



## **CaDNAP Proficiency Tests**

organized by the CaDNAP ISFG working group

## **Manual**

CaDNAP Proficiency Test 2022/2023

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# 1. Introduction

The Canine DNA Profiling (CaDNAP) group was founded in 2003 as a collaboration of the Institute of Legal Medicine, Medical University of Innsbruck (Austria) and the Federal Criminal Police Office, Bundeskriminalamt Wiesbaden, (Germany) to drive international harmonization of canine DNA-analysis and data interpretation. In 2008 the Institute of Veterinary Pathology Justus-Liebig-University, Giessen (Germany) and in 2015 the Institute of Forensic Medicine, University of Zurich (Switzerland) joined the group. In 2017 CaDNAP was recognized by the International Society for Forensic Genetics (ISFG) as an official ISFG working group. For further information see <https://cadnap.org/> and <https://www.isfg.org/Working+Groups/CaDNAP>.

## 1.1 Basic principles

Forensic molecular genetic methods provide evidence in a legal framework. Therefore, laboratories conducting forensic casework bear responsibility to comply with high standards required in the forensic field. Proficiency tests are a fundamental pillar for achieving and maintaining accreditation according to international standards, e.g. ISO 17025. The outcomes of the proficiency tests are an essential part of the internal quality control management of a laboratory and can provide an objective proof of the expertise of the participating laboratory. The participants are encouraged to self-critically review their methods, the obtained results and their laboratory organization.

Laboratories participating in proficiency testing need to make sure that their submitted analysis results are correct and meet the accepted quality standards. Organizers of proficiency testing programs are required to generate and distribute testing material (samples) of as identical as possible quality and quantity for examination by the participating laboratories in order to enable a direct comparison of the obtained experimental results.

The following criteria should be reviewed by a proficiency test:

- accuracy of the results
- precision of the results
- concordance of the results by inter laboratory comparisons

Furthermore, proficiency tests should not only examine the applied methodologies, but also review the analysis and the correct documentation of the data provided by the participating laboratories. Planning, realization and evaluation of the CaDNAP proficiency test are based on the same principal design as the GEDNAP proficiency tests, which is why this manual orients itself on the GEDNAP manual (Hohoff et al. 2013).

Consequently, the concept of the CaDNAP proficiency tests is based on following questions:

- Has the laboratory examined the correct sample?
- Has the laboratory achieved the correct experimental result?
- Has the laboratory interpreted the experimental result correctly?
- Was the result properly documented and correctly communicated to the organizer of the proficiency test?

Before employing a new methodology in forensic casework extensive validation is indispensable. The Short Tandem Repeat (STR) markers and the mitochondrial DNA (mtDNA) regions covered

by the CaDNAP proficiency test have undergone validation processes. For the canine STR markers and the two sex-specific markers (**Table 1**) a validation study was conducted (Berger et al. 2014) according to the ISFG recommendations (Linacre et al., 2011). The sequencing of the canine mitochondrial D-Loop has not been validated according the ISFG recommendations but several mitochondrial DNA sequencing strategies for forensic purposes, including internal validation studies were published (e.g., Van Asch B et al. 2009, Eichmann C, Parson W. 2007, Pereira L, Van Asch B, Amorim A. 2004, Wetton JH et al. 2003).

STR markers					Sex markers	mtDNA regions
C38	FH2054	FH2087ub	FH2137	FH2328	Amelogenin	HVS-I (15458-16129)
FH2611	FH2613	PEZ15	PEZ3	PEZ6	SRY	HVS-II (16430-16727)
FH2508	FH2361	WilmsTF				

**Table 1:** Autosomal STR loci, sex-specific markers and mtDNA regions covered by the CaDNAP proficiency test. Position numbers of the canine mitogenome are listed in brackets relative to the canine reference sequence (Kim et al. 1998)

The CaDNAP proficiency tests provide an external quality control and quality assurance scheme for forensic laboratories involving the following principles:

## 1.2 Aims

The main objectives of the CaDNAP proficiency tests are:

- The verification of the expertise of the participating laboratory to assess experimental results
- Identification of sources of error to minimize/eliminate errors

## 1.3 Information of the current offers of the CaNDAP proficiency test

Only dog samples and the above mentioned STR and sex markers as well as the mitochondrial DNA control region are offered for analyses and certification within the current CaDNAP proficiency testing program. At this stage, other animal species or further canine STR markers cannot be taken into the evaluation process.

# 2. Structure

## 2.1 Participants

The CaDNAP proficiency tests are open to every laboratory, including academic, governmental private and commercial partners.

## 2.2 Planning and coordination

The CaDNAP proficiency tests are organized by the CaDNAP group and are currently conducted in a bi-annual manner. The planning and the coordination of the proficiency tests are coordinated by the executive laboratory [see 2.3] and are offered in the year preceding a CaDNAP ISFG working group meeting. The result of the proficiency test will be presented to the participants at the next meeting of the CaDNAP-ISFG working group, where participants are invited to provide comments and offer suggestions.

## 2.3 Execution

Each participating laboratory receives two samples, originating from two different dogs. These two samples can derive from different source tissues (e.g. blood, saliva). The participating laboratories are free to choose among the canine DNA markers included in Table 1, but are asked to analyze at least 10 autosomal markers and 1 sex-specific marker for successful participation. For participation in mtDNA proficiency testing at least the HVS-I region (15458-16129) within the CR (15458-16727) is required.

The address of the executive laboratory and contact person for the CaDNAP proficiency tests can be found on the ISFG webpage <https://www.isfg.org/Working+Groups/CaDNAP>.

## 2.4 Proficiency Test commission

The CaDNAP group is striving for a maximum degree of fairness and integrity. Therefore, one representative of each CaDNAP founder Institute is nominated to join a proficiency test commission that aids solving issues that may arise in the course of the proficiency tests. Current nominees are:

Institute of Legal Medicine, Medical University of Innsbruck, Austria: Dr. Burkhard Berger

German Federal Criminal Institute, Wiesbaden, Germany: Dr. Uwe Schleenbecker

## 2.5 Registration

The proficiency tests will be announced every second year through the CaDNAP and ISFG websites. The required registration and reporting forms can be found here: <https://www.isfg.org/Working+Groups/CaDNAP>. Laboratories can register for the proficiency test by submitting the registration form (7.2) to the proficiency executive (see 2.3). The registration deadline is the October 31<sup>st</sup> of the year, in which the proficiency test was announced. Every laboratory has to report a contact person and the tax number of the Institute during registration. The shipment of the samples to the recipient will take place upon payment of the registration fee at the latest until December 31<sup>st</sup> of the said year.

Upon registration, each laboratory has to specify, which of the proposed canine markers will be typed:

1. Autosomal STR markers
2. Sex-specific markers: Amelogenin, SRY
3. Mitochondrial DNA regions

For further information see registration form (section 7.2).

## 2.6 Preparation and distribution of samples

The two test samples are prepared independently from each other. Prepared samples are dried overnight at room temperature. Further details regarding the sample preparation:

1. Blood sample collection: blood is collected in sterile citrate, heparin or EDTA containers.
2. Blood sample on carrier: identical volumes of well-mixed blood are applied on the carriers with a calibrated pipette.
3. Saliva samples, respectively buccal mucosa swabs: buccal mucosa cells are collected directly from the dog using buccal swabs.

4. Samples are preferably prepared in a way, that there is sufficient blank carrier present, to be used as a substrate control. If this is not possible, an additional blank carrier is sent alternatively.

Subsequently, the samples are placed in separate, adequate containers (usually parchment paper bags). For each of the participating laboratories a sample set is assembled by one person, checked for completeness and correctness by a second person, and packed in an envelope. The name and the address of the participating laboratory are added on the envelope and the envelope is sent to the recipient.

## 2.7 Typing of samples

Participating laboratories are expected to follow the international guidelines (Budowle et al. 2005, Linacre et al. 2011, Berger et al. 2014) for DNA typing and to include essential quality controls throughout the analysis. For DNA extraction as well as for amplification, a negative and a positive control should be included. For a possible second opinion testing, participating laboratories need to make sure to retain an adequate part of the sample. This procedure can solve doubts on the identity of a sample and clarify possible contamination prior to the sample being received by the participant.

### STR-Analysis:

STR-typing by capillary electrophoresis requires an internal size standard and an allelic ladder in order to unambiguously call the observed alleles. An aliquot of the canine positive control and allelic ladders are available from the CaDNAP group on request.

- The STR allele nomenclature described by Berger et al. 2014 applies.
- The number of repeats of an allele should not be rounded. Instead, alleles have to be reported with single base-pair precision. Rules and conventions established for human-specific STR typing are also valid for canine-specific STR analysis (Bär et al. 1997, Gill et al. 1997). Reporting amplicon sizes is not considered valid. Deviations from these guidelines will be regarded as incorrect results. Please enter only numerical allele values in the results forms, any other character (e.g. OL, R, ...) is considered as an error.
- Alleles outside of the specified marker range ("off-category") as defined in section 7.1 have to be reported using ">" (greater than) or "<" (smaller than) the longest or shortest allele of the specified marker range. For example, the off-category allele 25 at marker PEZ6 (allelic range from 14 to 23) is reported as ">23" In contrast, reporting the PEZ6 allele 20 as "<23" would be regarded as an error.

### MtDNA-Analysis:

The Control Region (CR) of the canine mtDNA genome consists of two hypervariable segments (HVS-I and HVS-II) separated by a variable number of tandem repeats (VNTR) region. The VNTR region is disregarded for forensic interpretation due to its high intra-individual variability and its complex interpretation (Eichmann et al. 2007). This also applies to proficiency test regulations.

- Typing and reporting of mtDNA haplotypes should be performed in agreement with the guidelines for human mtDNA typing (Parson et al. 2014).

- The sequences should be reported relative to the reference sequence NC\_002008.4 (Kim et al. 1998) and should include the interpretation range for HVS-I (15458-16129) and optionally HVS-II (16430-16727, excluding primer sequence information).
- Differences to the reference sequence should be reported noting the respective position and the variant with the reference base in preceding position (e.g. C15526T). Insertions and deletions should be reported following the canine mitochondrial phylogeny and in case of doubt at the 3' end with respect to the light strand. Insertions should be indicated by ".x" (e.g. - 15464.1C). Deletions should be indicated by "DEL" or "del" (e.g. A15931DEL).
- Reported consensus sequences must be based on redundant sequence information, using forward and reverse sequencing reactions whenever practical. In those cases, for which forward and reverse coverage are not possible, replicate coverage from the same strand, preferably obtained from different primers, is acceptable.
- Mixtures of nucleotides (e.g. point heteroplasmy) should be called according to the International Union of Pure and Applied Chemistry (IUPAC) code (Cornish-Bowden A. 1985, Johnson AD. 2010, e.g. C15526Y). The IUPAC code uses capital letters, which allows for the necessary extension of the existing nomenclature to small letters for describing (heteroplasmic) mixtures of deleted/undeleted and inserted/non-inserted bases, respectively (e.g. A15931a, which denotes a mixture of an A and the deletion at 15931). Length heteroplasmy in homopolymeric sequence stretches should be interpreted by calling the dominant variant, which can be determined by identifying the position with the highest representation of a non-repetitive peak downstream of the affected stretch. N-designations should only be used when all four bases are observed at a single position (or if no base call can be made at a given position). The minor component at a heteroplasmic position should only be indicated if it has a proportion of at least 20%. If length heteroplasmy was detected, please indicate it in the field "comments".

## 2.8 Returning results

Participants have to return their results until April 30<sup>th</sup> in the year following the CaDNAP proficiency test registration. For evaluation and certification, it is **obligatory** to include original laboratory data **electronically**. PDFs of the raw data (electropherograms) of the samples and the allelic ladders should be provided **electronically** for the autosomal and sex-specific markers. The allele scoring must be readily visible and unambiguous, and amplicon lengths and peak heights must be readable. MtDNA electropherograms should mark and name the observed variants. All these documents have to be sent **electronically by mail** to the organizer of the proficiency tests (2.3). For reporting the results, the specified result returning forms (see 7.3 autosomal marker analysis, 7.4 mtDNA analysis), provided by CaDNAP, have to be used. These EXCEL tables have to be sent electronically as .xls and PDF files including the validation date as well as the signature of the responsible participant. Other reporting forms are not accepted, and will not be evaluated. The participating laboratories are responsible to store and archive the submitted data.

## 3. Results

### 3.1 Possible causes of mistakes

The organizing laboratory reviews the submitted data, and compares the reported results with the submitted original laboratory data. Potential causes of errors can be assigned to:

- Low quality of the raw data
- Mis-interpreted stutter peaks
- Mis-interpreted weak peaks/artefacts
- Incorrect assignments of alleles to the allelic ladder
- Transcriptional errors
- Incorrect mtDNA reference sequence or nomenclature

### 3.2 Evaluation of reported results

#### STR-Analyses

Divergences in the reported STRs results could consist of wrong interpretation (e.g. improperly called alleles, reported alleles that lack raw data or artefacts that were reported as alleles).

#### MtDNA-Analyses

Divergences in the reported mtDNA results could consist of incorrectly called nucleotides in a non heteroplasmic region. The nucleotides at heteroplasmic sites have to be called according to the IUPAC code. If more than one “N” in a row is reported; it will count as a divergent result. Heteroplasmic regions are assessed as correct, if at least one of the reported nucleotides is identical to the nucleotides of the target sequence (e.g. target sequence nucleotide: G, reported nucleotide: R).

### 3.3 Certification

For each participating laboratory the organizing laboratory issues a certificate (7.5), which states the successful participation in the CaDNAP proficiency tests. First, all registered markers will be listed in the certificate, second these markers will be reported with a note “correct” or “incorrect”. Certificates are issued for the institution that actually analyzed the canine DNA samples; outsourcing of the analysis is not allowed. The certificates are reviewed and signed by the executive laboratory, respectively by the contact person for the CaDNAP proficiency tests. Certificates will be sent by regular mail to the participants.

Participants have the possibility to contest the validity of the certificate; the objection period starts immediately after the meeting and lasts for 2 weeks. For participants that receive their certificate by mail the incoming post stamp will start the objection period. In case of any disagreement, the stored samples can be retyped by another laboratory appointed by the proficiency test commission. Copies of the original laboratory data are not returned to the participating laboratories.



### 3.4 Proficiency test 2022/2023

The registration for the proficiency test 2022/2023 will start in **July 2022** and will end on **31<sup>st</sup> of October 2022**. Participants are required to report the tax number on registration. The executing laboratory will send an invoice to registered participants in the period from the 1<sup>st</sup> of November 2022 to the 16<sup>th</sup> of November 2022. The shipment of the two samples to the recipient will take place, after receiving payment, but not later than 31<sup>st</sup> December 2022. The results have to be returned until 30<sup>th</sup> of April 2023. The results of the proficiency test will be presented and discussed at the follow-up meeting of the CaDNAP working group at the ISFG conference.

## 4. Costs of participation

The costs of participation are € 350.00 for academic, governmental laboratories and € 750.00 for private/commercial laboratories. The costs are independent of the number of analyzed markers.

## 5. Versions of the CaDNAP Proficiency Test Manual

Version 2016
Version 2017
Version 2020
Version 2022

## 6. Literature

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(*Canis lupus familiaris*) mitochondrial DNA sequences: an inter-laboratory study of the GEP-ISFG working group. *Forensic Sci Int Genet.* Dec;4(1):49-54.

Wetton JH, Higgs JE, Spriggs AC, Roney CA, Tsang CS, Foster AP. (2003) Mitochondrial profiling of dog hairs. *Forensic Sci Int.* 2003 May 5;133(3):235-41.

## 7. Appendix

### 7.1 Allelic ladder range

<b>Autosomal STR marker</b>	<b>Allelic ladder range*</b>
C38	11 to 32.1
FH2054	9 to 18
FH2087ub	7 to 15
FH2137	18 to 27
FH2328	12 to 21
FH2361	13 to 36
FH2508	9 to 14.1
FH2611	14 to 25
FH2613	8 to 28.1
PEZ15	6 to 22.2
PEZ3	21 to 30
PEZ6	14 to 23
WilmsTF	8 to 19.3
<b>Sex-specific marker</b>	
Amelogenin	Y, X
SRY	SRY

\* The numbers indicate the range in which the classification of alleles must be made

## 7.2 Registration form

<b>Institution</b>	
Please indicate	
Commercial <input type="checkbox"/> Non commercial <input type="checkbox"/>	
Tax number:	
<b>Contact person</b>	
Name:	
Email:	
<b>Please specify which markers will be tested</b>	
<b>Autosomal STR marker</b>	Mark with a cross
C38	
FH2054	
FH2087ub	
FH2137	
FH2328	
FH2361	
FH2508	
FH2611	
FH2613	
PEZ15	
PEZ3	
PEZ6	
WilmsTF	
<b>Sex-specific marker</b>	Mark with a cross
Amelogenin	
SRY	
<b>Mitochondrial DNA</b>	Mark with a cross
HVS-I	
From 15458	
To 16129	
HVS-II	
From 16430	
To 16727	

### 7.3 Returning results form for autosomal STR markers and sex-specific markers

<b>Institution</b>				
<b>Contact person</b>				
Name:				
Email:				
<b>Autosomal STR marker</b>				
	<b>Sample 1</b>		<b>Sample 2</b>	
	Allele 1	Allele 2	Allele1	Allele 2
C38				
FH2054				
FH2087ub				
FH2137				
FH2328				
FH2361				
FH2508				
FH2611				
FH2613				
PEZ15				
PEZ3				
PEZ6				
WilmsTF				
<b>Sex-specific marker</b>				
	<b>Sample 1</b>		<b>Sample 2</b>	
	Allele 1	Allele 2	Allele1	Allele 2
Amelogenin				
SRY				

#### 7.4 Returning results form for mitochondrial DNA (Control Region)

Institution	
Contact person	
Name:	
Email:	
Mitochondrial DNA	
Sample 1	Sample 2
Analysis range	Analysis range
Variants <sup>1</sup>	Variants <sup>1</sup>
Comments:	

<sup>1</sup>To report the deviations from the reference sequence (NC\_002008.4, Kim et al., 1998), the nucleotide present in the reference sequence is written in front of the position of the variant, and the nucleotide of the variant is written after the position (e.g. C15526T). If possible, insertions and deletions shall be reported phylogenetically and in case of doubt of the position, they shall be added at the 3' end. Nucleotides at heteroplasmic sites have to be called according to the IUPAC code (e.g. C15526Y).

## 7.5 Certificate



## Certificate

**CaDNAP proficiency test 20xx**

The laboratory xxxxxxxxxxxxxxxx participated the CaDNAP proficiency test(s) 20xx.

**The canine STR proficiency test** included the following markers:

FH2054, FH2087ub, FH2137, FH2328, FH2611, FH2361, FH2508, FH2613, PEZ3, PEZ6, PEZ15, C38, WilmsTF, Amelogenin, SRY

The correctness or incorrectness of the analyzed and reported results is listed for the following canine markers:

<b>FH2054</b>	<b>correct</b>	<b>FH2361</b>	<b>correct</b>	<b>PEZ15</b>	<b>correct</b>
<b>FH2087ub</b>	<b>incorrect</b>	<b>FH2508</b>	<b>correct</b>	<b>C38</b>	<b>correct</b>
<b>FH2137</b>	<b>correct</b>	<b>FH2613</b>	<b>correct</b>	<b>WilmsTF</b>	<b>correct</b>
<b>FH2328</b>	<b>correct</b>	<b>PEZ3</b>	<b>correct</b>	<b>Amelogenin</b>	<b>correct</b>
<b>FH2611</b>	<b>correct</b>	<b>PEZ6</b>	<b>correct</b>	<b>SRY</b>	<b>correct</b>

Date and Signature



# Certificate

## CaDNAP proficiency test 20xx

The laboratory xxxxxxxxxxxxxxxx participated the CaDNAP proficiency test(s) 20xx.

**The canine mitochondrial DNA proficiency test** included the following mitochondrial sequence:

HVS-I 15458-16129, HVS-II 16430-16727

The correctness or incorrectness of the analyzed and reported results is listed for the following ranges of canine mitochondrial DNA

HVS-I	15458-16129	correct
HVS-II	16430-16727	incorrect

Date and Signature