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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 polymerase chain reaction (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 items or from individuals external to the DNA testing laboratory, including first responders, crime scene technicians, and laboratory personnel from other sections. Contamination may also occur within the biology/DNA testing laboratory from individuals, supplies and reagents used in the testing, or from cross-contamination from other items, DNA extracts or amplified products being processed or tested at the same or later time. 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 appropriate controls and quality assurance measures, including the use of elimination databases which may 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 and analysis software capabilities may be useful to detect contamination events. Such capabilities include the deconvolution of mixtures, enabling database searches, or performing comparisons between 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 events. This affects the interpretation of DNA profile data and its comparison to DNA profiles from known individuals. Increased vigilance throughout all phases of evidence collection, storage, preservation, handling and testing, and the monitoring for and detection of contamination in the DNA test results, is critical.

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@aafs.org or 410 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.

ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

Keywords: *DNA contamination, DNA elimination database.*

Table of Contents

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 polymerase chain reaction (PCR)-based human DNA analysis conducted within a forensic laboratory (i.e., casework including Rapid DNA 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; present before the sample is collected or introduced during collection or testing of the sample.

3.3
DNA contamination monitoring program
Activities (e.g., swabbing relevant equipment and surfaces) to evaluate the background levels of DNA in the laboratory to assess the risk of contamination.

3.4
elimination database
Searchable collection of elimination profiles.

3.5
elimination profile
DNA profile from an individual whose access, role, or activities might result in DNA contamination; includes profiles associated with consumables and positive controls; but not case-specific known DNA reference standards or exemplars.

3.6
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
negative control
An analytical control that consists of the reagents used in various stages of testing without the introduction of sample; no results are expected from a negative control.

NOTE For DNA testing, negative controls include extraction blanks/reagent blanks and amplification blanks. A negative control in DNA testing is used to detect contamination 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).

3.9
positive control
An analytical control sample that is used to determine if a test performed properly; this control consists of the test reagents and a known sample that will provide an expected positive response with the test.

NOTE For DNA testing, positive controls include positive amplification controls and may include extraction positive controls.

3.8
rapid DNA analysis
Fully automated, "swab in – profile out" process of developing a DNA profile from a sample 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.

4.2.2 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.

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

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

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

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

4.2.4 Evidence and evidence derivative /work product shall be packaged and handled in a manner to minimize the transfer of biological material. An analyst shall only handle one evidence item or derivative/work product at a time.

NOTE See The *Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*[a] for additional information

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

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

4.2.5.2 Applicable reagents, consumables, and PCR products shall be stored separately in post-PCR areas.

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

4.2.7 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.

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

4.2.9 When possible, the laboratory shall purchase reagents and consumables from a manufacturer compliant with the current version of ISO 18385 [9].

4.2.10 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, and bleach (or commercial equivalent) treatments, as appropriate.

4.2.11 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.

[a] Available from: https://www.nist.gov/system/files/documents/forensics/NIST-IR-7928.pdf

4.3.1.1 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 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 decontamination and/or cleaning agents or processes before new evidentiary items are examined;

c) handle and package evidence and evidence derivative/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 *SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories*[b] for detailed steps on how to evaluate and mitigate contamination.

4.3.4 For non-databasing laboratories, the separation by time or space used for requirements 4.3.3 e) and f) shall be defined in the laboratory protocol.

4.3.5 The choice of time or space shall be supported by validation data.

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

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

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[b] Available on the SWGDAM website: https://www.swgdam.org/

4.3.8 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 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.

4.3.9 The laboratory shall document, maintain, and periodically evaluate a log containing exogenous DNA (contamination) found in any sample or control.

4.3.9.1 This log shall include the source of the contamination (if known), step of processing in which the contamination likely occurred, and other information that would inform procedures to prevent future contamination events.

4.3.9.2 This log shall be made available for inspection upon request.

4.3.10 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. Exceptions can be made and shall be documented.

4.3.10.1 At a minimum, this database shall include biology/DNA staff and positive control samples from donors and kits, contamination elimination profiles such as unknown DNA profiles obtained from 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, janitorial staff and other associated workers such as medical examiners, law enforcement, sexual assault nurses, service personnel, laboratory vendors, and employees involved in any criminal proceedings who may have handled evidence in post-conviction cases).

4.3.10.2 Collection and confidentiality of DNA profiles within the elimination database shall follow applicable laws and regulations.

4.3.10.3 DNA elimination database profiles shall be added in a defined timeframe in order to maximize the potential of detecting a contamination event.

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

4.3.12 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.

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

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

4.3.13 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) determining a likelihood ratio threshold value (or defined threshold value for laboratories using non-probabilistic genotyping software) to report for comparisons to an elimination database.

4.3.14 The threshold value should be documented in the case record.

4.3.15 Suspected contamination events shall be investigated and referenced or documented within the case record or sample record.

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

4.3.17 Records of contamination events shall be maintained in accordance with the laboratory's documented retention schedule and applicable laws/regulations 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.

4.3.18 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.

4.4 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 and documented:

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) handling and packaging of evidence and evidence derivatives;

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 Except as provided in 4.6.3, Rapid DNA instrumentation and cartridges/chips shall be maintained in dedicated spaces away from other laboratory areas used for evidence and DNA extract storage, examination, and testing, including rooms containing amplified DNA.

4.6.3 If maintained inside a room containing amplified DNA, the sample cartridge/chip shall be loaded in an area that does not contain amplified DNA.

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

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

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

Annex A
(informative)

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