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**Forensic Laboratory Standard for Prevention,
Monitoring, and Mitigation of Human DNA
Contamination**



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Forensic Laboratory Standard for Prevention, Monitoring, and Mitigation of Human DNA Contamination

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Foreword

This document discusses the standards required for a laboratory conducting PCR-based analysis to limit, detect, assess the source of, and mitigate contamination events as they pertain to human forensic DNA analysis. This standard includes provisions for Rapid DNA analysis performed in an accredited forensic DNA laboratory and does not cover the use of Rapid DNA instrumentation outside of an accredited forensic DNA laboratory environment.

Some, but not all, contamination events in casework and database samples can be detected. Contamination can occur from individuals such as first responders, laboratory personnel, or crime scene technicians transferring DNA to evidence. Contamination can also occur when objects or surfaces transfer DNA to evidence. It can never be known with certainty that a casework or database sample is contamination-free, but detection and tracing efforts facilitated through the use of elimination databases which contain the DNA profiles of laboratory personnel, first responders, law enforcement, and medical personnel can assist in the identification of contamination.

Certain probabilistic genotyping software capabilities may be useful to detect contamination events, including deconvolution of mixtures enabling database searches and performing comparisons between unknown mixtures to assess the likelihood of a common donor.

While contamination has always been an issue in forensic laboratories, the sensitivity of testing instrumentation and methods in human forensic DNA laboratories has steadily increased and has resulted in a greater chance of detecting low-level contamination and drop-in events. This affects the interpretation of the sample, including comparisons to known individuals.

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2015 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI), and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practices, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

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 Biological Methods Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aaafs.org or 401 N 21st Street, Colorado Springs, CO 80904.

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

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Keywords: *DNA contamination, DNA elimination database.*

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Forensic Laboratory Standard for Prevention, Monitoring, and Mitigation of Human DNA Contamination

1 Scope

This standard provides requirements for limiting, detecting, assessing the source of, and mitigating the effect of DNA contamination as applied to PCR-based human DNA analysis conducted within a forensic laboratory (i.e., casework and DNA database).

2 Normative References

There are no normative reference documents. Annex A, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1

comparison (comparable DNA profile)

The process of examining two or more DNA data sets to assess the degree of similarity or difference.

3.2

contamination

Exogenous DNA or other biological material in a DNA sample, PCR reaction, or item of evidence, which may be present before the sample is collected or introduced during collection or testing of the sample.

3.3

controls

Samples of known type, run in parallel with experimental, reference, or evidence samples that are used to evaluate whether a procedure is working correctly.

- A **positive control** is a sample that is used to determine if a test performed as expected. This control consists of the test reagents and a known DNA sample that will provide a known DNA profile as a result of the test.
- A **negative control** (e.g., extraction blanks, reagent blanks and amplification blanks) consists of the reagents used in various stages of testing without the introduction of sample; no results are expected from a negative control.

3.4

DNA elimination database

Collection of DNA profiles, held in a searchable format, from individuals whose access, role, or activities present a potential DNA contamination risk including possible contamination DNA profiles recognized by the laboratory.

NOTE A DNA elimination database cannot detect all forms of contamination, but with DNA profiles of first responders including law enforcement and medical personnel, and with the production of likelihood ratio distributions for elimination database profiles, more contamination events can be detected.

3.5

DNA laboratory monitoring

Activities (e.g., swabbing relevant equipment and surfaces) to evaluate the background levels of DNA in the laboratory to assess the risk of contamination.

3.6

drop-in

Presence of a low number of nonreproducible alleles (as determined by validation) in DNA data where each allele may be interpreted as coming from different individuals whereas contamination consists of multiple alleles from one or more individuals. **interpretation (interpretable DNA profile)**

The process of evaluating DNA data for purposes including defining assumptions related to mixtures and single source profiles, distinguishing between alleles and artifacts, assessing the possibility of degradation, inhibition, and stochastic effects, and determining whether the data are suitable for comparison.

3.7

Rapid DNA analysis

Fully automated, “swab in – profile out” process of developing a DNA profile from samples without human intervention.

4 Requirements

4.1 General

The laboratory shall develop and follow appropriate documented laboratory procedures and policies to address each of the requirements in this standard. The DNA technical leader (or equivalent role, position, or title as designated by the laboratory) shall ensure the laboratory follows all requirements.

4.2 Physical Requirements for Laboratory Areas, Evidence Processing, Reagents, Consumables, Storage, and Personal Protective Equipment

4.2.1 Access to laboratory areas shall be restricted to authorized individuals to reduce the risk of introducing extraneous DNA into work areas and samples. The laboratory shall have separate work areas with dedicated equipment and supplies for pre- and post-PCR activities to reduce the risk of introducing amplified DNA into samples. Pre-PCR includes all activities prior to the amplification of the DNA. Post-PCR includes PCR and all activities involving amplified DNA.

4.2.1.1 Separation of pre- and post-PCR areas shall be accomplished by use of physical barriers (this requires floor-to-ceiling walls and closed doors).

4.2.1.2 Equipment, tools, and supplies dedicated to post-amplification areas shall not be moved outside the post-amplification area without first being decontaminated.

4.2.1.3 Separate personal protective equipment shall be dedicated to and worn in pre- and post-amplification areas.

4.2.2 Evidence shall be stored in pre-PCR areas separate from reagents, consumables, and work products.

4.2.3 Evidence items and evidence derivatives and/or work products shall be packaged and handled in a manner to minimize the transfer of biological material (see <https://www.nist.gov/system/files/documents/forensics/NIST-IR-7928.pdf> for additional information). An analyst shall only handle one evidence item or derivative/work product at a time.

4.2.4 Separate storage areas shall exist for reagents, consumables, DNA extracts, and PCR products.

4.2.4.1 Applicable reagents, consumables, and DNA extracts shall be stored separately in pre-PCR areas.

4.2.4.2 Applicable reagents, consumables, and PCR product shall be stored separately in post-PCR areas.

4.2.5 The laboratory shall arrange the working environment to mitigate potential contamination.

4.2.6 The laboratory shall have and follow a written, regularly scheduled decontamination procedure to include laboratory areas, items to be decontaminated, and decontamination frequency. The decontamination schedule shall be determined by volume, frequency, and nature of use.

4.2.7 The laboratory shall have and follow a written, regularly scheduled laboratory DNA contamination monitoring program. The results from the program shall be documented and made available for inspection upon request.

4.2.8 When possible, the laboratory shall purchase reagents and consumables from an ISO 18385:2016^[9] compliant manufacturer.

4.2.9 The laboratory shall institute procedures to minimize the possibility of contamination from laboratory equipment, glassware, reagents, and consumables. These procedures may include UV irradiation, ethylene oxide treatments, bleach (or commercial equivalent) treatments, as appropriate.

4.2.10 The laboratory shall have a system to track reagent lot numbers and consumables to assist in the investigation of a contamination event.

4.3 Procedural Requirements

4.3.1 The laboratory shall define and use appropriate decontamination and/or cleaning agents, or procedures for each method, technology, tool and instrument, and laboratory area. Decontamination agents or procedures known to destroy DNA shall be used as appropriate on the items and surfaces being cleaned.

4.3.2 The laboratory shall have procedures and policies for the proper disposal of post-PCR waste.

4.3.2.1 Post-PCR waste shall not be stored in pre-PCR spaces.

4.3.2.2 Post-PCR waste shall not be transported through pre-PCR areas without adequate precautions (e.g., double bagging).

4.3.3 The laboratory shall have procedures and policies defined to reduce potential contamination events during evidence and evidence derivative/work product processing to include the following requirements:

- a) the use of personal protective equipment;
- b) the decontamination of work surfaces and examination tools that are not single use with DNA destroying reagents or processes before new evidentiary items are examined;
- c) handle and package evidence and evidence derivatives/work product to limit the possibility of contamination;
- d) limit the opening and examination to one item of evidence at a time at each workstation;
- e) separate in time or space the processing of reference samples from evidentiary items;
- f) examine potential high template evidence (e.g., blood, semen, saliva) separately in time or space and independently from potential low-template evidence (e.g., trace amounts of DNA), when possible;
- g) have validated procedures to mitigate the contamination risk associated with concurrently extracting high template DNA and low template DNA evidence.

NOTE See *SWGDM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories^a* for detailed steps on how to evaluate and mitigate contamination.

4.3.4 The laboratory shall document in the case record when items of evidence are received packaged together and how they were packaged.

4.3.5 The laboratory shall perform quality checks of extraction and PCR reagents prior to use in forensic DNA analysis to monitor for contamination.

4.3.6 The laboratory shall document, maintain, and periodically evaluate a log containing exogenous DNA (contamination and drop-in) found in any sample or control. This log shall include the source of the contamination (if known), step of processing the contamination likely occurred, and other information that would inform procedures to prevent future contamination events. This log shall be made available for audit purposes.

4.3.7 The laboratory shall maintain and use a searchable DNA elimination database to detect contamination of casework and database samples. These searches shall occur for every interpretable/comparable DNA profile obtained, and all results shall be documented in the case record.

4.3.7.1 At a minimum, this database shall include biology staff and positive control samples from donors and kits, contamination elimination profiles such as unknown DNA profiles obtained from

^a Available on the SWGDAM website: <https://www.swgdam.org/>

negative or positive controls, or profiles that have been putatively assigned as possible contaminant profiles (e.g., from consumables). To the extent possible, typing shall use the same genetic markers/amplification test kit(s) utilized by the laboratory. Where possible, the laboratory shall include profiles from any DNA laboratory visitors and individuals who are involved in the collection and handling of evidence, work samples, reagents, equipment, or consumables (e.g., staff, agency personnel and other associated workers such as medical examiners, law enforcement, sexual assault nurses, service personnel, and laboratory vendors).

4.3.7.2 Confidentiality of DNA profiles within the elimination database shall follow applicable laws and regulations.

4.3.7.3 DNA elimination database profiles shall be added in a defined timeframe .

4.3.8 The laboratory shall conduct intra-batch comparisons (i.e., samples processed concurrently) to detect contamination in a defined timeframe.

4.3.9 The laboratory shall assess the occurrence of contamination and its possibility when conducting a validation project (internal or developmental), and determine the extent of decontamination/cleaning necessary for reagents, consumables, surfaces, tools, equipment, and sample set up, etc., to produce acceptable genetic data.

4.3.9.1 The laboratory shall include the contamination assessment and underlying data in the validation documentation.

4.3.9.2 The laboratory shall conduct a contamination assessment when a laboratory method/technology is modified.

4.3.10 If the laboratory uses probabilistic genotyping software (or other software), the laboratory shall use such software within its validated capabilities to detect contamination in casework and database samples to include:

- a) searching all interpretable/comparable mixtures, single source profiles, or deduced profiles to profiles contained within the DNA elimination database;
- b) performing mixture-to-mixture comparisons to detect common sources;
- c) performing contamination and cross-contamination checks;
- d) performing batch comparisons;
- e) each laboratory should determine a likelihood ratio threshold value to report for comparisons to an elimination database. This should be documented in the case record and in the report.

4.3.11 Potential contamination events shall be investigated and referenced or documented within the case record or sample record.

4.3.12 When contamination is identified, a root cause analysis^[12] shall be conducted, documented, and referenced within the case record or sample record.

4.3.13 Records of contamination events shall be maintained indefinitely in a centralized manner that allows such events to be tracked across cases/batches and over time. Tracking information

shall include a general description of the event, identifying information (date, case number, individuals involved), and outcome.

4.3.14 The laboratory shall have and follow protocols requiring reporting and communicating contamination events to customers including legal parties (e.g., prosecution and defense), if known.

4.3.15 The laboratory shall use positive and negative controls for the detection of contamination.

NOTE A negative control in DNA testing is used to detect contamination and drop-in introduced into the assay during the testing process via reagents, disposables or handling errors (which may impact the results observed from samples tested at the same time). The use of negative controls helps assess the overall robustness of the testing process but cannot be used to determine whether a particular sample is free from contamination.

4.4 Corrective Measures

4.4.1 The laboratory shall mitigate and address the impact of the contamination event. The type of corrective measure shall be determined by the root cause analysis.

4.4.2 The laboratory shall, at a minimum, have policies and protocols defining when each action is warranted:

- a) suspension of casework;
- b) decontamination;
- c) review of casework;
- d) reevaluation of procedures/protocols;
- e) retraining.

4.5 Personnel and Training Requirements

4.5.1 Personnel defined by the laboratory shall receive documented practical training to include the detection and minimization of contamination.

4.5.2 The laboratory shall have and follow documented policies and protocols to include:

- a) use of personal protective equipment;
- b) evidence and evidence derivatives handling and packaging;
- c) cleaning and decontamination;
- d) quality control measures used to detect and minimize contamination; and
- e) documentation, investigation, and reporting of contamination events.

4.6 Requirements Specific to Use of Rapid DNA Instruments and Consumables in a Laboratory

4.6.1 All previous requirements outlined in this document shall be followed.

4.6.2 Rapid DNA instrumentation shall be maintained in rooms outside of evidence examination areas and those containing amplified DNA..

4.6.3 Rapid DNA consumables shall be monitored for extraneous DNA through the use of a positive and negative controls per lot.

4.6.4 Personal protective equipment shall be worn while preparing samples for and using Rapid DNA instrumentation.

4.6.5 Lot numbers for Rapid DNA consumables shall be recorded and monitored.

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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 goal of the bibliography is to provide examples of publications addressed in the standard.

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^c Available from: <https://enfsi.eu/wp-content/uploads/2017/09/DNA-contamination-prevention-guidelines-v2.pdf>

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ⁱ Available on the SWGDAM website: <https://www.swgdam.org/>

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