



GUIDELINE FOR DNA CONTAMINATION MINIMIZATION IN DNA LABORATORIES			
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1. AIMS

This document aims to provide guidelines on the minimization (i.e., avoidance, control, detection and monitoring) of DNA contamination in a forensic DNA laboratory. This Guideline can be used by forensic laboratories of ENFSI as well as other laboratories to improve and harmonise their working practices regarding DNA contamination minimization. It may be used in combination with other quality management documents and procedures.

This Guideline reflects the scientifically accepted principles and applications at the time of creating.

2. SCOPE

The scope of this guideline is to provide the requirements and recommendations for forensic biology examinations and DNA analyses in order to prevent or minimize as well as to detect, monitor and handle the occurrence of DNA contamination in DNA laboratories.

This Guideline is aimed at experts in the field and assumes prior knowledge in the discipline.

3. TERMS AND DEFINITIONS

For the purposes of this Guideline, the relevant terms and definitions given in other ENFSI documents, the ILAC G19 “*Modules in a Forensic Science Process*”, as in standards like ISO 9000, ISO 17000 and 17025 apply as well as ISO 21043 [1–5].

Allelic drop in:

Additional random alleles present in a profile originating from fragmented sources and regarded as independent events.

Background

DNA that is present from unknown sources and unknown activities. It can be described as ‘foreign’ (non-self). It is not known how or why it is there. For example:

- 1) DNA underneath fingernails from unknown sources/activities.
- 2) Non-self-DNA on clothing from unknown sources/activities.
- 3) Non-self-DNA on a surface from unknown sources/activities.

Background does not include DNA from known individuals – this is known as prevalent DNA.

Contamination

Undesirable introduction of a substance to an item at any point in the forensic process.

Note 1 to entry: This includes undesirable transfer of a substance within an item or between items (also referred to as cross-contamination).

Note 2 to entry: Contamination may arise from various sources: 1) items, DNA samples 2) persons 3) consumables 4) laboratory environment such as equipment and benches.

Elimination data base (EDB)

Collection of DNA profiles held in a searchable format from personnel and visitors (e.g., service engineers) whose access/role/activities are deemed to be a potential DNA contamination risk. The data are used to identify instances of contamination. Also included are possible still unsourced contaminations identified by the DNA laboratory.

Environmental monitoring

A sampling and analytical process for monitoring the background levels of DNA on for example laboratory equipment, work areas and surfaces and also the effectiveness of the cleaning and decontamination procedures.

Equipment

Includes but it is not limited to measuring instruments, reference materials, reagents or consumables that is required for the correct performance of laboratory activities and that can influence the results. (Modified from ISO 17025 [4]).

Examination

Act or process of observing, searching, detecting, recording, prioritizing, collecting, analysing, measuring, comparing and/or interpreting.

Note 1 to entry: Examination can include collecting items from persons.

Incompatible activities

Activities that need to be separated spatially or temporally in order to prevent one from compromising the other.

Note 1 to entry: examples include: pre- and post-PCR procedures, analysis of high and low yield DNA items or samples, analysis of reference and crime scene samples.

Item

Item, object, substance or material that is collected, derived or sampled as part of the forensic process [5].

4. BACKGROUND

Due to the sensitivity of DNA analysis methods, DNA contamination risks need to be minimized and contamination monitored and managed.

This Guideline outlines the general principles and recommendations pertinent to the minimization of DNA contamination of items received, handled and analysed in the DNA laboratory in order to preserve the integrity of the items and the results. This is intended to prevent the consequences of reporting erroneous data attributed to contamination incidents.

In this Guideline the term “should” is used to indicate good practice and “shall” to indicate a strong recommendation. The word “shall” is also used where it relates to the accreditation requirements of the ISO 17025 Quality Assurance Standard [4].

5. GUIDELINES

5.1 General Guidelines

The DNA laboratory shall have a policy and procedure in place which defines what is considered to be a DNA contamination in various categories of samples analysed as well as the management of these contamination events. These sample categories should include negative controls to detect contamination at various stages throughout the process (for example: reagent blank, extraction blank, quantitation blank, PCR negative).

Defined limits should be in place also for environmental monitoring samples.

5.2 Staff

DNA contamination minimization procedures shall be included in staff education and training.

Competency testing of staff should include verification for compliance with DNA contamination minimization procedures and contamination assessments as documented in protocols and SOPs and used for training.

5.3 Facility

The facilities of an organisation are required to meet the “ENFSI DNA Working Group Quality Assurance Programme for DNA Laboratories” requirements in terms of separation for reference and crime scene items/samples [6]. More specifically:

- a) Separation of reference material/samples and crime scene items/samples collected from crime scene(s) during storage in the laboratory.
- b) Separation of reference material/samples and crime scene items/samples during processing (i.e. separation physically or in time).

When separation of working areas is not possible or dedicated equipment is not available, then decontamination of working areas and equipment between analyses and separation of reference and crime scene items/samples processing by time shall be implemented.

- a) Dedicated changing rooms/areas close to the laboratories are recommended.
- b) Laboratories, storage rooms and equipment should be easy to clean (i.e., smooth walls, floors).
- c) Laboratories should only be equipped with essential, frequently used equipment to minimize contamination risk.
- d) Access to laboratories and storage rooms shall be restricted to authorized personnel.

Post-PCR rooms shall be separated from the rest of the laboratory areas. Provision of negative air pressure or an airlock space between post-PCR and other laboratory areas should be considered.

Every laboratory area should have its own dedicated cleaning equipment.

In order to further minimize risk for contamination a sequential order or other solution reaching the same objective for cleaning the laboratory areas should be deployed.

Note 1 to entry: E.g. staff cleaning post-PCR areas will not continue with cleaning trace recovery or pre-PCR areas.

The laboratory should demonstrate that the cleaning and decontamination methods used are effective.

Cleaning personnel (internal or external) shall be trained in the use of laboratory specified cleaning methods.

5.4 Equipment

Each section/room/designated area of a forensic DNA laboratory should have its own dedicated equipment/reagents.

Equipment transferred between different laboratory areas/rooms shall be decontaminated before transfer and before use in the new location.

Equipment preventive maintenance procedures should be scheduled and implemented in order to prevent cross-contamination events as a result of instrument malfunction.

Automated systems using open tubes/plates shall include contamination risk analysis as determined through validation [7,8] before being placed in the routine workflow.

MPS systems should be placed in post-PCR areas.

A Rapid DNA system i.e., with integrated extraction, pre-/post-PCR processing and genetic analysis can be placed in either a dedicated area or in a post-PCR area.

The Rapid DNA system should not be placed in a pre-PCR area where other sample preparation and extraction is conducted that is not intended for RAPID DNA analysis.

When a Rapid DNA system is placed in a dedicated area, steps should be taken to separate crime scene and reference sample preparation to minimize cross-contamination of samples.

When a Rapid DNA system is placed in a post-PCR area, to minimize the potential risk of exposure to post-PCR products, the sample loading of the cartridge should be performed in a trace recovery area for crime scene samples or another area for reference samples, sealed and then taken to the Rapid DNA system [9–12].

5.5 Procedures

There shall be written procedures in relation to the following activities:
(see also section 5.9.1).

- a) Cleaning and decontaminating DNA designated working areas and equipment with attention also to robotic systems including accessories.
- b) Handling, transporting and storage of items and samples.
- c) Risk assessment and mitigation for DNA contamination.
- d) Detection and monitoring for DNA contamination (including environmental monitoring).
- e) Investigation of DNA contamination events.
- f) Follow up with corrective actions for minimization of DNA contamination.
- g) Management & use of the Elimination database, if in place.

5.6 Protective Clothing

Protective clothing shall be used throughout the workflow in the laboratory and changed when engaging in incompatible activities such as transfer from pre-PCR to post-PCR areas. This should include as appropriate: lab coats, gloves, arm sleeve covers, face mask, hair cover, snoods or beard covers and for post-PCR areas, shoe covers or designated shoes. Protective clothing should be changed frequently and shall be changed when there is a risk that they have been compromised. Gloves should be changed frequently and between items and before/after opening tubes and reaction plates containing samples.

A gowning/disrobing procedure should be in place in order to prevent contamination of the protective clothing used.

- a) If disposable lab coats are not used then each laboratory section should have its own dedicated lab coats. Dedicated lab coats should be laundered separately (e.g., item examination, pre-PCR, post-PCR) using an agreed effective cleaning and decontamination procedure. Swabs/tape-lifts may be taken from lab coats as part of the environmental monitoring procedure.

5.7 Consumables/Reagents

Every laboratory section should have its own dedicated consumables and reagents.

Molecular biology grade reagents and consumables should be used where necessary and if available.

Forensic DNA grade reagents and (disposable) consumables should be procured from manufacturers who are accredited or comply with ISO 18385:2016 [13] or comply with PAS 377:2023 [14].

Where possible, reagents should be divided and stored in small aliquots to minimize the frequency of use and contamination risk of bulk volumes.

Batch contamination testing should be performed before use as defined by the laboratory, in particular where testing results or certification is not available for commercial and in-house consumables and reagents, including those for RAPID DNA analysis.

5.8 Contamination Prevention in the Reception, Examination, Sampling and Analysis of Items

5.8.1 General considerations

Guidance should be made available to other departments/agencies/organizations for contamination prevention through optimization/testing and validation of proper packaging, transport and storage methods.

Guidance should also be made available for contamination prevention to other departments/agencies/organizations if items have to be sampled and/or examined before submission to the DNA laboratory.

The time that an item is placed on an examination table should be minimized.

The time that items/samples are held in open receptacles, tubes or containers shall be minimised.

5.8.2 Reception

Items received shall be checked to ensure that they conform with the requirements for preservation of their integrity and identity and shall include the inspection to verify that packaging has not been compromised in order to identify any contamination risks. Any deviation detected shall be appropriately handled and documented. This includes documentation of items that are delivered in the same packaging entailing a high risk of cross-contamination.

Reference and crime scene items received shall be kept separated after submission and stored in appropriate conditions throughout their retention in the facility.

5.8.3 Examination and Sampling

Precautions shall be taken to avoid contamination from the outside of the packaging to the item to be examined.

Reference and crime scene samples shall be examined and sampled as separate batches based on their origin (see section 5.3).

Items within the same case that are collected from different sites/crime scenes or from different persons (victim or suspect) should be examined separately in time or space (different rooms).

When possible, based on the case information and the nature of the items, an assessment shall be done to categorize items for examination and sampling in accordance with expected DNA yield, i.e. shall be separated into high yield and low yield DNA categories.

When examining an item, ideally low yield areas should be sampled first from those yielding high quantities of DNA which should be sampled last.

The handling and reopening of packaging should be minimized.

5.8.4 Analysis of Items

Reference and crime scene samples shall be analyzed in separate batches.

Items within the same case that are collected from different sites/crime scenes or from different persons (victim or suspect) should be analyzed separately.

High and low yield DNA categories should be analyzed as separate batches based on their origin (see section 5.3).

A unidirectional workflow should be in place for processing items and samples. Movement of a sample, equipment or person from a post-PCR area to a pre-PCR area should be minimised and shall only be allowed after thorough decontamination of equipment or change of protective clothing by the person (see also section 5.6), this also includes cleaning staff. In addition, where there is cross-over of incompatible activities there shall be a decontamination procedure in place.

5.9 Detection - Investigating Contamination - Monitoring

5.9.1 Detection of Contamination

Detection of contamination using quality control measures includes the following:

- a) A sufficient/representative number of reagent/extraction blanks (carried from start to end of the analytical process) and negative PCR controls shall be used for every series/run/batch of analyses (tests) as determined by the laboratory's validation studies and risk assessments.
- b) Intra- and inter-batch contamination assessments should be done using profile comparison tools (i.e. any software used to analyze casework results) in accordance with the laboratory's SOPs and risk assessments.
- c) DNA profile results should be checked against an elimination database (EDB) in accordance with the laboratory SOPs, if national legislation permits the use of data for this purpose.

- d) In relation to point (c) above, the laboratory should:
- Establish, maintain and process the EDB and handle “matches” with elimination profiles.
 - In the EDB, include biology/DNA laboratory staff, crime scene investigators, police officers transporting items to the DNA Laboratory, cleaning staff, service technicians and other visitors.
 - Consider including persons in the EDB that handle items prior to submission to the biology/DNA laboratory.

The laboratory shall define what is considered a DNA contamination that would interfere with the validity of the results (see section 5.1).

5.9.2 Investigating Contamination

The laboratory shall attempt to determine the following:

- a) The step where the contamination has most likely occurred (e.g. at the scene, during item handling, packaging, examination, sampling, extraction, quantification, amplification, electrophoresis).
- b) The potential activity that led to the contamination (e.g. procedure, human factor or equipment related causes).
- c) The potential source/root/origin of the contaminant (e.g. personnel, reference sample, scene item, or consumable).

Information from the root cause analysis shall be used for the formulation of mitigation/improvement steps.

Unknown profile contaminations originating from consumables should be shared with the ICMP Elimination Database.

5.9.3 Monitoring

The laboratory should at least record the following information concerning the traceability of contaminations for downstream investigations:

- a) Contamination sources concerning point (5.9.2) above should be identified where possible and recorded.
- b) Contamination count vs. total number of samples processed (i.e. contamination rate) should be recorded in the sample categories:
 - Negative controls
 - Reagent blanks
 - Reference samples
 - Crime scene samples

5.10 Contamination Trend Monitoring

Trend monitoring procedures shall be in place and documented. Test results from monitoring shall be recorded, reviewed and any detected contamination managed in accordance with section 5.9. This should include the following categories:

- a) Evaluate contamination trends for control samples.
- b) Evaluate contamination trends for traces and references.

- c) Evaluate environmental monitoring data.

In case of a trend of contamination events, the lab shall implement mitigation/corrective actions as necessary.

5.11 Implementing New Methods and Techniques

Validation tests shall incorporate the evaluation of the risk of contamination.

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7. AMENDMENTS TO PREVIOUS VERSION

This guideline is a complete re-write of the previous version. For traceability on changes made we refer the reader to the previous version numbered “002” that is available on the ENFSI website.

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