAP proficiency test is obligatory.

In principle, participation in the GEDNAP proficiency test is open to any laboratory, whether it represents a private institute, a university institute or a governmental laboratory from any country. Although GEDNAP was originally a German working group, laboratories from non-German-speaking countries have gradually been included due to the fact that opportunities to participate in proficiency testing were lacking elsewhere.

2.2 Organisation

For the GEDNAP proficiency tests each participating laboratory usually receives 2 sets of samples 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 agreement among the participants. Since a number of years, the following format has been established: 3 reference samples and 4 stains.

The DNA typing systems included in the proficiency test are selected based on the unanimous decision within the Stain Commission and an agreement with the participants. For the present proficiency tests, the following STR systems (Tab.1 – 3) are available for evaluation and certification. This selection is based on the German DNA database (DNA-Analyse-Datei, DAD) and the European Standard Set (ESS) of STR systems, which were extended by five additional STR systems in 2009 by a European Council resolution³.

Table 1: Core autosomal STR systems and amelogenin

TH01	FGA	ACTBP2	D8S1179	D16S539	D19S433	D2S441	D22S1045	Amelogenin
VWA	D21S11	D3S1358	D18S51	D2S1338	D12S391	D10S1248	D1S1656	

Table 2: Supplementary autosomal STR systems

TPOX	CSF1PO	D5S818	D13S317	D7S820	Penta D	Penta E	D6S1043
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Table 3: Y-STR systems

DYS19	DYS389I	DYS390	DYS392	DYS437	DYS439	DYS456	DYS635	DYS481	DYS533	DYS576
DYS385	DYS389II	DYS391	DYS393	DYS438	DYS448	DYS458	GATAH4	DYS549	DYS570	DYS643

³ COUNCIL RESOLUTION of 30 November 2009 on the exchange of DNA analysis results (2009/C 296/01)

The nuclear DNA systems are all components of commercially available kits such as e.g. the Yfiler, Identifiler, NGM SElect kits from Life Technologies (Foster City, CA), PowerPlex Y23 and PowerPlex 21 from Promega (Madison, Wisc.), MPX-5 ESS from Serac (Bad Homburg, Germany) or ESSplex SE and Nonaplex ESS from Qiagen (Hilden, Germany). At present, laboratories receive a total of 7 samples for each trial, consisting of 3 reference samples (usually blood on cotton fabric) from known and tested individuals and 4 stains of unknown origin, typically composed from blood, saliva, or semen stains. Mixed stains are composed from samples of up to three different persons.

In general, the type and size of the stains to be tested are designed to reflect the state of the art of DNA typing, and to reflect real casework as closely as possible.

There are also some laboratories not engaged in stain analysis but wishing to participate in the proficiency test. These are mostly typing reference samples for the national DNA database and will type only the control samples (i.e. 28 labs in 2012).

2.2.1 Planning

The planning of proficiency tests is carried out by the organising laboratory (Institut für Forensische Genetik Münster) in consultation with members of the Stain Commission, and also with the participants.

The true DNA profiles of the proficiency tests are revealed and discussed at a national DNA stain workshop taking place each year in February. Prior to the workshop, the Stain Commission convenes to evaluate the results and to make suggestions for the subsequent proficiency tests based in part on the outcome of the previous proficiency tests and on the latest relevant developments in the field.

At the annual workshop the participants will be asked for their opinions regarding these aspects. This will then be taken into consideration when the Stain Commission decides about the final planning for future proficiency tests. A period of approximately 2 months then allows all possible comments to be registered before the final decision is made.

2.2.2 Registration

The registration is web-based (www.gednap.org). The applicants can select modules for which they want to be certified (these may change if new markers or methods are included):

1. Core autosomal STRs and amelogenin (17 STR systems)

TH01, VWA, FGA, D21S11, ACTBP2, D3S1358, D8S1179, D18S51, D16S539, D2S1338, D19S433, D12S391, D2S441, D10S1248, D22S1045, D1S1656, Amelogenin

2. Supplementary autosomal STRs (8 STR systems)

TPOX, CSF1PO, D5S818, D13S317, D7S820, Penta D, Penta E, D6S1043

3. Y-STRs (23 STR systems)

DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4, DYS481, DYS533, DYS549, DYS570, DYS576, DYS643

4. mtDNA sequence analysis of D-Loop/control region

5. Biostatistical evaluation of a mixed stain

6. Stain characterization (presumptive and confirmatory tests for blood, semen, saliva)

The registered participants receive a number ('lab code') which is used instead of the name of the participating institution in all subsequent evaluation and communication steps. This procedure ensures that there is no bias towards any participating laboratory. In subsequent GEDNAP trials, laboratories receive new random numbers, maintaining the anonymity of the system.

2.2.3 Preparation of samples

The samples are prepared by the organising laboratory according to the recommendations laid down by the ISFG and other organisations to include a maximum of integrity of the samples and a minimum of contamination.

In general, samples are donated voluntarily by members and guests of the organising laboratory (Institut für Forensische Genetik Münster), and by other volunteers. For each proficiency test, individual body fluid samples or mixtures from different persons are used to avoid duplication (and de facto recognition). When preparing the samples, the following principles are observed:

1. Lab coats, disposable gloves, hairnets and face-masks are worn at all times. All containers and utensils are sterile and/or DNA-free, if possible, and used only once.
2. Stains are prepared in such a way that there is sufficient blank cloth for negative blank cloth controls.
3. Blood is collected into sterile citrate, heparin or EDTA containers and the appropriate volume is dispensed using a calibrated pipette.
4. Saliva is collected into 50 mL tubes and vortexed continuously to maintain homogeneity. The appropriate volume is dispensed using a calibrated pipette.
5. 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 mixture ratio between components in a mixture is checked by several procedures, e.g.:

- cell count,
- determination of the DNA content in the different components of a mixture,
- comparison of the peak heights/ area after amplification and software-based fragment length analysis.

While these procedures may not provide an absolute value, in particular the third method does reflect the actual mixture ratio.

Some examples of stains prepared for the GEDNAP proficiency tests 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 GEDNAP 44 proficiency test, the following samples were prepared:

Persons A-C:	10 µl blood on a cellulose swab each
Stain 1:	20 µl blood on rock sugar
Stain 2:	20 µl blood / blood mixture on a piece of dish cloth
Stain 3:	20 µl saliva on a Sarstedt Forensic Swab L
Stain 4:	20 µl blood / blood / blood mixture on a cellulose swab

A single sample type is prepared at a time by spotting the respective body fluid or mixture onto the substrate which is then air-dried overnight. The individual stains are placed in adequate containers (usually parchment paper bags) labelled with the corresponding number, and sealed.

For each laboratory a set of samples is prepared and stapled together. Each package is then checked by another 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 a third assistant prior to shipping.

2.2.4 Typing of samples

The laboratories are expected to follow the international guidelines for forensic DNA analyses and to include all the necessary controls. For the DNA extraction and amplification, positive and negative controls must be processed in parallel. For electrophoretic typing, an allelic ladder is essential. For the currently used standard method of capillary gel electrophoresis with Laser-induced fluorescence, an internal length standard must be added to every sample. The Stain Commission recommends the use of a separation control to ensure that two alleles differing by one bp can be detected properly. For this purpose, a particular allele mix (e.g., commercially available for ACTBP2/SE33) or a suitable allelic ladder can be used.

If an allele is detected outside its defined allelic range ('off-category') it can be reported using the "smaller than" (<) or "greater than" (>) signs relative to the shortest or longest allele of the defined allelic range.

Example: the allele 6.2 at D19S433 could be reported as "<9" or ">6.2", both options would be regarded as correct (group I). In contrast, reporting the allele 20.2 at DYS458 as ">20" would be regarded as incorrect (group IV).

The participants are requested to use the STR allele nomenclature as described in the ISFG guidelines [1-4]. Allele numbers should not be rounded and should be given with a 1bp precision. Giving the amplicon length in bp alone cannot be accepted and this will be regarded as incorrect result (group IV).

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

Off-ladder alleles or other allelic variants will be sequenced prior to being used in the GEDNAP proficiency test by the organizers.

One mixed stain per proficiency test is provided for biostatistical analysis, and a certificate is provided when correct results are submitted. The exact task description is included with the samples. The samples must be typed correctly by the participant and the calculation should follow the guidelines of the Stain Commission [5]. Common allele frequencies will be provided to the participants and must be used for the calculations. The use of a suitable software for mixture interpretation is allowed. The calculation must be done without Theta correction for ease of comparability.

2.2.5 Returning results

Participants are requested to return the results by the closing deadline at the beginning of December of each year (the exact date will be announced) to allow the organising laboratory sufficient time to evaluate and present the results at the Workshop in February of the following year.

For evaluation and certification it is obligatory to include original laboratory data, i.e., copies of the electropherograms of the samples *and* the allelic ladders.

Participants submit their results on the internet (www.gednap.org). The details (i.e., login, password) are sent by e-mail to the participants. After entering the results the participants must print the data forms and return them signed and stamped (the website allows to create a pdf file) together with the original laboratory data by regular mail to the organizer, the Institut für Forensische Genetik Münster. The use of other result forms is prohibited; an evaluation cannot be carried out.

3. Results

3.1 Error sources

The submitted results are checked by the organiser, and compared with the original data. This enables to identify most sources of errors.

In the past, the most frequent sources of errors have been observed as described below:

- poor quality of the electropherogram
- overinterpretation of stutter peaks
- overinterpretation of very weak peaks
- incorrect assignment of alleles to the allele ladder
- transcriptional errors

Due to the fact that the detection limit has been reduced in recent years due to the development of more sensitive typing kits, the Stain Commission has decided that alleles constituting $\geq 15\%$ in mixtures must be detected and reported.

3.2 Criteria for classifying accuracy of results

After checking all results, each individual result for each STR system and for each stain is categorised according to the following criteria. Note that these criteria were modified a few years ago:

Group I:

- The participant obtained correct results.

Group II:

- An allele in a mixture was not detected/reported but constituted at least 15% of the total amount of all mixture components, and was detected by less than 90% of the participants.
- An allele was not detected/reported, and more than 90% of the participants did not report this allele correctly.
- No result was reported for individual STR systems of a stain sample, if correct results were reported for this sample by less than 90% of the participants.

Group III:

This group has been removed. The option “reportable” or “no reportable results” is no longer available.

Group IV:

- All incorrect results.
- An allele in a mixture was not detected/reported but constituted at least 15% of the total amount of all mixture components, and was detected/ reported by more than 90% of the participants.
- An allele in was not detected/reported, and more than 90% of the participants did report this allele correctly.
- No result was reported for an individual STR system of a stain sample, although more than 90% of the participants did report results for this sample.

Only **group IV** errors are treated as real errors regarding the final evaluation. In other words, the affected STR system is excluded from the list of successfully analyzed systems on the certificate.

3.3 Informing participants of results

The results are presented at the Spurenworkshop by the GEDNAP organizers announce the results in the form of an oral presentation. The various error classes are explained and the laboratory code numbers where errors have been made are given, usually together with examples (display of original data/electropherograms), so that the audience has the opportunity to understand the problem, and to receive guidance for avoiding such errors. After the presentation each laboratory receives a copy of the relevant evaluation tables displaying errors and correct results.

Laboratories have the opportunity to appeal an error decision if they feel that their results have been incorrectly classified or if they have been unfairly treated. If a participant requests that the sample in question be retested, the following procedure can be 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 upon request).
2. The selected member of the commission should be from an academic institute of legal medicine if the participant is a government laboratory, and vice versa.
3. A private laboratory has a choice between these two options.
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.

The organizer, the Institut für Forensische Genetik Münster, retains a number of original samples that can be provided if necessary.

In addition, in case of an error there is the opportunity to request the organizer to provide a new set of similar samples at cost. If correct results are reported

for these samples (usually three) the certificate (see below) can be changed accordingly.

4. Certification

A certificate is issued by the organising laboratory in which it states that the laboratory in question has successfully completed the proficiency test for the specified STR systems. Certificates of participation will be issued for the Institute which has actually performed the laboratory analysis. Any investigation by a substitute or affiliated laboratory not disclosed to the organizer is not valid. False results (i.e., errors) are not explicitly named but the affected system(s) is/are not included in the list. The certificates will be reviewed and signed by the Technical Director of the GEDNAP Proficiency Tests and the Chairman of the GEDNAP Proficiency Testing Program.

All the supporting paper documentation sent to the organising laboratory will be destroyed one month after the certificates have been sent out unless the participant has asked for its return. The participating laboratory is responsible for archiving and storage of the submitted data for an as yet undefined period of time.

5. Future developments

At present, STR systems form the backbone of the GEDNAP proficiency test and are expected to do so for some time to come. Newly developed and/or possible candidate markers for inclusion in the proficiency test system will be considered by the Stain Commission and a decision will be reached after consultation with the participants.

In 2005 a certification of mtDNA sequencing of reference samples was offered for the first time. It became obvious that different thresholds for the report of heteroplasmic nucleotide positions exist and that there is a demand for harmonisation of the nomenclature. The evaluation of the received data is based mainly on the nomenclature rules set by the EMPOP database⁴ [6].

Up to now the organising laboratory has also participated in the proficiency test. 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(s) not involved in the preparation of the proficiency test samples. The report on the GEDNAP proficiency testing programme by B. Budowle (FBI) suggested that in addition an independent governmental laboratory sends other unknown samples to the organising laboratory for testing in a similar way to the official proficiency test. The Stain Commission adopted this proposal and it was unanimously decided that additional samples would be provided to the organising laboratory by Dr. Bastisch (KT31, BKA Wiesbaden) in a similar fashion and in the same period as the common proficiency test samples. These samples would then be tested and evaluated using the same criteria as employed in the common proficiency test. The organising laboratory also participates in the other proficiency tests (e.g., organized by the Deutschen Gesellschaft für Abstammungsbegutachtung, DGAB⁵) so that the quality of results produced in the organizing institute is also open to official scrutiny from external sources.

⁴ www.empop.org

⁵ www.dgab.org

6. Summary

In the past, there have been many changes in the selection and implementation of several generations of DNA systems, while the general scheme of the proficiency testing has been maintained. 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 stain workshop, has proved to be a successful combination and will be maintained as long as agreed with all participants.

7. Review of the GEDNAP proficiency testing programme

In November 2000, a review of the GEDNAP procedure was carried out by Dr. B. Budowle (then employed at the FBI) as part of an overall review of the database 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, Prof. Dr. Kube.

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 proficiency test system. Every change of this document will be made available to the participants via the GEDNAP website.

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9. Appendix

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