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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, ~~to~~ detect, ~~to~~ assess the source of, and ~~to~~ 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 the evidence. Contamination can also occur when objects transfer DNA to the 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 ~~use 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.

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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, \[asb@aaafs.org\]\(mailto:asb@aaafs.org\)](#)~~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.~~

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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 **background DNA**

~~DNA that is present from unknown sources and unknown activities. It can be described as 'foreign' (non-self). It is unknown how or why it is there.~~

3.2 **contamination**

~~Introduction of DNA onto the evidence after responders arrive.~~

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 properly. This control consists of the test reagents and a known DNA sample that will provide a known DNA profile ~~was a result of~~ the test.
- A **negative control** (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. ~~For DNA testing, negative controls include extraction blanks, reagent blanks, and amplification blanks.~~

3.4

DNA elimination database

~~A collection~~Collection of DNA profiles, held in a searchable format, from individuals whose access, role, and/or activities ~~are deemed~~present a potential DNA contamination risk. ~~Also included are including~~ possible contamination DNA profiles identified/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 can be detected.

3.43.5

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. ~~Such activities include swabbing at-risk equipment (e.g., in pre- and post-amplification testing areas) and surfaces to demonstrate they are DNA free.~~

3.6

drop-in

Presence of ~~spurious~~approximately 1 to 3 nonreproducible alleles. ~~Drop-in alleles are in DNA data where each allele is~~ assumed to originate/come from different individuals, whereas contamination consists of multiple alleles from one or more individuals.

3.53.7 interpretation (interpretable DNA profile)

~~The occurrence of drop-in alleles may be minimized~~process of evaluating DNA data for purposes including, but ~~cannot be avoided completely. If drop-in occurs then approximately 1 to 3 alleles are expected in addition to~~ not limited to, defining assumptions related to mixtures and single source profiles, distinguishing between alleles and artifacts, assessing the main profile(s). The numberpossibility of alleles which can reasonably be assumed to be drop-in as opposed to 'contamination' can be estimated by plotting a Poisson distribution^[16]. ~~The data are taken from negative controls. These data can be used to inform probabilistic genotyping models that take account of degradation, inhibition, and stochastic effects, and determining whether the phenomenon~~data are suitable for comparison.

3.63.8

Rapid DNA analysis

~~The fully~~Fully automated (hands-free) process of developing a STR profile. The, "swab in – profile out" process ~~consists of automated extraction, amplification, separation, detection and allele calling of developing a DNA profile from samples~~ without human intervention. ~~A modified Rapid DNA analysis requires human interpretation and technical review.~~

4 Requirements

4.1 General

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 [actively involved with laboratory procedures](#) 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 [cleaned and](#) 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 [sample evidence derivatives](#) shall be packaged and handled in a manner to minimize unwanted transfer of biological material.

4.2.4 Separate storage areas shall exist for reagents [and](#), 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 in post-PCR areas.

4.2.5 ~~Laboratory~~[The laboratory](#) shall arrange the working environment to mitigate potential contamination.

4.2.6 The laboratory shall have and follow a written, regularly scheduled, cleaning procedure to include laboratory areas, items to be cleaned, and [cleaning](#) frequency. The cleaning schedule shall be determined by volume, frequency, and nature of use.

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

4.2.8 When possible, the forensic 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 ~~for from~~ laboratory equipment, glassware, reagents, and consumables. These procedures may include UV irradiation, ethylene oxide treatments, bleach (or commercial equivalent) treatments etc., as appropriate.

4.2.10 The laboratory shall have a system to track ~~the reagent~~ lot numbers ~~of reagents~~ 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 cleaning agents or procedures for each method, technology, tool and instrument, and laboratory area. Cleaning 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 (~~i.e.g.~~, double bagging).

4.3.3 The laboratory shall have procedures and policies defined to reduce potential contamination events during evidence and ~~sample evidence derivative~~ processing to include the following.

a) The ~~laboratory shall require the~~ use of personal protective equipment.

~~b) The handling and packaging of evidence and samples to limit the possibility of contamination.~~

~~c) a) The laboratory shall examine and extract high template evidence (e.g., blood, semen, saliva) separately in time or space and independently from low template evidence (e.g., epithelial cells/touch DNA).~~

~~d) The laboratory shall separate in time or space the processing of reference samples from evidentiary items during the entirety of laboratory processing from screening through genetic analysis.~~

~~e) The laboratory shall limit the opening and examining of no more than one item of evidence at a time.~~

~~f) b) They require the~~ cleaning of work surfaces and examination tools with DNA destroying reagents or processes before new evidentiary items are examined.

c) The laboratory shall handle and package evidence and evidence derivatives to limit the possibility of contamination.

- d) The laboratory shall limit the opening and examination to one item of evidence at a time at each workstation
- e) The laboratory shall separate in time or space the processing of reference samples from evidentiary items
- f) The laboratory shall examine potential high template evidence (e.g., blood, semen, saliva) separately in time or space and independently from potential low-template evidence (e.g., epithelial cells/touch DNA).
- g) The laboratory shall 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 casefile when ~~and how~~ items of evidence are packaged together and ~~include such information in the casefile~~ how they were packaged.

4.3.5 The laboratory shall perform quality checks of extraction and PCR reagents prior to use in ~~testing~~ 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), stage of contamination 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 file.

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 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 timely manner.

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

4.3.8 Intra-batch comparisons (i.e., samples processed concurrently) to detect contamination shall be conducted.

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 A contamination assessment shall be conducted 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/or database samples to include:

- a) ~~comparing all~~[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) ~~documenting all each lab should determine a~~ likelihood ~~ratios (greater than 1) produced by~~ [comparison ratio threshold value to the report for comparisons to an](#) elimination database. [This should be documented](#) in the casefile and ~~tabulate them~~ in the report; ~~and~~.
- f) ~~identifying any potential contamination events when compared to a non-contributor (elimination database test^[1]).~~

4.3.11 Potential contamination events shall be investigated and referenced or documented within the case record. When contamination is identified, a root cause analysis^[12] shall be conducted and documented [and included within a casefile](#).

4.3.12 Records of contamination events shall ~~also~~ 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.

4.3.13 The laboratory shall have and follow protocols requiring reporting and communicating contamination events to [customers including](#) legal parties (i.e., prosecution and defense); ~~if known~~.

4.3.14 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 [health/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. 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;~~
- ~~f) post-contamination review.~~

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 the use of:

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

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 a pre-amplification room.

4.6.3 Rapid DNA consumables shall be monitored for extraneous DNA through the use of a positive sample control and negative sample control 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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