Forensic Laboratory Standards for Prevention, Monitoring, and Mitigation of DNA Contamination

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

OSAC Proposed Standard

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Prepared by
Biological Methods Subcommittee
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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 was revised, prepared and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board (ASB). The Biological Methods Subcommittee of the Organization of Scientific Area Committees developed the initial draft document.

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

Keywords: Forensic DNA contamination, elimination database

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1 Scope

This standard covers aspects of limiting, detecting, assessing the source of, and mitigating DNA contamination as applied to forensic and DNA database STR analysis via capillary electrophoresis and Rapid DNA analysis conducted in a laboratory. This standard does not cover methods of STR analysis specific to low-copy DNA samples or use of Rapid instrumentation outside of a laboratory environment.

2 Normative References

No applicable normative references.

3 Terms and Definitions

3.1. **Contamination** Exogenous DNA (deposited from the point of collection onwards) or other biological material in a DNA sample, PCR reaction, or item of evidence; extraneous DNA or biological material introduced during collection or testing of the sample.

3.2. **Controls** Samples of known types (positive and negative), run in parallel with experimental, reference, or evidence samples that are used to demonstrate that a procedure is working correctly.

3.3. **Developmental Validation** The acquisition of test data and determination of conditions and limitations of a new methodology; this generally occurs while the conditions and parameters are being worked out prior to the establishment of a defined assay, procedure, or product. Internal validation studies typically follow developmental validation studies. See internal validation.

3.4. **Elimination Database** A collection of DNA profiles held in a searchable format from individuals whose access, role, and/or activities are deemed a potential DNA contamination risk. Also included are possible contamination profiles recognized by the laboratory. The profiles, known as **contamination elimination profiles**, are used to detect instances of contamination.

3.5. **Internal Validation** The accumulation of test data within the laboratory for developing the laboratory standard operating procedures and demonstrating that the established protocols for the technical steps of the test and for data interpretation perform as expected in the laboratory.

3.6. **Rapid DNA Analysis** The fully automated (hands-free) process of developing an STR profile from a known reference sample. The "swab in – profile out" process consists of

automated extraction, amplification, separation, detection and allele calling without human intervention. A **modified Rapid DNA analysis** requires human interpretation and technical review.

3.7. **Tolerance Level** A contamination tolerance is the level of detection at or below which contamination does not interfere with a confident interpretation of the data.

4 Requirements

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

4.1.1. Access to laboratory areas shall be restricted to individuals actively focused on laboratory procedures to reduce the risk of introducing extraneous DNA into work areas and samples.

4.1.2. 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.

4.1.2.1. Separation of pre-and post-PCR areas shall be accomplished in part by the use of physical barriers (this includes floor-to-ceiling walls and closed doors).

4.1.2.2. Equipment, tools, and supplies dedicated to post-amplification areas shall not be moved outside the post-amplification area without first being cleaned/decontaminated. Such treatment will be appropriate to the items being moved.

4.1.2.3. Separate personal protective equipment will be dedicated to pre- and post-amplification areas.

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

4.1.3.1. Evidence items and samples shall be packaged and manipulated in a manner to minimize unwanted transfer of biological material.

4.1.4. Separate storage areas shall exist for reagents and consumables, DNA extracts, and PCR product (see also annex B1).

4.1.4.1. DNA extracts shall be stored in pre-PCR areas.

4.1.4.2. PCR products shall be stored in post-PCR areas.

4.1.5. The laboratory shall have and follow a written, regularly scheduled, cleaning

procedure to include lab areas, items to be cleaned, and the frequency of cleaning.

4.1.5.1. Laboratory work area surfaces and furnishings shall be able to withstand frequent cleaning and/or decontamination, *e.g.* bleaching.

4.1.5.2. Cleaning agents known to destroy DNA shall be used as appropriate to the items/surfaces being cleaned.

4.1.6. When purchasing amplification kits and the option exists, the laboratory shall procure them from an ISO 18385 compliant manufacturer. Other reagents and consumables shall be of a grade suitable for PCR applications.

Note: In addition to kit manufacturing precautions, the laboratory should institute procedures to minimize the possibility of contamination for other reagents and consumables. These procedures may include UV irradiation, ethylene oxide treatments, autoclaving, etc.

4.1.7. The laboratory shall have a system to track lot numbers of pre-amplification reagents and consumables involved in a potential contamination event.

4.2. Procedural Requirements

4.2.1. The laboratory shall define and use appropriate cleaning procedures for each method/technology/laboratory area.

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

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

4.2.2.2. Post-PCR waste shall not be transported through pre-PCR areas without adequate precautions (*i.e.* double bagging).

4.2.3. The laboratory shall have procedures and policies defined to reduce potential contamination events during evidence and sample processing that include the following:

4.2.3.1. Use of personal protective equipment.

4.2.3.2. Handling and packaging of samples/evidence to limit the possibility of contamination.

4.2.3.3. Quality checks of extraction and PCR reagents conducted prior to use in testing to monitor for contamination (*i.e.* checks of positive and negative controls for new lots prior to use in testing).

4.2.3.4. Cleaning procedures specific to tools and instruments (as indicated by validation and experience).

4.2.4. 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, and equipment, etc. to produce acceptable genetic data.

4.2.5. The laboratory shall establish a contamination tolerance level for each procedure/technology used. Tolerance levels shall be based on internal validation and be reassessed as applicable when a method /technology is modified (see Annex B).

4.2.6. The laboratory shall have procedures to detect contamination.

4.2.6.1. The laboratory shall use appropriate controls to monitor/detect contamination.

4.2.7. Procedures shall provide guidance on steps undertaken when contamination tolerances are exceeded.

4.2.8. Contamination events shall be investigated (see Annex B).

4.2.8.1. Guidance will also cover steps taken when a reagent, consumable, or critical instrument has been contaminated

4.2.9. The laboratory shall maintain and use a contamination elimination database to aid in identification of profiles not consistent with provided reference samples.

4.2.9.1. At a minimum, this database shall include biology staff and positive control samples. To the extent possible, typing shall use the same genetic markers/amplification test kit(s) utilized by the laboratory (see Annex B).

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

4.2.10. Contamination elimination profiles shall be added in a timely manner.

4.2.11. The laboratory shall conduct contamination and cross-contamination checks, and batch comparisons when profiles inconsistent with reference profiles are obtained, or when no reference samples are available. During a contamination event investigation, intra-batch comparisons should be undertaken as appropriate.

4.2.12. Records of contamination events shall be maintained in a centralized manner that allows such events to be tracked across cases, batches, and over time (see

Annex B).

4.2.13. The laboratory shall have procedures to address contamination events, and to document such events within appropriate test/case records (see Annex B).

4.2.14. The laboratory shall have and follow protocols for reporting and communicating contaminated results (those results outside of protocol tolerances). It is imperative that the customer be alerted to situations where contamination has or may have affected results (see Annex B).

4.3. Personnel and Training Requirements

4.3.1. Personnel defined by the laboratory shall receive documented practical training on those aspects of the laboratory's protocol covering the detection and minimization of contamination. Competency can be assessed based on satisfactory processing of forensic-type samples.

4.3.2. The laboratory shall have and follow a training policy covering the use of personal protective equipment, evidence/sample handling and packaging, cleaning/decontamination protocols, and quality control measures used to detect and minimize contamination.

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

4.4.1. Rapid DNA instrumentation shall be maintained in a pre-amplification room.

4.4.2. Rapid DNA consumables shall be monitored for extraneous DNA through a positive and negative control conducted with each received lot of reagents.

4.4.3. Personal protective equipment shall be worn while engaging in testing and use of Rapid DNA instrumentation.

4.4.4. Lot numbers for Rapid DNA consumables will be recorded and monitored.

Annex A
(informative)

Bibliography

The following information provides a limited list of literature resources related to DNA contamination prevention, monitoring, and mitigation. This list is not meant to be all-inclusive.[1] The laboratory shall develop a list tailored to its specific needs. Updated references shall be added to the laboratory's list as needed.

1] AABB, *Standards for Relationship Testing Laboratories*

2] ENFSI, Contamination prevention guidelines. Issue No 001, issue date: November 2010

3] FBI, *Quality Assurance Standards for DNA Databasing Laboratories, effective September 1, 2011. It is available at* https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view. Accessed on August 13, 2019.

4] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective July 1, 2020. It is available at https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf Accessed on August 13, 2019.

5] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS), effective September 11, 2011. It is available at* https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view. Accessed on August 13, 2019.

6] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, effective July 1, 2020. It is available at https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf Accessed on August 13, 2019.

7] FBI, Addendum to the *Quality Assurance Standards for DNA Databasing Laboratories performing Rapid DNA analysis or modified Rapid DNA analysis using a Rapid DNA instrument*

8] ISO Standard *Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes – Requirements,* ISO 19385:2016(E).

9] SWGDAM, *Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories.* It is available on the SWGDAM website, https://www.swgdam.org/

10] Kloosterman, Sjerps & Quak (2014). Error rates in forensic DNA analysis: Definition, numbers, impact and communication. FSI: Genetics, 12: 77-85.

11] Kloosterman, A.D. (2001). Credibility of forensic DNA typing is driven by stringent quality standards. Accreditation and Quality Assurance, 6: 409-414.

[1] A more comprehensive bibliography may be found within the SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories

12] Rutty, Watson & Davison (2000). DNA contamination of mortuary instruments and work surfaces: as significant problem in forensic practice. International Journal of Legal Medicine, 114: 56-60.

13] Thompson, W.C. Forensic DNA Evidence: The Myth of Infallibility. In Sheldon Krimsky & Jeremy Gruber (Eds.), Genetic Explanations: Sense and Nonsense. Harvard University Press, 2013, pp. 227-255.

14] ISO Standard *Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes – Requirements,* ISO 19385:2016(E).

Annex B
(normative)

Requirements – Supporting Information

Requirement-Specific Supporting Information

4.1.4 The intention of this standard on storage is to cause separation of DNA containing items from non-DNA containing items. When DNA is present, the intention is to distance those items that may have low quantities from those expected to have higher quantities. Within the strictures of the standards, the laboratory shall define appropriate use of storage locations.

4.2.5 Contamination tolerance levels are defined based on the methodology, technology, sensitivity requirements, and sample types as applicable to laboratory protocols (*e.g. autosomal STR, Y-STR,* negative controls and/ or reagent blank controls, positive controls, unknown samples, and reference samples). Genetic data detected below a laboratory's tolerance level has been determined not to interfere and may be disregarded. Tolerance levels may also allow different acceptability criteria of sample data based on presence or absence of specific exogenous data (match/ no match) observed in negative/reagent blank controls.

4.2.11 Documentation of contamination incidents within case records is critical to transparency and preparation for discovery.

4.2.7 Investigation of a contamination event typically includes actions taken to determine the procedural step where the contamination occurred during processing. The actions involve stepping sequentially backwards through the process and repeating a step to determine if the contaminant was reproducible and present at that point in the process.

4.2.8.1. Profiles added to a contamination elimination database (as legally allowable) should not be limited to biology staff and positive control samples. More effective use would be to include profiles from any individuals who are involved in the collection and handling of evidence, work samples, reagents, or consumables (*e.g.* staff, agency and other associated workers such as medical examiners, sexual assault nurses, service personnel and lab visitors.). It is also important to include contamination elimination profiles: unknown DNA profiles obtained from negative or positive controls, or profiles that have been putatively assigned as possible contaminant profiles (*e.g.* from consumables).

4.2.12 Tracking information should include a general description of the event, identifying information (date, case number), and outcome. This can aid in trend recognition, particularly for low-level events. It may provide information to prevent case-threatening situations.

4.2.13 Documentation of contamination incidents within case records is critical to transparency and preparation for discovery.

4.2.14 Documented contamination events shall be reported. As appropriate, laboratory reports and communications shall include an alert to potential contamination events. This may be accomplished by a statement specific to a particular event and/or by a more general statement reminding the reader that a report is a summary document (*e.g.* "This is a summary report and additional supporting information is available.").