

Standard for Training in Forensic DNA Quantification Methods

*Biological Methods Subcommittee
Biology/DNA Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science*



OSAC Proposed Standard

Standard for Training in Forensic DNA Quantification Methods

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This document has not been published by an SDO. Its contents are subject to change during the standards development process. All interested groups or individuals are strongly encouraged to submit comments on this proposed document during the open comment period administered by the Academy Standards Board (www.asbstandardsboard.org).

Foreword

This standard defines the minimum requirements that shall be met in a Forensic DNA Analyst training program for DNA quantification methods. The aim is to provide a framework for quality training that will result in consistency in the forensic DNA community.

This standard was revised, prepared and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board (ASB). The initial draft document was developed by the Biological Methods Subcommittee of the Organization of Scientific Area Committees. All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

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1 Scope

This standard provides the requirements for a forensic DNA laboratory's training program in DNA quantification.

2 Normative References

ASB Standard 022 - *Standard for Forensic DNA Analysis Training Programs*¹

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1

DNA quantification

A process by which the DNA concentration in a sample is determined.

3.2

Cycle threshold

Cycle number (in quantitative PCR) at which the fluorescence generated within a reaction exceeds a defined threshold; this value is converted to a DNA concentration for each sample tested using a standard curve developed from DNA samples of known concentrations.

3.3

Degradation

The fragmenting, or breakdown, of DNA or protein (e.g. loss of enzymatic-activity) by chemical, physical, or biological means; a common occurrence when biological samples containing DNA encounter warm moist environments or excessive UV light.

3.4

Polymerase chain reaction (PCR)

An enzymatic process by which a specific region of DNA is replicated during repetitive cycles that consist of the following: denaturation of the template; annealing of primers to complementary sequences at an empirically determined temperature; and extension of the bound primers by a DNA polymerase. The goal of the PCR process is to generate many copies (termed products or amplicons) of a specific region of DNA for further analysis.

3.5

Quantitative PCR (qPCR)

A means for quantifying the amount of nucleic acid present in a sample using PCR

4 Requirements

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4.1 Knowledge-based training

The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of the theory behind various DNA quantification methods, the function of the reagents and other components used in each method, the information generated by each assay, the limitations of each method, and the laboratory's own DNA quantification guidelines.

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

- a) The laboratory's protocols for DNA quantification
- b) The laboratory's applicable validation studies
- c) Literature used to support validation
- d) Applicable literature as assigned by the trainer

4.1.2 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. In-depth understanding of these methods may not be required for successful training.

- a) Principles of non-PCR based DNA quantification methods
 - i. Spectrophotometric analysis
 - ii. Fluorometric methods
 - iii. Yield gels
 - iv. Southern blots (e.g., slot blots)
- b) Principles of quantitative PCR (qPCR) DNA quantification methods
 - i. qPCR assays based on fluorescence quenching using specific probes
 - ii. qPCR assays based on non-specific double-stranded DNA intercalating dye
 - iii. Digital PCR (dPCR)
 - iv. End-point PCR assays
 - v. Standards and standard curves
 - vi. Cycle threshold (Ct) establishment and its role in the quantification process
- c) Characteristics, performance, and information provided by PCR and non-PCR based methods of DNA quantification

- i. Sensitivity (limit of detection and reliable concentration range)
 - ii. Specificity (non-human, higher primate, human, male/female)
 - iii. Single-copy vs. multi-copy qPCR targets
 - iv. qPCR target length and consequent performance on degraded DNA
 - v. qPCR multiplex capabilities
 - a. Limits of detection
 - b. Internal positive control for the co-detection of PCR inhibitors
 - c. Autosomal and Y chromosome co-quantification
 - d. Detection and characterization of degradation based on targeting fragments of different lengths in a multiplex reaction
- d) Characteristics of results of different methods of DNA quantification
- i. Amount of total DNA or target DNA
 - ii. Sample purity
 - iii. Detection of male contributor(s)
 - iv. Presence of PCR inhibitors
 - v. DNA degradation levels
 - vi. Suitability of DNA for downstream DNA typing methods
 - vii. Impact of the source of the DNA standard (e.g., cell line, single source genomic DNA, pooled genomic DNA) on the quantification estimate
- e) Interpretation of results
- i. Evaluation of the standard curve (slope, R^2 , Y intercept)
 - ii. DNA concentration in the sample
 - iii. Presence of PCR inhibitors (if applicable)
 - iv. Ratio between male and female contributors (if applicable)
 - v. Level of sample degradation (if applicable)
 - vi. Determining whether the DNA should be further purified, concentrated, or re-extracted

- vii. Determining whether the sample has detectable amounts of DNA
- viii. Determining whether it is appropriate to proceed with the amplification step
- ix. Determining the amount of DNA to be added to the amplification reaction
- x. Method limitations (e.g., non-targeted detection methods, specificity, sensitivity)
- f) Instrumentation and reagents
 - i. DNA quantification instruments and parameters
 - ii. Software parameters associated with instruments
 - iii. Maintenance and calibration
 - iv. Storage and handling of quantification kit reagents
- g) Troubleshooting
 - i. Spectral calibration failure
 - ii. Standard quantification curve failure
 - iii. Non-specific detection (e.g., background fluorescence, cellular debris, pigments)
 - iv. General equipment failure

4.2 Practical training

The laboratory's training program shall provide the trainee with sufficient practical instruction for the trainee to obtain the skills for performing DNA quantification protocols used by the laboratory.

- 4.2.1 At a minimum, the practical portion of the training program shall include exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These include:
- a) DNA quantification methods to be utilized by the trainee
 - b) Documentation of the process
- 4.2.2 Practical exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These shall include
- a) DNA quantification methods to be utilized by the trainee
 - b) Documentation of the process

- c) The number and quality of samples processed by the trainee shall be appropriate to demonstrate the ability to follow the laboratory's DNA quantification protocol(s) and to produce reliable and accurate results.

4.3 Competency

The laboratory's training program shall include knowledge-based and practical competency testing in the application of DNA quantification. The format of the test(s) shall meet section 4.3 of ASB 022.

4.3.1 Knowledge-based competency

The trainee shall successfully complete a knowledge-based test covering the critical information obtained during the training of DNA quantification methods. The test(s) shall cover, at a minimum:

- a) The theoretical and scientific bases of DNA quantification
- b) The function of the reagents and other components used in each method
- c) The proper application of each method
- d) The quality control steps pertaining to DNA quantification
- e) The laboratory's analytical procedures pertaining to DNA quantification methods

4.3.2 Practical competency

The trainee shall successfully complete a practical test covering each of the DNA quantification protocol(s) for which he or she will be independently authorized. The trainee shall be able to satisfactorily perform the following, as applicable:

- a) Properly and accurately execute the analytical procedures related to DNA quantification
- b) Apply the laboratory's analytical procedures to a variety of evidentiary casework- or database-type samples
- c) Operate relevant equipment and instrumentation used in the laboratory
- d) 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 ASB 022.

Annex A **(informative)**

Bibliography

The following information provides a list of the literature resources that may assist the DNA technical leader in defining the breadth and scope of the materials to be reviewed by the trainee. This list is not meant to be all inclusive. The laboratory shall develop a list tailored to its specific needs. Updated references shall be added to the laboratory's list as new methods or technologies are incorporated into the laboratory's protocols.

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