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Standard for Training in Forensic Human Mitochondrial DNA Analysis, Interpretation, Comparison, Statistical Evaluation, and Reporting

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Foreword

This standard defines the minimum requirements for a Forensic DNA Analyst training program for human mitochondrial DNA analysis, interpretation, comparison, statistical evaluation, and reporting. The aim is to provide a framework for training that will result in quality and consistency in the forensic DNA community.

This document is part of a series of training documents under ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*.

This document was revised, prepared, and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Biology/DNA Biological Data Interpretation and Reporting Subcommittee, the Biological Methods Subcommittee, and the Wildlife Forensic Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

The AAFS Standards Board (ASB) is an ANSI-accredited Standards Developing Organization with the purpose of providing accessible, high quality science-based consensus forensic standards. The ASB is a wholly owned subsidiary of the American Academy of Forensic Sciences (AAFS), established in 2015 and accredited by the American National Standards Institute (ANSI) in 2016. The ASB consists of Consensus Bodies (CB), which are open to all materially interested and affected individuals, companies, and organizations; a Board of Directors; and Staff.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

Keywords: *training, human Mitochondrial DNA, interpretation.*

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Standard for Training in Forensic Human Mitochondrial DNA Analysis, Interpretation, Comparison, Statistical Evaluation, and Reporting

1 Scope

This document provides the requirements for a forensic DNA laboratory's training program in forensic human mitochondrial DNA (mtDNA) analysis, interpretation, comparison, statistical evaluation, and reporting.

2 Normative References

The following reference is indispensable for the application of the standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs,* First Edition 2019[a].

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
control region
A presumed non-coding portion of the mitochondrial DNA molecule analyzed through DNA sequencing, which may be used to determine an individual's mitochondrial haplotype or for taxonomic discrimination. The control region often contains hypervariable regions (in humans: HV1 and HV2) that differ in sequence among non-maternally related individuals. The control region encompasses the D-loop region in humans and other species.

3.2
haplogroup
A group of similar haplotypes that share a common ancestor with a single-nucleotide polymorphism mutation.

3.3
haplotype
A set of linked DNA variations, or polymorphisms, that tend to be inherited together (e.g., commonly used for human Y-chromosome or mitochondrial analysis). A haplotype can refer to a combination of alleles or to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome.

3.4
heteroplasmy
The presence of more than one mitochondrial DNA (mtDNA) sequence or type within a single individual.

[a] Available from: https://www.asbstandardsboard.org/published-documents/

3.5
homopolymeric stretches
A segment of DNA consisting of repeats of a single nucleotide; may cause slippage during amplification and sequencing.

3.6
hypervariable region 1
HVI
A section of the human mtDNA control region spanning nucleotide positions 16024-16365, that often differs among non-maternally related individuals.

3.7
hypervariable region 2
HVII
A section of the human mtDNA control region spanning nucleotide positions 73-340, that often differs among non-maternally related individuals.

3.8
nuclear mitochondrial DNA segment
NUMT
A transposition of any type of cytoplasmic mitochondrial DNA into the nuclear genome of a eukaryotic organism.

3.9
Revised Cambridge Reference Sequence
rCRS
A fully corrected version of the original Cambridge Reference Sequence (GenBank# J01415 0 gi:337188) of Anderson et al. (1981) of the human mitochondrial genome (mtDNA), also referred to as the Anderson sequence. The rCRS is GenBank sequence NC_012920; it differs from the original CRS and other complete mtDNA GenBank sequences in that it has eighteen corrected or confirmed nucleotides. It is used for reporting human mtDNA sequences, generally by denoting the differences observed from the rCRS.

3.10
transition
A mutation that results in a change from one purine to the other purine (e.g., A-to-G) or one pyrimidine to the other pyrimidine (e.g., C-to-T).

3.11
transversion
A mutation that results in a change from a purine to pyrimidine or vice versa (e.g., A-to-T).

4 Requirements

4.1 General

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs* shall be used in conjunction with this document because ANSI/ASB Standard 022 provides the foundational training program requirements upon which additional specific requirements, such as this document, will be based.

4.2 Knowledge-based Training

4.2.1 The laboratory's training program shall provide the trainee with an understanding of the fundamental principles and limitations of forensic human mtDNA analysis to include technical procedures for generating a mitochondrial DNA sequence, interpretation, comparison, statistical evaluation, and reporting.

4.2.2 At a minimum, the knowledge-based portion of the training program shall require training and review of the following:

a) the laboratory's protocols for mtDNA sequence analysis;

b) the laboratory's applicable validation studies;

c) literature used to support validation and the test methods in the laboratory; and

d) applicable literature as assigned.

4.2.3 At a minimum, the knowledge-based portion of the training program shall cover the following topics.

NOTE Knowledge of historical methods is intended to provide an educated perspective on current methods.

a) General biology of human mtDNA including:

    1) structure,

    2) control region [hypervariable region 1 (HVI); hypervariable region 2 (HVII)],

    3) gene composition of the mtDNA genome,

    4) copy number of mtDNA,

    5) mtDNA related diseases,

    6) homopolymeric stretches,

    7) Nuclear Mitochondrial DNA segment (NUMT), and

    8) differences between nuclear DNA (nDNA) and mtDNA (e.g., copy number, location, structure, size, inheritance mode, recombination).

b) Inheritance of mtDNA, including:

    1) maternal,

    2) transmission of mtDNA from sperm,

    3) mutation:

        i) rates,

ii) hotspots,

iii) transitions/transversions,

iv) insertions/deletions.

c) Heteroplasmy, including:

1) types,

2) rate,

3) prevalence in various tissues,

4) family studies with heteroplasmy,

5) effect of heteroplasmy on forensic mtDNA analysis.

d) Sequence alignment, including:

1) Revised Cambridge Reference Sequence (rCRS),

2) interpretation of length heteroplasmic sequences,

3) nomenclature.

e) Evaluation of controls, including:

1) reagent blanks,

2) negative controls,

3) positive controls.

f) Contamination, including:

1) sources (environmental, procedural),

2) sample handling strategies and preventative methods,

3) decontamination procedures,

4) root cause analysis, corrective action when contamination occurs,

5) interpretation, comparison, statistical evaluation, and reporting of data when a contamination event has occurred.

g) Comparison of evidentiary data to known sample data, including:

1) cannot exclude,

2) exclusion,

3) inconclusive,

4) not suitable for comparison.

h) Databases, including:

1) diversity among and within ethnic groups,

2) haplogroup,

3) composition of mtDNA sequence databases:

i) SWGDAM (forensic),

ii) The European DNA Profiling Group Mitochondrial DNA Population Database (EMPOP) (forensic),

iii) other published databases;

4) mtDNA sequence database searches:

i) difference-coded haplotype searches,

ii) string based searches.

i) Statistics and reporting (as established and used by the laboratory), including:

1) calculation of frequencies and confidence intervals (profile probabilities),

2) calculation of a population correction factor, match probabilities, and confidence intervals (credible intervals),

3) calculation of likelihood ratios,

4) reporting of results:

i) profile probabilities,

ii) match probabilities,

iii) likelihood ratios;

5) reporting of conclusions:

i) cannot exclude,

ii) exclusion,

iii) inconclusive,

iv) not suitable for comparison.

4.3 Practical Training

4.3.1 The laboratory's training program shall provide the trainee with sufficient practical instruction for the trainee to obtain the skills for performing forensic human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting protocols used by the laboratory.

4.3.2 At a minimum, the practical portion of the training program shall include the observation of the process at least once or until clearly understood with exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These include:

a) mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting to be utilized by the trainee;

b) the use of appropriate controls;

c) proper documentation of the process.

4.3.3 At a minimum, the practical portion of the training program shall include hands-on or file review exercises representative of the range, type, and complexity of casework or database samples processed by the laboratory. These include:

a) mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting to be utilized by the trainee;

b) evaluation of controls and expected results;

c) proper documentation of the process;

d) the number and quality of samples processed by the trainee shall be appropriate to demonstrate the ability to follow the laboratory's mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting protocol(s) and to produce reliable and accurate results.

4.4 Competency Testing

4.4.1 General

The laboratory's training program shall include knowledge-based and practical competency in the application of human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting. The format of the test(s) shall meet Section 4.3 of ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*.

4.4.2 Knowledge-based Competency

The trainee shall successfully complete a knowledge-based test covering the critical information obtained during the training of human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting methods. The test(s) shall cover, at a minimum:

a) the theoretical and scientific bases of human mtDNA interpretation, comparison, statistical evaluation, and reporting;

b) the function of the reagents and other components used in each method;

c) the function of the controls used in human mtDNA analysis with regard to interpretation, comparison, statistical evaluation, and reporting of the sample results;

d) the quality control steps pertaining to human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting;

e) the laboratory's procedures pertaining to mtDNA interpretation, comparison, statistical evaluation, and reporting.

4.4.3 Practical Competency

The trainee shall successfully complete a practical test covering human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting protocols for which he or she will be independently authorized to perform. The trainee, at a minimum, shall be able to satisfactorily perform the following:

a) properly and accurately execute human mtDNA analysis, interpretation, comparison, statistical evaluation, and reporting procedures;

b) apply the laboratory's interpretation and comparison procedures to a variety of evidentiary casework -or database-type samples, as applicable to the trainee;

c) operate relevant equipment, instrumentation, and software used in the laboratory for human mtDNA interpretation and comparison;

d) correctly document work performed in accordance with laboratory procedures.

5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall meet Section 5 of the ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs* and all the requirements set forth in this document.

Annex A
(informative)

Bibliography

The following bibliography is not intended to be an all-inclusive list, review, or endorsement of literature on this topic. The laboratory shall develop a list tailored to its specific needs. The goal of the bibliography is to provide examples of publications addressed in the standard.

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