# THE CaDNAP PROFICIENCY TEST
# A Quality Control Instrument for Performance Monitoring the Forensic Analysis of Canine DNA

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## Introduction

Identity testing of domestic dogs (*Canis familiaris*) has repeatedly demonstrated its capability to add relevant information to forensic cases and plays a leading role in setting quality standards for non-human DNA typing. However, canine DNA analysis is miles away from being widespread practice. That in turn means that the laboratories concerned operate isolated from others and have limited opportunities to compare their competence. Errors, bias or significant methodical differences might have serious consequences. Therefore, appropriate procedures are necessary to counteract these potential risks, if possible in advance. In order to meet this issue the Canine DNA Profiling (CaDNAP) group has successfully performed validation studies including interlaboratory comparisons for specified canine STRs [1]. According to ISO/IEC 17025 a laboratory shall have quality control procedures in place such as the participation in proficiency tests (PT). Proficiency testing is defined as the determination of a laboratory's testing performance against pre-established criteria by means of interlaboratory comparisons providing an independent appraisal to reference values [2].

## General outline

In the 2014-CaDNAP meeting the group decided to institutionalize a biannual PT and therefore to develop a framework of rules and criteria for forensic canine DNA competence testing encompassing STRs and the mtDNA control region. The general policy of the PT closely follows the model provided by GEDNAP (German DNA profiling group). The basic principles are in accordance to the ISFG recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations [3]. In particular, canine STR analysis is described in [1] and mtDNA analysis in [4 - 6].

**Aims:**

1. Harmonization of the canine STR marker set
2. Standardization of a repeat-based STR nomenclature
3. Standardization of canine mtDNA nomenclature
4. Monitoring the performance of methods/procedures
5. Evaluation of the competence of the institutions
6. Detection of error sources

**Participation:**

The CaDNAP-PT is open to all group members and to any laboratory invited by CaDNAP. The participants can select mtDNA, autosomal STRs and sex-specific markers (**Table 1**).

**Organisation:**

The planning and implementation of the PT is carried out by the organizing laboratory (*Institute of Veterinary Pathology, Justus-Liebig-University Giessen, Germany*) in consultation with the CaDNAP group. At the annual meetings the PT is scheduled or the results are presented and discussed.

**Frequency:**

It was specified by the CaDNAP group to organize the PT on a biannual basis, starting in 2014/15.

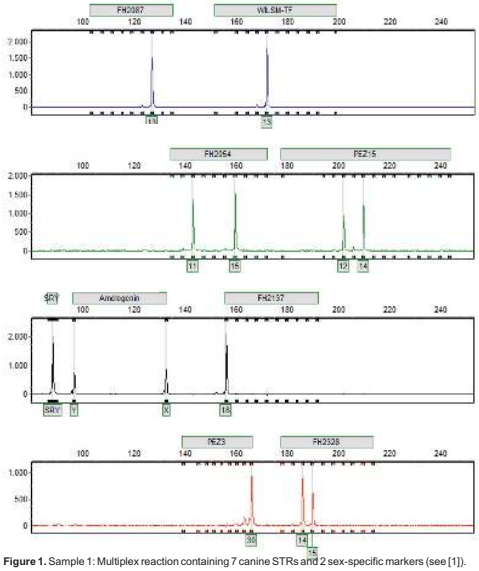


**Figure 1.** Sample 1: Multiplex reaction containing 7 canine STRs and 2 sex-specific markers (see [1]).


**Samples:**

The samples are prepared by the organizing laboratory. The number and type of samples for the first CaDNAP-PT was defined as follows:

- 2 samples originating from 2 different dogs
- Body fluids tested: blood and saliva
- No mixtures

**Typing of samples:**

The laboratories are expected to follow the international guidelines for forensic DNA analyses and to include all necessary controls. The use of allelic ladders and of positive control DNA DH82-D3167 is highly recommended. The latter derives from a permanent canine cell line and all alleles were confirmed by sequence analysis (for details see [1]).

The mtDNA is notated with respect to the reference sequence U96639.2 [5] according to the recommendations of [6].

**Returning of results:**

Results are submitted by using an EXCEL spreadsheet provided by the organizing laboratory. For evaluation laboratory data (e.g. electropherograms) have to be included.

**Certification:**

A certificate is issued by the organizing laboratory in which is stated that the participant has successfully completed the PT.

| Marker | Allelic range | Sample 1 | Sample 2 | Labs (n) | Correct (n) | Method* | Nomencl. |
|---|---|---|---|---|---|---|---|
| C38 | 11 - 32.1 | 15.2/20.2 | 16.2/17.2 | 2 | 2 | M-M | [7] |
| FHC 2010 | 9 - 12 | 9/10 | 10/13 | 1 | 1 | T | [8] |
| FHC 2054 | 9 - 18 | 11/15 | 15/16 | 3 | 3 | M-M-D | [8] |
| FHC 2079 | 4 - 10 | 6 | 5/11 | 1 | 1 | D | [8] |
| FHC 2087 | 7 - 15 | 13 | 11/13 | 3 | 3 | M-M-S | [8] |
| FHC 2137 | 18 - 27 | 18 | 19.3 | 2 | 2 | M-M | [9] |
| FHC 2161 | 12 - 21 | 14 | 17/18 | 1 | 1 | D | [10] |
| FHC 2328 | 12 - 21 | 14/15 | 15 | 3 | 3 | M-M-D | [10] |
| FHC 2361 | 13 - 36 | 15.2/16 | 15.2 | 3 | 3 | M-M-S | [7] |
| FHC 2508 | 9 - 14.1 | 10 | 11.1 | 3 | 3 | M-M-S | [1] |
| FHC 2611 | 14 - 25 | 23.2 | 23.2 | 3 | 3 | M-M-D | [8] |
| FHC 2613 | 8 - 28.1 | 12.3/15 | 12.3/17 | 2 | 2 | M-M | [1] |
| PEZ 1 | 11 - 16 | 12 | 11/13 | 1 | 1 | T | [11] |
| PEZ 10 | 19 - 35 | 25 | 27/29 | 1 | 1 | D | [10] |
| PEZ 15 | 6 - 22.2 | 12/14 | 14/20.2 | 3 | 3 | M-M-S | [8] |
| PEZ 3 | 22 - 29 | 30 | 25 | 3 | 3 | M-M-D | [10] |
| PEZ 5 | 7 - 12 | 9 | 13 | 1 | 1 | D | [11] |
| PEZ 6 | 14 - 23 | 17/21.1 | 19/20.1 | 3 | 3 | M-M-T | [10] |
| Wilms TF | 8 - 19.3 | 13 | 13/15 | 3 | 3 | M-M-S | [8] |
| Amelogenin | | X/Y | X/Y | 3 | 3 | M-M-S | [12] |
| SRY | | SRY | SRY | 2 | 2 | M-M | [8] |

**Table 1.** Summary of the results of the CaDNAP-PT 2014/15: The 19 canine STR markers available for evaluation and certification are listed as well as the two sex-specific markers. All participating labs submitted correct results. *Methods applied by the participants : M - Multiplex 1&2 as described in [1], T - Triplex, D - Duplex, S - Singleplex

## Results CaDNAP-PT 2014/15

**Participants:**

Three laboratories from three different countries (Austria, Germany and Switzerland) participated. The organizing laboratory typed all STR markers and the entire mtDNA control region of the samples.

**Samples:**

Two samples from two different unrelated male dog for individual analysis and subsequent comparison of the results:
Sample 1 – blood spotted on a piece of linen cloth
Sample 2 – saliva on a FLOQ Swab (Copan)

**STR typing:**

A total of 19 different canine STR markers were available for analysis. Of these ten (53%) were typed by all three participating laboratories, three (16%) by two and six (32%) by one laboratory.

The genotypes of samples 1 and 2 are listed in **Table 1**. Different PCR-Methods were applied by the participants ranging from singleplex reactions to multiplexes containing seven STR markers (see **Figure 1**). Concordant results were obtained by all participants for all STRs (**Table 1**).

**Sex-specific markers:**

Amelogenin was typed correctly by all participants and confirmed the male sex of both dogs tested.
Additionally two laboratories tested SRY supporting the amelogenin results (**Figure 1**).

**mtDNA:**

One laboratory sequenced the hypervariable region of the canine mtDNA control region ranging from position 15458 to 16727. At all positions concordant results compared to the organizing lab were obtained.

**References**

[1] Berger *et al.* Forensic Sci Int Genet 8 (2014): 90-100
[2] ILAC-P9: 06/2014
[3] Linacre *et al.* Forensic Sci Int Genet 5 (2011): 501-505
[4] Eichmann & Parson Int J Legal Med 121 (2007): 411-416
[5] Kim *et al.* Mol Phylogenet Evol 10 (1998): 210-220
[6] Pereira *et al.* Forensic Sci Int 141 (2004): 99-108
[7] Van, Asch *et al.* Electrophoresis 30 (2009): 417-23.
[8] Eichmann *et al.* Int J Legal Med 118 (2004): 249-66
[9] Francisco *et al.* Mamm.Genome 7 (1996): 359-62
[10] Hellmann *et al.* J Forensic Sci 51 (2006): 274-81
[11] Zenke *et al.* For Sci Int Genet 5 (2010): e31-e36
[12] Delgado *et al.* Mol Phyl Evol 47(2008): 865-69