GEDNAP · c/o Institut für Forensische Genetik GmbH · Im Derdel 8 · 48161 Münster

August 07, 2020

**GEDNAP 60 & 61**

Dear colleague,

Please find enclosed the samples for the GEDNAP Proficiency Tests 60 and 61, and some important explanations, instructions and conditions. Please read them carefully and forward them also to the person(s) carrying out the practical steps of the analyses.

This information will also be available as well as the letter from the Stain Commission concerning the 'Rules for publicising the participation in the GEDNAP Proficiency Tests' and the associated declaration to be signed by the authorised participant in the „Download service center" section of the GEDNAP Homepage:

https://www.gednap.org/gednap-proficiency-tests/download-servicecenter/

Please return this document stamped and signed by an authorized person until **04 December 2020** (date of postmark). For further details see para IV and VII.

If you have any queries or problems, please do not hesitate to contact us.

Sincerely

**Dr. rer. nat. C. Hohoff**

Executive Director of the
GEDNAP Proficiency Tests

**Prof. Dr. med. B. Brinkmann**

Chairman of the GEDNAP
Proficiency Testing Program

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## I. Notes on the Samples:

The proficiency tests are composed of three reference samples and four stains:

GEDNAP 60: Person A – C; stain 1 – 4
GEDNAP 61: Person A – C; stain 1 – 4

GEDNAP participants that have registered for the module *age estimation* will receive in addition Person D – F (see VI.).

GEDNAP participants that have registered for the module *extraction efficiency* will receive in addition stain 5 (see VI.).

Please bear in mind that any of the stains could consist of the DNA of a single person ("single source stain") or of the DNA from up to 3 different persons ("mixed stain"), and that the stain material might consist of saliva, blood or semen (as well as mixtures of these materials). In principle, the stains in these Proficiency Tests could simulate any stains encountered in routine casework.

N.B.: Each participating laboratory must retain some material from every stain (except for stain 5) to allow a reanalysis if necessary.

## II. DNA loci that may be included in the certificate(s) for GEDNAP 60 and 61:

*Please note that compared to previous years there might occur changes of the allele ranges due to the launch of new available kits.*

Tab. 1: autosomal core STRs and Amelogenin *- allelic ranges

| locus | TH01 | VWA | FGA | D21S11 | ACTBP2 | D3S1358 | D8S1179 | D18S51 | D16S539 |
|---|---|---|---|---|---|---|---|---|---|
| allele range* | 2-14.3 | 9-25 | 12-34.2, 41.2-52.2 | 23-39 | 3.2-43, 48-50 | 8-21 | 4-20 | 6-28 | 3-17 |

| locus | D2S1338 | D19S433 | D12S391 | D2S441 | D10S1248 | D22S1045 | D1S1656 | Amelogenin |
|---|---|---|---|---|---|---|---|---|
| allele range * | 9-29 | 4.2-20.2 | 13-28 | 7-18 | 7-20 | 6-21 | 8-21.3 | X/X; X/Y |

Tab. 2: supplementary autosomal STRs *- allelic ranges

| locus | TPOX | CSF1PO | D5S818 | D13S317 | D7S820 | Penta D | Penta E | D6S1043 |
|---|---|---|---|---|---|---|---|---|
| allele range * | 3-17 | 4-17 | 5-19 | 4-18 | 4-17 | 1.2-18 | 4-25 | 6-26 |

Tab 3: Y-STRs *- allelic ranges

| System | DYS19 | DYS385 | DYS389I | DYS389II | DYS390 | DYS391 | DYS392 | DYS393 | DYS437 |
|---|---|---|---|---|---|---|---|---|---|
| allele range * | 8-20 | 5-29 | 8-18 | 23-36 | 16-30 | 4-17 | 3-21 | 6-19 | 9-19 |

| System | DYS438 | DYS439 | DYS448 | DYS449 | DYS456 | DYS458 | DYS460 | DYS481 | DYS518 |
|---|---|---|---|---|---|---|---|---|---|
| allele range * | 5-17 | 5-18 | 13-25 | 21-41 | 9-25 | 9-25 | 6-15 | 16-33 | 31-50 |

| System | DYS533 | DYS549 | DYS570 | DYS576 | DYS627 | DYS635 | DYS643 | GATAH4 | DYF387S1 |
|---|---|---|---|---|---|---|---|---|---|
| allele range * | 6-18 | 6-18 | 9-27 | 9-26 | 10-28 | 14-31 | 5-18 | 7-19 | 29-45 |

Tab. 4: additional autosomal STRs #*- allelic ranges

| System | D2S1360 | D3S1744 | D4S2366 | D5S2500 | D6S474 |
|---|---|---|---|---|---|
| allele range * | 18-33 | 12-22 | 8-16 | 8-19 | 12-20 |

| System | D7S1517 | D8S1132 | D10S2325 | D21S2055 |
|---|---|---|---|---|
| allele range * | 15-29 | 11.1-28 | 5-20 | 15.1-40 |

Tab. 5: X-STRs [#] [*] - allelic ranges

| locus | DXS8378 | HPRTB | DXS7423 | DXS7132 | DXS10134 | DXS10074 | DXS10101 |
|---|---|---|---|---|---|---|---|
| allele range * | 7-16 | 7-18 | 11-19 | 9-18 | 27-45.3 | 3-22 | 20-36 |

| locus | DXS10135 | DXS10079 | DXS10103 | DXS10148 | DXS10146 |
|---|---|---|---|---|---|
| allele range * | 12-40.2 | 13-26 | 14-23 | 12.3-34.1, 37.1-39.1 | 20-48.2 |

Notes on the tables 1 - 5

*: the numbers indicate the allelic range in which the classification of alleles must be made.
for further explanations see para **VII**.

#: the certification of these markers is not carried out in conjunction with the Stain Commission

6. mtDNA

The sequence range np 16024 – 576 will be evaluated. If a participating lab will analyze a differing sequence range (e.g., np 16024-16365, 73 – 340 and/or 438 – 576), we will evaluate the results in these ranges and these ranges will be indicated in the certificate.

## III. Biostatistical calculation

In the course of both GEDNAP Proficiency Tests GEDNAP 60 and 61 a paper challenge will be conducted for the biostatistics module. The necessary DNA typing results will be sent by eMail. In case of a mixed stain please use **both** calculation methods that are recommended by the German Stain Commission (P. Schneider et al. (2009) Int J Legal Med 123:1–5), i.e., LR and RMNE. Please use only the allele frequencies that were observed in the course of an ENFSI population study (source: "Europe" in STRidER_frequencies_2019-08-02, https://STRider.online). They can be

downloaded from the GEDNAP website (http://www.gednap.org). For rare alleles please follow the recent recommendations (W. Ulbrich et al. (2016) Rechtsmedizin 26: 291-298):

The frequency of rare alleles ($f_{min}$) shall be estimated according to the NRC report (1996). If the estimated $f_{min}$ is below 0.001, one shall use the value 0.001.

There will be a particular module for *probabilistic genotyping* in 2020. A paper challenge for the biostatistical interpretation of a mixed stain that might show *drop in* and/or *drop out* events will be conducted. The participant receives digital documents (e.g., .fsa files, DNA profile of a person of interest), analyzes the findings by application of a software that follows a fully continuous model, and later uploads the results (LR, deconvoluted DNA profiles of mixture components) in addition to proper documentation.

The calculation steps must be documented by a print-out. The employed software version must be named. Please note that the calculations have to be executed without the correction factor 'theta' (i.e. with a theta value of zero).

## IV. Instructions for submitting the results

- To submit your results (e.g., stain characterization, extraction details, genotyping, mixed-person stain calculation), please exclusively use the web forms on the GEDNAP homepage (https://gednap.qualitype.de). The submission option will be deactivated on the deadline of **04 December 2020 at 23:59 CET**. Detailed information as well as your login and password have already been provided to you in separate emails.

- After submitting your results electronically we request you to send a signed and stamped printed copy (the website allows you to create a PDF file), which has to be posted to us together with your original laboratory data (e.g., print outs of the relevant electropherograms) as well. The deadline is the **04 December 2020** (date of postmark).

- Also for the submission of your mtDNA results please use the form on the GEDNAP homepage (https://www.gednap.org). As in previous years, you are requested to score the differences of the sequences of Persons A-C and single source stains to the revised Cambridge Reference Sequence (rCRS) using the nomenclature recommendations of the DNA commission of the ISFG (W. Parson

et al. 2014, PMID: 25117402). In the case of a point heteroplasmy the np shall be reported according to the IUPAC code, provided the minor component represents at least 20 %. In the case of a length heteroplasmy, „LHP“ shall be reported in the field ‘remarks‘; the shorter variant shall be named if there are two dominant types. The second dominant variant shall be reported in the field ‘remarks‘ as well.

## V. Module Extraction Efficiency

In the course of both Proficiency Tests GEDNAP 60 and 61 a fifth stain will be sent out in triplicate for the module “extraction efficiency”. The participant shall extract the **three sub-samples (stain 5.1, 5.2 and 5.3) of each proficiency test separately by the same methodology** without prior presumptive testing **and return the three DNA extracts per proficiency tests** at his own expense and on his own responsibility to the IFG Münster (within 6 weeks after receiving of the samples but no later than **22 November 2020**). The postal address is as follows:

Institut für Forensische Genetik GmbH
GEDNAP XF
Im Derdel 8
48161 Münster
Germany

The choice of transport is left to the participant (on ice, frozen, lyophilized with or without a stabilizer). The IFG asks for details of the extraction techniques (i.e., analytical platform/hardware & chemistry [if applicable], elution buffer and elution volume) and performs the DNA quantitation by real-time PCR. The individual reporting will be provided in conjunction with the other report forms in February 2020.

## VI. Modules for forensic DNA phenotyping

In the course of the GEDNAP Proficiency Tests GEDNAP 60 und 61 there will be two new modules entitled „Pigmentation Analysis“ and „Age Estimation“. While for „Pigmentation Analysis“ the reference samples A – C are to be investigated, for the „Age Estimation“ module there will be three extra blood samples (D – F) that contain a sufficient amount of nuclear DNA. While for „Pigmentation Analysis“ 41 established markers can be investigated (see https://hirisplex.erasmusmc.nl/), the markers for the module „Age Estimation“ have not been specified.

## VII. General Information

- Please enter only numerical allele values in the web-based results' forms; we would consider any other character (e.g., OL, F, ?) as an error, except for < and > (see below).

- 'Off-category' alleles, i.e. those alleles that are smaller than the smallest allele or longer than the longest allele in the STR systems listed in table 1 - 5, can be reported using the "smaller than" (<) or "greater than" (>) signs relative to the shortest or longest allele. Example: allele 18 at TPOX can be given as '**>17**' or as '**18**' – both would be considered correct. Please note that allele numbers must be given with a 1bp precision (this does not however mean that the example allele above should be scored as *18.0*). Allele designations not adhering to these instructions will be considered erroneous.

- For evaluation and certification it is obligatory to include original laboratory data, i.e., copies of the electropherograms of the samples **and** the corresponding allelic ladders. The allele scoring must be readily visible and unambiguous, and amplicon lengths and peak heights must be readable. The printed copies must be clearly marked with the Proficiency Test series (GEDNAP 60 or GEDNAP 61, respectively), with the sample name and with its laboratory code (e.g. G60_987). Printed sequence electropherograms must be labelled likewise, and the evaluated range must clearly be indicated by the nucleotide positions (np). The relevant np must be indicated. Furthermore, the steps from the electropherogram to the scoring as deviation(s) from the rCRS must be documented (among other things by mentioning the software for generating the consensus sequence from sequencing both strands). Examples of a GeneMapper analysis as well as an exemplary print-out of a sequencing electropherogram with proper labels are available upon request.

- If the original laboratory data are not included in the submission, the results will not be evaluated and subsequently a certificate will not be issued.

- If you wish to send your original data in digital form (e.g. USB stick, DVD/CD-ROM, e-mail attachment) please ensure that the files are clearly labelled and comprehensible. Electropherograms have to be sent as PDF files. Please provide your user name (also known as lab code)

- Certificates of participation will be issued for those modules for which you have registered (stain characterisation, common and supplementary STR loci, Y-STRs, sequence analysis of the mtDNA control region, biostatistics of mixed-person

stains, additional autosomal STRs and X-STRs, the last two modules being evaluated and certified without involvement by the Stain Commission).

- Certificates of participation can be issued only in the name of the institute which has actually undertaken the analysis. An analysis by a third party is not permissible. In accordance to the Stain Commission ruling, all participants have to sign a self-declaration stating that their GEDNAP certification may not be used by third parties, for example for advertising purposes. If this self-declaration has been submitted in the previous year it can be omitted this year. If the self-declaration has not been received by us until **20 December 2020**, we will neither evaluate the results nor subsequently issue the certificates.

- The error categories are defined as in the previous years. Details are given on the GEDNAP website (http://www.gednap.org).

## VIII. Stain Workshop in Bielefeld, Germany

The results of the Proficiency Tests GEDNAP 60 and 61 will be presented during the 41st Stain Workshop in Bielefeld (Germany; February 25 - 27, 2021), organized by Prof. Dr. Carsten Tiemann, LABCON-OWL (https://www.r-km.de/Spurenworkshop/), in conjunction with the German Society of Legal Medicine and the Stain Commission – if applicable during the recent SARV-CoV-2-pandemic.

Oral contributions (also in English) are encouraged.

## IX. Fulfilment of Conditions

The executive of the proficiency tests, who is appointed by the Stain Commission, agrees to provide test samples, evaluate submitted results and to issue certificates if the participating laboratory meets the above conditions and furthermore pays the current participation fee, signs the enclosed self-declaration by an authorized member of the participating laboratory and sends it back to the executive such that it arrives at his address. If any of these requirements is not fulfilled, then the submitted results will not be evaluated and a certificate will not be issued.