Canine DNA profiling group

CaDNAP Proficiency Tests

organized by the CaDNAP ISFG working Group

Manual

4. CaDNAP Proficiency Test 2018/2019

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Contents

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 German Federal Criminal Police Office (Bundeskriminalamt Wiesbaden [BKA]) to drive international harmonization of 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 the group was officially recognized by the ISFG as an ISFG working group. For further information see http://gerichtsmedizin.at/cadnap.html and https://www.isfg.org/Working%20Groups.

1.1 Basic principles

Forensic molecular genetic methods provide evidence for legal framework; therefore laboratories conducting forensic casework bear responsibility to comply high standards in accuracy and precision. Performance tests like proficiency tests provide a basis for accreditation procedure according to international standards, e.g. ISO 17025. The outcomes of the proficiency tests are an essential part of the internal quality management of a laboratory and can give an objective proof of the expertise of the participating laboratory. The participants are encouraged to self critically review their internal methods, the obtained results and their laboratory organization.

Laboratories participating in a proficiency test need to make sure that their submitted analysis results are correct and meet accepted quality standards. Especially important is this for laboratories, which work with forensic casework samples, because the results of such an analysis can have consequences for the public. The main precondition for an appropriate proficiency test is the distribution of exactly the same material to be tested to the participating laboratories, to enable a direct comparison of the obtained experimental results.

Following criteria should be reviewed by a proficiency test:

- accuracy of the results
- precision of the results
- reproducibility, by inter laboratory comparisons

Furthermore, proficiency tests should not only review the applied methodologies, but also the analysis, and the correct documentation of the data, done 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. Therefore, this manual is strongly oriented 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 obtained result correctly?
- Were the results properly documented and correctly communicated to the organizer of the proficiency test?

Before employing a new methodology in forensic casework an extensive validation study has to be conducted. The STR markers and the mitochondrial DNA markers used in the CaDNAP proficiency test have undergone such a validation step. For two sets of canine genotyping markers including two sex specific markers (**Table 1**) a validation study was done by Berger et al. (2014) according to the ISFG recommendations (Linacre et al., 2011). An additional marker set was validated by Hellmann et al. (2006). Forensic sequencing of the canine mitochondrial D-loop was done by Pereira et al. (2004), Eichmann and Parson (2007), Gundry et al. (2007) and Webb and Allard (2009).

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

Table 1: Autosomal STR markers, sex specific markers and mitochondrial DNA markers used for the CaDNAP proficiency test**.** Position numbers (in brackets) are relative to the canine reference sequence (Kim et al. 1998)

The CaDNAP proficiency tests provide an external quality control and quality assurance for laboratories. For the CaDNAP proficiency tests, the basic principles are the same as for any other system of quality control.

1.2 Aims

The main objectives of the CaDNAP proficiency tests are:

- The verification of the expertise of the participating laboratory to assess the experimental results
- Identification of sources of error and thereby the elimination of typing errors

1.3 Information of the current offers of the CaNDAP proficiency test

Only dog samples and the above mentioned STR markers as well as the mitochondrial DNA control region are currently offered for analyses and certification within the CaDNAP proficiency test. Other animal species and further STR marker cannot be currently taken into the evaluation process.

2. Structure

2.1 Participants

The CaDNAP proficiency tests are open to every laboratory, whether it represents a University institute, a governmental laboratory or a private institute.

2.2 Planning and coordination

The CaDNAP proficiency tests are organized by the CaDNAP group and are conducted every two years. The planning and the coordination of the proficiency tests take place at the annual CaDNAP meetings. The outcome of the proficiency test is presented to the participants at the follow-up meeting. At this occasion, participants can express their suggestions, opinions and criticism regarding the CaDNAP proficiency tests.

2.3 Execution

Each participating laboratory receives two samples, originating from two different dogs. These two samples can for example consist of a blood and a saliva sample. The participating laboratories are free to choose the canine DNA markers included in Table 1. The execution of the CaDNAP proficiency tests is currently done by the Institute of Veterinary Pathology, University Giessen, Germany.

Executive laboratory, respectively contact person for the CaDNAP proficiency tests:

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2.4 Proficiency Test commission

The CaDNAP group makes efforts to treat the results with a maximum degree of fairness and a high level of integrity. Therefore, a representative of each CaDNAP founder institute is nominated to form a Proficiency test commission that aids solving issues that may arise from the executive or participating laboratories. 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 (http://gerichtsmedizin.at/cadnap.html/ https://www.isfg.org/Working%20Groups). Laboratories can register for the proficiency test by contacting the responsible person mentioned above (2.3). The registration deadline is the October 31st of the year, in which the proficiency test was announced. Every participant has to report the tax number of the institute on registration. The shipment of the samples to the recipient will take place upon payment of the registration fee at the latest until December 31st .

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

1. Autosomal STR markers
2. Sex specific markers: amelogenine, SRY
3. Sequencing analysis of mitochondrial DNA

For further information see Registration form (section 6.2).

2.6 Preparation and distribution of samples

The two test samples are prepared independently from each other, by pipetting the liquids on the particular carrier or letting the carrier absorb the liquids. Prepared samples are dried overnight at room temperature. Further detail regarding the sample preparation:

1. Blood sample collection: Blood is collected into 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 a dog, using buccal swabs.
4. Samples are preferably prepared in a way, that there is sufficient blank carrier present, to be used as a negative control. If not possible, alternatively an additional blank carrier is sent.

Subsequently the samples are placed in separate, adequate containers (usually parchment paper bags). For each of the participating laboratories a sample set is assembled, checked for completeness and correctness by an assistant, 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 for DNA typing and to include essential quality controls throughout the analysis. For the DNA extraction, respectively the amplification procedure, a negative and a positive control should be carried along. For a possible second opinion testing, participating laboratories need to make sure to retain an adequate part of the sample. Therewith doubts of identity of a sample can be cleared, and possible contamination prior to the sample being received by the participant, can be identified.

STR-Analysis:

STR-typing the samples by capillary electrophoresis, requires an internal size standard and an allelic ladder, to unambiguously call the different alleles. A canine positive control and allelic ladders are available from the CaDNAP group on request.

The STR allele nomenclature described by Berger B *et al.* 2014 applies. The number of repeats of an allele should not be rounded; therefore the alleles have to be reported with single bp precision. This does not however mean that an allele should be scored as x.0. Reporting just the amplicon length in bp is not permissible; 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) would be considered as an error.

If there is an allele detected outside of the specified marker range (“off-category”) as defined in section 7.1, the allele has to be reported using the signs “>” (“greater than”) or “<”(“smaller

than") relative to the longest or shortest allele of the specified marker range. For example, the off-category allele 25 at marker PEZ6 (Allel range 14 to 23) is reported as ">23" In contrast, reporting the PEZ6 allele 20 as "<23" would be regarded as incorrect.

mtDNA Typing:

The Control Region (CR) of the canine mtDNA genome consists of two hypervariable segments (HVS-I and HVS-II) separated by a VNTR region (see Table 1). The VNTR region is usually disregarded for forensic interpretation due to its high intra-individual variability (Eichmann et al. 2007).

Typing and reporting of mtDNA haplotypes should be performed in agreement with the guidelines for human mtDNA typing (Pereira et al. 2004 and Parson et al. 2014). The sequences should be aligned and reported relative to the reference sequence NC_002008.4 (Kim et al., 1998) and should include the interpretation range for HVS-I and/or HVS-II and/or CR (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 (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 30th in the year following the registration to the CaDNAP proficiency test. For evaluation and certification it is obligatory to include original laboratory data, i.e., PDFs of the electropherograms of the samples and the allelic ladders. The allele scoring must be readily visible and unambiguous, and amplicon lengths and peak heights must be readable. All these documents have to be sent to the organizer of the proficiency tests (2.3). For reporting the results, the specified result returning forms (see 6.3, 6.4), provided by CaDNAP, have to be used. These EXCEL tables have to be sent electronically to the organizing laboratory; any other reporting forms are not accepted, and will not be evaluated. The participating laboratories are responsible to store and archive the submitted data for an as yet undefined period of time.

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. In that process possible mistakes are found and most of the potential causes of mistakes can be identified:

- Poor quality of the electropherogram
- Over interpreted stutter peaks
- Over 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

If divergent alleles or sequences are reported, the particular systems are excluded from the list of successfully analyzed systems on the certificate. Divergences concerning STRs could consist of improperly called alleles, an allele that was not detected, or an artefact that was reported as an allele.

Divergences concerning mtDNA 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, which states the successful participation in the CaDNAP proficiency tests. The correctly reported systems are specifically listed on the certificate, whereas incorrect results are not explicitly named but the affected system(s) is/are not stated on the certificate. Certificates are issued for the institution that actually analyzed the canine DNA samples; outsourcing of the sample analysis is not allowed. The certificates are reviewed and signed by the executive laboratory, respectively by the contact person for the CaDNAP proficiency tests. The certificate will be handed over after the

presentation of the proficiency test results at the follow-up meeting. For laboratories that cannot attend the meeting, the certificate will be sent by post.

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

3.4 Proficiency test 2018/2019

The registration for the proficiency test 2018/2019 will start in January 2018 and will end on 31st of October 2018. Every participant has to report his tax number on registration. The executing laboratory will send an invoice to registered participants in the period from the 1st of November 2018 to the 16th of November 2018. The shipment of the two samples to the recipient will take place, after receiving payment, latest until 31st December 2018. The results have to be returned until 30th of April 2019. The results of the proficiency test will be presented and discussed at the follow-up meeting (to be determined).

4. Costs of participation

The costs of participation are for non-commercial laboratories € 350.00, whereas for commercial laboratories € 750.00. The costs are independent of the number of analyzed markers.

5. Versions of the CaDNAP Proficiency Test Manual

Version 1 and 2	2016/10/04
Version 3.1	2017/10/12
Version 3.2	2017/11/08
Version 3.3	

6. Literature

Berger, B., Berger, C., Hecht, W., Hellmann, A., Rohleder, U., Schleenbecker, U., & Parson, W. (2014). Validation of two canine STR multiplex-assays following the ISFG recommendations for non-human DNA analysis. [Validation Studies]. *Forensic Sci Int Genet, 8*(1), 90-100. doi: 10.1016/j.fsigen.2013.07.002

Eichmann, C., & Parson, W. (2007). Molecular characterization of the canine mitochondrial DNA control region for forensic applications. *International Journal of Legal Medicine, 121*(5), 411-416. doi: 10.1007/s00414-006-0143-5

Gundry, R. L., Allard, M. W., Moretti, T. R., Honeycutt, R. L., Wilson, M. R., Monson, K. L., & Foran, D. R. (2007). Mitochondrial DNA analysis of the domestic dog: Control region variation within and among breeds. *Journal of Forensic Sciences, 52*(3), 562-572. doi: 10.1111/j.1556-4029.2007.00425.x

Hellmann, A. P., Rohleder, U., Eichmann, C., Pfeiffer, I., Parson, W., & Schleenbecker, U. (2006). A proposal for standardization in forensic canine DNA typing: Allele nomenclature of six canine-specific STR loci. *Journal of Forensic Sciences, 51*(2), 274-281. doi: 10.1111/j.1556-4029.2006.00049.x

Hohoff, C., Schnöink, K., Brinkmann, B., Schneider, P. M. (2013) The GEDNAP (German DNA profiling group) proficiency testing system. 21pp

Kim, K. S., Lee, S. E., Jeong, H. W., & Ha, J. H. (1998). The complete nucleotide sequence of the domestic dog (Canis familiaris) mitochondrial genome. *Molecular Phylogenetics and Evolution, 10*(2), 210-220. doi: DOI 10.1006/mpev.1998.0513

Linacre, A., Gusmao, L., Hecht, W., Hellmann, A. P., Mayr, W. R., Parson, W., . . . Morling, N. (2011). ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International-Genetics, 5*(5), 501-505. doi: 10.1016/j.fsigen.2010.10.017

Parson, W., Gusmão, L., Hares, D.R., Irwin, J.A., Mayr, W.R., Morling, N., Pokorak, E., Prinz, M., Salas, A., Schneider, P.M., Parsons, T.J. (2014). DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. *Forensic Sci Int Genet,* 13, 134-42. doi: 10.1016/j.fsigen.2014.07.010. Epub 2014 Jul 29.

Pereira, L.,Van Asch, B.,Amorim A.(2004) Standardisation of nomenclature for dog mtDNA D-loop: a prerequisite for launching a Canis familiaris database. *Forensic Sci Int* 141(2-3):99-108.

Webb, K. M., & Allard, M. W. (2009). Identification of Forensically Informative SNPs in the Domestic Dog Mitochondrial Control Region. *Journal of Forensic Sciences, 54*(2), 289-304. doi: 10.1111/j.1556-4029.2008.00953.x

7. Appendix

7.1 Allelic ladder range

Autosomal STR marker	**Allelic ladder range***
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	22 to 29
PEZ6	14 to 23
WilmsTF	8 to 19.3
Sex specific marker	
Amelogenine	Y, X
SRY	SRY

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

7.2 Registration form

Institution	
Please indicate	
Commercial ☐ Non commercial☐	
Tax number	
Contact person	
Name:	
Email:	
Autosomal STR marker	Mark with a cross
C38	
FH2054	
FH2087ub	
FH2137	
FH2328	
FH2361	
FH2508	
FH2611	
FH2613	
PEZ15	
PEZ3	
PEZ6	
WilmsTF	
Sex specific marker	Mark with a cross
Amelogenine	
SRY	
Mitochondrial DNA	Specify range
HVS-I	
From	
To	
HVS-II	
From	
To	
CR	
From	
To	

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

Institution				
Contact person				
Name:				
Email:				
Autosomal STR marker				
	Sample 1		**Sample 2**	
	Allele 1	Allele 2	Allele1	Allele 2
C38				
FH2054				
FH2087ub				
FH2137				
FH2328				
FH2361				
FH2508				
FH2611				
FH2613				
PEZ15				
PEZ3				
PEZ6				
WilmsTF				
Sex specific marker				
	Sample 1		**Sample 2**	
	Allele 1	Allele 2	Allele1	Allele 2
Amelogenine				
SRY				

Returning results form for mitochondrial DNA (Control Region)

7.4

Institution	
Contact person	
Name:	
Email:	
Mitochondrial DNA	
Sample 1	**Sample 2**
Analysis range	**Analysis range**
Variants[1]	**Variants[1]**
Comments:	

[1]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

Canine DNA profiling group

CaDNAP proficiency test 20xx

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

The canine STR proficiency test included following STR markers:

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

The correctness of the analyzed and reported results is confirmed for following canine STR markers:

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

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

Control Region

The correctness of the analyzed and reported results is confirmed for the range of canine mitochondrial DNA from **position xxxxxx** to **xxxxx.**

Date and Signature