Scientific Working Group on DNA Analysis Methods

Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories

Table of Contents

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of scientists representing Federal, State, and Local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, Committees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. These guidelines were presented to the SWGDAM membership and received approval on XXXX.

This document provides best practices and guidance for the prevention and detection of DNA contamination as applied to forensic casework and DNA databasing. This document is intended to apply to, but not limited to, laboratories that employ autosomal STRs, Y-STRs, mitochondrial DNA analysis, and enhanced detection methods. The FBI Quality Assurance Standards for Forensic DNA Testing Laboratories and DNA Databasing Laboratories (QAS) define contamination as the unintentional introduction of exogenous DNA into a DNA sample or PCR reaction; therefore, this document refers to

contamination introduced at or after the start of a controlled forensic process. The start of a controlled forensic process can be defined by a laboratory as the arrival of the item at the laboratory, the moment a processor (analyst or technician) handles an item or another defined control point in the process. As human DNA is pervasive throughout the environment, contamination may not be completely avoided. In addition, the improved sensitivity of DNA methodologies and the introduction of new DNA technologies may allow low-level or previously undetected contamination to be detected and potentially cause DNA interpretational difficulties. The QAS requires laboratories to have and follow a documented policy for the detection and control of contamination. These recommendations are intended to provide guidance in meeting this standard for all laboratories that conduct forensic serological and/or DNA analysis. Because these are guidelines and not minimum standards, in the event of a conflict between the QAS and these guidelines, the QAS has precedence over these guidelines. Additionally, to avoid any such conflict, the term 'shall' has been used when that term is similarly used in the QAS and the use of the term 'shall' is not intended to transform these guidelines into standards. Laboratories conducting forensic serological and/or DNA analysis are encouraged to review their standard operating procedures and validation protocols in light of these guidelines and to update their procedures as needed. Some recommendations as written are not feasible or necessary for every laboratory, particularly when considering limitations of laboratory spaces and buildings, procedures, and the sensitivity of the DNA testing being performed. If these recommendations are not currently feasible, a laboratory should consider other mechanisms for achieving the intent of these recommendations.

These guidelines should not be applied retroactively. It is anticipated that these guidelines will be updated as needed.

Introduction

Refer to the QAS for definitions and standards that relate to contamination. In accordance with the QAS, accredited laboratories shall have procedures to minimize contamination, perform

contamination assessments during validations and have policies for the detection and control of contamination.

1. Contamination Sources

1.1 A contaminant is unintentionally introduced into a sample by various means. A sample that is expected to be a mixture of DNA from more than one individual given the sample context (e.g., vaginal swab containing semen) does not constitute a contaminated sample. Sources of contamination include, but are not limited to:

- 1.1.1 DNA from laboratory personnel to an evidentiary item or DNA sample.
- 1.1.2 DNA from contaminated reagents or consumables to an evidentiary item or DNA sample.
- 1.1.3 Cross contamination of an evidentiary item or DNA sample to another evidentiary item or DNA sample.
- 1.1.4 Laboratory environment (e.g., surfaces, equipment, ventilation system) to an evidentiary item or DNA sample.

1.2 Contamination can occur directly or indirectly.

- 1.2.1 Direct contamination involves the transfer of DNA from the source of the contamination to the evidentiary item or DNA sample. This may occur when laboratory personnel handle an evidentiary item or DNA sample but may also occur without direct physical contact, such as speaking, sneezing or coughing on an evidentiary item or DNA sample.
- 1.2.2 Indirect contamination (i.e., secondary transfer) is a result of the transfer of DNA from the source of contamination to the evidentiary item or DNA sample through an intermediary such as pens, packaging and laboratory surfaces.

2. Contamination Prevention and Control

2.1 Laboratory design

When designing a laboratory and the flow of activities within, several features can contribute to the prevention of contamination. These features include, but are not limited to:

2.1.1 Separate work areas. Due to the high concentration of amplified DNA in a PCR sample, laboratories must have designated spaces for pre- and post- PCR activities (refer to QAS regarding facilities). Evidence examination, DNA extraction, and pre-amplification set-up activities must be restricted to the pre-PCR area while the post-PCR area is limited to PCR amplification and all analytical processes using the products of PCR amplification. For guidance regarding Rapid DNA instruments, refer to the FBI Rapid DNA Addendum to QAS document. Other instruments that perform both pre- and post-PCR activities may require a dedicated space.

2.1.1.1 Recommended features of the pre-PCR area include, but are not limited to:

2.1.1.1.1 Restricted access to appropriate laboratory personnel only.

2.1.1.1.2 Activity limited to conducting laboratory procedures. Laboratory personnel should avoid casual conversations and loitering.

2.1.1.1.3 Designated areas that house all necessary personal protective equipment, hooks for lab coats and operational sinks with soap and disposable towels. These areas may be immediately adjacent to but physically separated from the pre-PCR area.

2.1.1.1.4 Walls and floors made of materials that are easy to clean and can withstand bleaching or other cleaning methods.

2.1.1.1.5 Laboratory furniture, benches and chairs that can withstand frequent cleaning. Chairs should be covered in a non-porous material (e.g., vinyl).

2.1.1.1.6 Dedicated equipment and tools (e.g., centrifuges, pens, tube racks). These can be dedicated for use by a particular processor, for a specific piece of equipment, or for a defined location.

2.1.1.1.7 Separate the preparation and storage areas for reagents away from DNA extracts and evidentiary items.

2.1.1.1.8 Circulation-free or filtered air. This can be accomplished by the use of a dead-air hood or laminar flow hood at a location dedicated to a particular task(s) or incorporated into laboratory-wide ventilation system.

2.1.1.1.9 Positive air pressure that is higher than the positive or ambient pressure in the adjacent common area or hallway. Air should flow from clean spaces to less clean spaces. This recommendation can refer to the entire laboratory space or a specific area.

2.1.1.2 Recommended features of the post-PCR area include, but are not limited to:

2.1.1.2.1 Filtered outgoing air. Refer to laboratory air quality documents listed in the references section.

2.1.1.2.2 Negative or lower positive air pressure than the air pressure in the adjacent common area or hallway.

2.1.1.2.3 Designated areas that house all necessary personal protective equipment, hooks for lab coats and operational sinks with soap and disposable towels.

2.1.1.2.4 Dedicated equipment and tools (e.g., pipettors, pens). These can be dedicated for use by a particular processor, a particular methodology or technology, for a specific piece of equipment, or for a defined area.

2.1.1.2.5 Separate the preparation and storage areas for reagents and PCR products.

2.1.2 Separate processing in pre-PCR areas by case type. References should be processed separately from evidentiary items by area and/or time. High copy evidentiary samples (e.g., blood) should be processed separately from low copy evidentiary samples (e.g., touch, hair, bone) by area and/or time. If the same work area is used, it should be thoroughly cleaned between the processing of different case types.

2.1.3 To the extent possible, limit the examination of evidentiary items in a DNA clean area to items that will require DNA extraction and analysis (i.e., exclude items intended for other forensic disciplines).

2.1.4 To the extent possible, limit the movement of laboratory personnel:

2.1.4.1 From a post-PCR area back into a pre-PCR area within a single work day.

2.1.4.2 From entering an area dedicated to the processing of high copy or reference samples to an area dedicated to the processing of low copy or evidentiary items within a single work day.

2.2 Procedure planning

When implementing or revising a procedure, the risk of contamination at each step needs to be assessed. This contamination assessment may include:

2.2.1 Determining the impact of implementing new technologies that increase the sensitivity of DNA detection and in turn, the detection of contamination. The laboratory should consider the impact of the flow of work processes, laboratory space configurations or analysis procedures to reduce and detect contamination. When assessing contamination risks with a new technology, a laboratory can research the current literature, consult with other laboratories using the new technology and/or perform preliminary work with the new technology.

2.2.2 Defining the tolerance level of contamination for each procedure and technology through validation. See Appendix 1.

2.2.3 Determining the extent of decontamination necessary for reagents, consumables, surfaces, tools, etc. for the procedure and that the method of decontamination performed is effective.

2.2.4 Incorporating the following quality measures into a validation:

2.2.4.1 Assessing all controls (negative, reagent blank, and positive) for the presence of any source of contamination.

2.2.4.1.1 Assessing the possibility of carry-over contamination on robotic systems by alternating a known sample or positive control and negative controls, for example using a zebra and/or checkerboard pattern.

2.2.4.2 Performing a contamination investigation, if contamination is detected. Refer to section 3 on contamination detection for guidance.

2.2.4.3 Implementing modifications to a procedure as necessary to minimize the risk of contamination. These modifications can be re-assessed to determine if implementation is successful. Modifications to be considered include, but are not limited to:

2.2.4.3.1 Items listed in section 2.1.

2.2.4.3.2 Limit the movement of equipment and tools from a post-PCR area back into a pre-PCR area. If movement of equipment and tools back into a pre-PCR area is required, it should only occur after decontamination.

2.2.4.3.3 Limit access to DNA areas to persons included in the laboratory's elimination database.

2.2.4.3.4 Use only aerosol-resistant pipet tips for DNA extraction, pre-amplification set-up and post-PCR processing. This may not be possible with automated or robotic systems that use fixed or non-aerosol-resistant tips.

2.2.4.3.5 Consider the number of samples processed in a batch. Several factors may be considered in determining batch size: method (manual or automated), minimizing processor fatigue, experience level of processor and type of samples.

2.2.4.3.6 Reduce the number of transfers or sample manipulations, so as to minimize the creation of aerosols and drips. Incorporation of liquid robotic handlers may be used for sample transfers and minimizing the creation of aerosols.

2.2.4.3.7 Incorporate additional cleaning or decontamination steps such as wiping the exterior of tubes, racks, tools (e.g., scalpels, tweezers, punchers) and equipment with bleach or ethanol. Incorporate UV crosslinking of pre-PCR hoods or areas.

2.2.4.3.8 Limit the amount of time and number of uncapped tubes open at one time. 96-well plates should be covered or sealed as soon as possible.

2.2.4.3.9 Incorporate robotics to reduce human contamination; however, programs should be designed that avoid moving pipet tips that contain or previously contained a sample over other samples.

2.2.4.3.10 Minimize risk of cross contamination when opening seals on 96-well plates by first centrifuging and then slowly removing the seal (or use puncture method to collect sample directly from an individual well).

2.3 Personal Protective Equipment

A variety of personal protective equipment (PPE) can be employed to not only protect laboratory personnel from hazardous chemicals and biological substances, but also to control or minimize contamination. The donning of and the removal of PPE should occur upon entrance into and exit out of a common area of the laboratory. PPE should be worn

by all individuals upon entering a DNA work space. These PPE items include, but are not limited to:

2.3.1 Laboratory coats

2.3.1.1 Coats should be dedicated to specific areas, such as pre- and post-PCR areas and can be dedicated to specific activities (evidence examination and DNA extraction separate from those used for pre-amplification set-up).

2.3.1.2 Coats can be made of disposable material that are discarded after each use or after a defined amount of time or number of cases/samples processed or of cotton fabric that undergo frequent cleaning.

2.3.1.3 If examining heavily soiled evidentiary items, coats should be changed immediately after examination and before continuing to examine other evidentiary items or cases. Soiled coats should be disposed of or washed before next use.

2.3.1.4 Coats dedicated to a particular area and/or activity should not be worn outside the dedicated area.

2.3.1.5 Other PPE, such as disposable coat sleeves or aprons, can be worn over a laboratory coat to provide an additional measure of contamination control and personal protection. If these items are used, they should be disposed of after examining each case, extracting a batch or setting up amplifications. The underlying laboratory coat may not need to be changed as frequently if these additional types of PPE are used.

2.3.2 Gloves

2.3.2.1 All laboratory personnel working in a DNA work space should wear disposable gloves at all times.

2.3.2.2 Gloves may be wiped with bleach after donning.

2.3.2.3 Gloves should be changed or wiped with bleach frequently throughout each activity. This is especially important after contact with a potentially

contaminated surface or item such as packages, phones, pens, door handles, face, eye glasses, etc.

2.3.2.4 Multiple layers of gloves can be worn during each activity. The outer set of gloves should be changed or removed after contact with potentially contaminated surfaces.

2.3.2.5 The cuff of the gloves should be of sufficient length to reach and cover the end of the coat sleeve. Disposable laboratory sleeves can be used if the gap cannot be covered.

2.3.2.6 Gloves may need to be changed during the examination of an item if an item is heavily soiled or wet.

2.3.2.7 Gloves should be changed after a set of transfer steps within a procedure (e.g., after all supernatants have been transferred to concentrators in an organic extraction) and between DNA extraction batches. Gloves may need to be changed during DNA extraction if an incident such as a cracked tube, dripping or spilling occurs.

2.3.3 Face masks or shields

2.3.3.1 A disposable face mask should completely cover the mouth and nose.

2.3.3.2 A disposable or non-disposable face shield should completely cover the mouth, nose and eyes.

2.3.3.3 A face mask or shield should be dedicated to a specific activity and discarded after the activity is complete. If using a non-disposable face shield, the shield should be thoroughly cleaned between uses with an appropriate cleaner, such as, but not limited to, bleach or ethanol.

2.3.3.4 If a face mask or shield is adjusted with a gloved hand, the glove should be changed before proceeding to the next procedural step.

2.3.4 Hair covers

2.3.4.1 A laboratory can utilize disposable hair and beard covers as an additional precaution against contamination of laboratory personnel to evidentiary items or DNA samples.

2.3.4.2 Hair covers should completely cover head and facial hair.

2.3.4.3 Hair covers should be dedicated to a specific activity and discarded after the activity is complete.

2.4 Personnel

2.4.1 All laboratory personnel should be trained to recognize their role in contamination prevention and control. This training can be included in the training manual and assessed during training exercises.

2.4.2 If a laboratory has the opportunity, contamination prevention and control training can extend outside of the DNA laboratory to other personnel who participate in the collection or processing of evidentiary items.

2.5 Evidence Examination and Sampling

2.5.1 Limit the examination of evidentiary items in a DNA clean area to items that will require DNA extraction and analysis (i.e., exclude items intended for other forensic disciplines). If a DNA clean area is not available for very large items, a separate examination area can be identified and decontaminated before proceeding.

2.5.2 Any issue with the integrity of the packaging (e.g., tears, leaks, unusual stains) should be noted. If the issue is of concern, the laboratory may choose not to accept an item for testing. Reuse of evidence packaging or biological stains on the exterior of packaging may pose a contamination issue.

2.5.3 Depending on the type of material, the outer packaging can be wiped down using pre-moistened disinfecting cloths or bleach.

2.5.4 To reduce transfer from the outer package(s) to the evidentiary items, gloves should be changed before proceeding with examination.

2.5.5 Non-disposable tools (e.g., scissors, razors, forceps, etc.) coming into contact with packaging should be cleaned before and after use. The same tool should not be in contact with evidence before cleaning.

2.5.6 Items can be examined on disposable paper or pads where practical. These should be discarded immediately after use and before the examination of a new item.

2.5.7 Gloves should be changed between examinations of different evidentiary items.

2.5.8 Non-disposable tools used to collect each evidentiary sample should be cleaned before and after collection.

2.6 Cleaning

Routine cleaning is critical for reducing contamination. There are a variety of cleaning and detergent products and procedures available to assist in removing or damaging DNA so that it cannot be amplified. The most commonly used chemicals for cleaning are sodium hypochlorite (i.e., bleach) and ethanol. When cleaning with bleach, use a freshly prepared dilution as its effectiveness declines over time.

2.6.1 Pre-PCR areas:

2.6.1.1 Entire pre-PCR areas should be decontaminated on a routine basis as dictated by the volume and frequency of use. This cleaning can include, but is not limited to:

2.6.1.1.1 Bench surfaces.

2.6.1.1.2 Equipment such as centrifuges, microscopes, automated instruments, keyboards and hoods.

2.6.1.1.3 Handles on doors, refrigerators, freezers and evidence lockers.

2.6.1.2 On a daily basis or immediately before and after use, equipment such as dead-air hoods, laminar flow hoods, centrifuges, and pipettors should be decontaminated.

2.6.1.3 Items such as bench surfaces, examination hoods and other equipment (e.g., microscopes, pens, keyboards, etc.) should be decontaminated.

2.6.1.4 Multiple cleaning schedules (e.g., weekly, monthly) can be incorporated for additional measures of contamination control. Dedicated cleaning equipment (e.g., mops) should be used. This less frequent cleaning can include, but is not limited to:

2.6.1.4.1 Chairs

2.6.1.4.2 Furniture (shelving, drawers, handles on drawers)

2.6.1.4.3 Floors

2.6.1.4.4 Walls

2.6.1.4.5 Doors

2.6.1.4.6 Vents

2.6.1.5 Any repaired equipment should be decontaminated before resuming use.

2.6.2 Post-PCR areas:

2.6.2.1 Post-PCR areas may be decontaminated with dedicated cleaning equipment on a routine basis as dictated by the volume and frequency of use.

2.6.2.2 In accordance with laboratory policy, discard amplification products and plate preparations.

2.6.2.3 Any repaired equipment should be decontaminated before resuming use.

2.7 Reagents and Consumables

Reagents and consumables used in sample collection, DNA extraction or amplification can become contaminated during the manufacturing process or packaging. When feasible, reagents and consumables should be purchased from an ISO 18385 compliant manufacturer. Laboratories can have procedures in place to detect this type of contamination before the reagent or consumable is used in casework; however, recognize it is impossible to completely guard against this type of contamination as the level of manufacturer contamination may vary across a single lot or package of reagent or

consumable product. Laboratories should document lot numbers of reagents and consumables for the purpose of tracking potential contamination.

2.7.1 Any in-house prepared or purchased reagent involved in DNA collection, extraction or amplification, should undergo a quality check using the procedure for which the reagent is intended.

2.7.1.1 If possible, outer packaging and reagent bottles should be wiped down before opening with pre-moistened disinfecting cloths or bleach.

2.7.1.2 In-house reagents should be prepared in a designated reagent preparation area with thoroughly cleaned glassware or disposable single-use utensils and containers. In-house reagents may be autoclaved before use.

2.7.1.3 Reagents should be verified to be free of contaminants or below the laboratory's established tolerance level with the relevant procedure/ technology using the most sensitive parameters.

2.7.1.4 A collection of reagents used for a particular procedure can be verified together; however, if any individual reagent is replenished with a new lot, another performance check should be conducted.

2.7.1.5 If contamination is detected, additional testing may be performed to determine which reagent(s) is (are) the source of the contamination or the collection of reagents can be considered contaminated as a whole.

2.7.2 Depending on the chemical composition, some consumables may be autoclaved or UV crosslinked before use.

2.7.2.1 Before use in casework, determine if new consumables can tolerate autoclave and/or UV exposure. This may be previously determined by the manufacturer or available in literature.

2.7.2.2 Routine performance check of UV crosslinkers should be performed. Follow operator's manual for procedure to test the intensity of the bulbs.

2.7.3 In an effort to offer DNA-free products, some manufacturers pre-sterilize consumables by a variety of methods, such as ethylene oxide or irradiation. If

a laboratory chooses to use pre-sterilized consumables, quality checks should be conducted to verify that the pre-sterilization method does not have an adverse effect on either the recovery or the amplification of DNA. Previously typed DNA extracts (e.g., proficiencies and other control or known samples) can be used to verify the performance of the new consumable.

3. Contamination Detection

Despite employing numerous measures to prevent contamination, contamination incidents will be encountered on occasion. Therefore, a laboratory should define a tolerance level based on each methodology, technology and sensitivity requirements. Any genetic data detected below a laboratory's tolerance level may be disregarded. Detecting contamination incidents is critical to improving laboratory procedures and ensuring the accuracy of reported genetic data.

3.1 Controls

Positive, negative and reagent blank controls are critical for detecting contamination.

3.1.1 Negative and reagent blank controls

3.1.1.1 Any detectable peaks or sequence data in negative and reagent blank controls may indicate contamination. Refer to Appendix 1 for examples regarding acceptability of associated data.

3.1.2 Positive controls

3.1.2.1 If any detectable peaks or sequence data are observed beyond the known profile of the positive control, the extraneous data may originate from a contaminant.

3.1.2.1.1 In STR systems, contamination is suspected when unexplained allelic peaks above the analytical threshold are observed. Caution should be used when considering peaks in positions of stutter, incomplete terminal nucleotide addition (minus A) and spectral pull-up.

3.1.2.1.2 In mitochondrial DNA sequencing, contamination is suspected if a mixture is present.

3.2 Material Controls/Blanks

Laboratories can request and process material controls/blanks (e.g., water used for swabbing) from crime scene investigators. Any detectable peaks or sequence data from a material control/blank may indicate contamination.

3.3 Samples

Samples that are expected to be single source may indicate a contamination event by producing a mixed genetic profile.

3.4 Unexpected results

Genetic data that do not conform to case circumstances (e.g., a male profile was obtained when a female profile was expected or a mixture was obtained from a single source sample) may indicate a contamination event has occurred. An attempt should be made to identify the source of the profile. A laboratory can conduct additional testing if circumstances warrant further investigation.

3.5 Comparisons

An attempt should be made to identify the source of a contaminant by comparing the genetic data from the contaminant to genetic data originating from various sources. As a result of identifying the source, a laboratory can take measures to prevent additional contamination events.

3.5.1 The following sources should be compared to the genetic data of the contaminant:

3.5.1.1 Samples processed in the same batch.

3.5.1.2 Samples from other batches processed at the same time by the same processor.

3.5.1.3 Samples from other batches processed at the same time by different processors.

3.5.1.4 Previously processed samples and batches.

3.5.1.5 A laboratory elimination database that contains the genetic profiles generated from all applicable technologies from the following (as applicable laws and policy permits):

3.5.1.5.1 Laboratory personnel.

3.5.1.5.2 Non-laboratory personnel that have access to the laboratory (e.g., maintenance and visitors).

3.5.1.5.3 Non-laboratory personnel that have had contact with the items prior to processing (e.g., law enforcement).

3.5.1.5.4 Previously observed contaminants including those from contaminated reagents and consumables.

3.5.2 Comparisons can be performed manually or with software that houses both the genetic profiles processed in the laboratory and the laboratory elimination database.

3.5.3 A laboratory may not be able to determine the true source or identity of a contaminant if the genetic data are sporadic, low-level or not represented in a laboratory elimination database.

4. Contamination Investigation and Management

4.1 Introduction of contaminant

Whether or not the source of the contaminant can be identified, actions can be taken to determine the procedural step in which the contaminant was introduced. Generally, repeating the procedural steps in reverse order will assist in this process and may even resolve the situation so that data originating from the evidentiary or reference item can be reported. The following actions can be used to investigate the introduction of the contaminant:

4.1.1 Re-injecting or re-loading a sample. This may resolve carry-over contamination from a neighboring well or a contaminant that was introduced while preparing the sample for detection/analysis on an instrument.

4.1.2 Repeat amplification and/or sequencing if sufficient extracted DNA is available. This may resolve contamination introduced from the processor or another sample amplified and/or sequenced in the same batch.

4.1.3 Repeat the DNA extraction if sufficient evidentiary material is available. This may resolve contamination introduced from the processor, cross-sample contamination from another sample extracted in the same batch or examined on the same day or contamination from a reagent or consumable.

4.1.4 Swipe or swab tests can be used to investigate if the source of a contaminant is part of the laboratory environment. This process can include, but is not limited to, the following aspects:

4.1.4.1 Swabbing of selected areas and equipment that make contact with processors, evidentiary items or DNA extracts and are suspected to be involved in the contamination event.

4.1.4.2 Swabs used for monitoring may be verified to be free of DNA using the laboratory's most relevant testing procedure and technology prior to commencing swabbing.

4.1.4.3 After swabbing, the laboratory's most relevant testing procedure and technology should be used to detect the contamination.

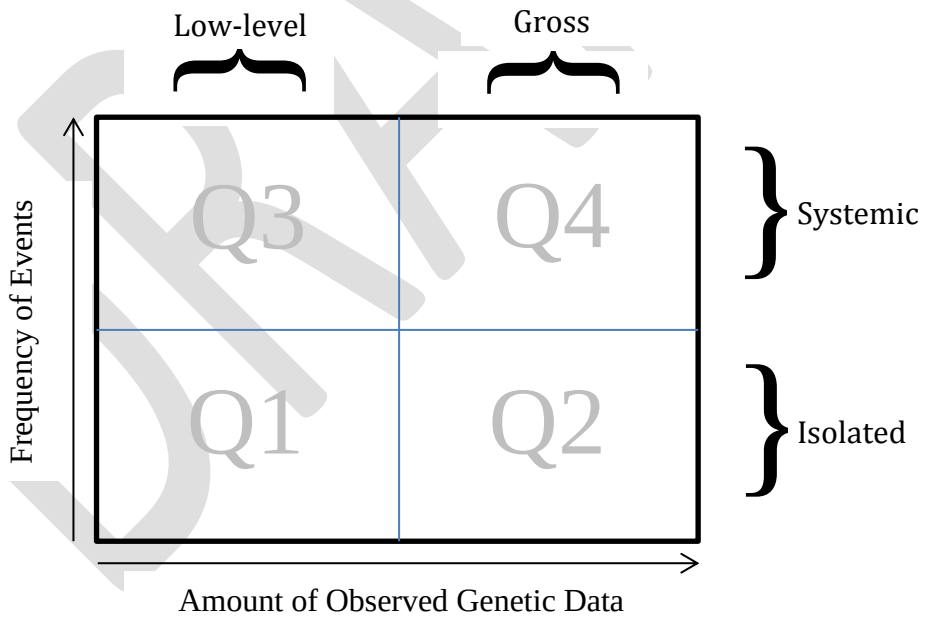
4.1.4.4 The amount of swabbing should be proportional to the amount of activity, the number of processors and/or items processed in a particular area suspected to be involved in the contamination event.

4.1.4.5 The results of swipe or swab tests should be documented. The documentation may include the locations of each swabbing, the genetic data observed at each location and the comparison of the genetic data against a laboratory's elimination database and contamination records.

4.1.4.6 Swipe and swab tests can be used as a preventive measure after maintenance has been performed, and after the reconfiguration or relocation of an entire laboratory or single laboratory space.

4.2 Investigate the problem

The level of a laboratory's response to a contamination event is generally determined by the amount of genetic data observed and the frequency with which the genetic data are being observed. Contamination events may be considered isolated events if the contamination only occurs once or infrequently as defined by the laboratory. If contamination events are repetitive (either by the same processor, the same procedure by multiple processors and/or the same contaminant is observed), this is indicative of a potential systemic problem and warrants further investigation. Contamination events may be considered low-level if the contaminant produces sporadic or small amounts of genetic data. If the contaminant produces full or nearly full genetic profiles above tolerance level, this is considered gross contamination due to an elevated concentration of contaminating DNA. This concept can be visualized in the table below.



4.2.1 Isolated contamination

Quadrants 1 and 2 represent random contamination events that occur infrequently and may be determined to originate from various sources such as the processor, another sample processed in the laboratory or an item contaminated before arriving at the laboratory. In some cases, particularly quadrant 1 contamination, the source of the contaminant cannot be determined. Depending on a laboratory's tolerance level and the sensitivity of the particular methodology and technology, these contamination events may warrant minimal investigation. However, tracking of these events is critical for the detection of systemic contamination.

4.2.2 Systemic contamination

If a contamination event that was originally classified as quadrant 1 or 2 contamination becomes repetitive over a particular timeframe, this is indicative of a systemic problem and warrants further investigation. The source of the systemic contamination, particularly quadrant 3 contamination, may be unidentified. A laboratory should define when contamination falls into quadrants 3 and 4.

4.2.2.1 Common denominator

The goal of a contamination investigation is to determine the common denominator contributing to the repetitive contamination events so that corrective measures can be taken to prevent reoccurrence. The common denominator is not always as obvious as a contaminant originating from the same source. Additionally, systemic contamination may have more than one common denominator. The following common denominators should be considered:

4.2.2.1.1 Common processor. Laboratory personnel may repeatedly fail to comply with contamination control measures. Systemic contamination of this type may manifest as the repeated

appearance of the processor's genetic profile in controls or samples or the processor may repeatedly cross contaminate samples within a batch.

4.2.2.1.2 Common procedure. Contamination may appear in controls or samples processed independently by different laboratory personnel using the same procedure. Systemic contamination of this type may be introduced at the same procedural step resulting in contaminants from various sources. A contamination assessment should be performed resulting in the implementation of procedural modifications as necessary (see section 2.2).

4.2.2.1.3 Common source. The same genetic profile may repeatedly appear in controls or samples processed by the same or different laboratory personnel using the same or different procedure. Low-level contamination (quadrant 3) may be difficult to assess if the genetic data do not overlap by common loci or sequence. Systemic contamination of this type may originate from contaminated reagents or consumables, the gross contamination of a high quantity sample, contamination from laboratory personnel or non-laboratory personnel or the laboratory environment.

4.2.2.2 Root cause analysis

A laboratory should define which level(s) of contamination warrant a root cause analysis. Documentation of all contamination events, isolated and systemic, is needed to accurately determine the root cause(s) of systemic contamination. The root cause may be a combination of several factors including single or multiple laboratory personnel, inadequate or ineffective contamination control measures, procedural deficiencies and manufacturer contamination.

4.3 Corrective measures

Once a systemic contamination event as defined by the laboratory is detected, the appropriate actions should be taken to mitigate the impact of the contamination event on casework. A laboratory may choose to implement one or more of the following actions:

4.3.1 Suspension of casework

As necessary, a laboratory may need to cease current and future processing involving a single processor, a particular procedure, laboratory space or the entire laboratory. Processing can resume once a contamination investigation is complete and contamination control measures are modified as necessary.

4.3.2 Decontamination

All affected laboratory areas should be thoroughly cleaned. Refer to section 2.6 for guidance on cleaning.

4.3.2.1 A thorough cleaning may be followed by monitoring the effectiveness of the cleaning using swipe or swab tests or through other mechanisms for monitoring contamination. Refer to section 4.1.4 for guidance.

4.3.3 Review of casework

As appropriate, the results of current and previous casework may need to be reviewed to ensure that a contaminant profile was not erroneously reported or did not hinder the reporting of the correct genetic data. Supplemental or amended reports may be warranted.

4.3.4 Reevaluation of procedures

Previous contamination assessments may not have adequately identified contamination risks and/or procedures may not have sufficiently addressed how to execute contamination control measures. Procedures should be revised as needed to include or clarify measures of contamination control. Refer to section 2 for guidance on minimizing contamination.

4.3.5 Retraining

Laboratory personnel, either individually or as a whole, may need to be retrained to understand and exercise contamination control measures. If possible, retraining may extend outside of the DNA laboratory to other individuals who participate in the collection or processing of evidentiary items and may have been the source of a contaminant.

4.3.6 Post contamination review

A laboratory should review the effectiveness of any procedural modifications and/or training that were implemented as a result of a contamination event.

4.4 Documentation

Contamination events that exceed the laboratory's established tolerance level, regardless of the severity, should be documented. This documentation is necessary in the event systemic contamination occurs. Documentation may be stored in individual case files, but should also reside in a single composite location, electronic or paper, for the purpose of detecting contamination trends.

4.4.1 Isolated contamination

4.4.1.1 A laboratory's documentation can include the contaminant profile, the source if known, the procedural step that may have introduced the contaminant and a list of the cases affected. Any corrective actions taken should also be documented.

4.4.2 Systemic contamination

4.4.2.1 A laboratory's documentation should include all items listed under isolated contamination section including the corrective actions taken, any procedural changes implemented, and a post contamination review.

4.4.3 Case file documentation

The contamination event should be documented in the individual case file(s) of the case(s) affected. Supplemental or amended reports may be warranted.

633 4.5 Management review

634 Laboratory management should periodically review contamination documentation in an
635 effort to continue any process improvements, identify emerging patterns that may need
636 monitoring and to detect any change in the rate of contamination events. Laboratory
637 management should encourage all laboratory personnel to participate in process
638 improvements regarding the prevention and minimization of contamination.

639

References

Quality Assurance Standards

Federal Bureau of Investigation (2011) *Quality Assurance Standards for Forensic DNA Testing Laboratories and Quality Assurance Standards for DNA Databasing Laboratories*, available at https://www.fbi.gov/about-us/lab/biometric-analysis/codis.

Federal Bureau of Investigation (2014) *Addendum to the Quality Assurance Standards for DNA Databasing Laboratories performing Rapid DNA Analysis and Modified Rapid DNA Analysis Using a Rapid DNA Instrument*, available at https://www.fbi.gov/about-us/lab/biometric-analysis/codis.

Laboratory Air Quality

International Organization for Standardization (ISO) Technical Committee 209 (1999) *14644 series: Cleanroom and associated controlled environments.*

American National Standards Institute (2012) *ANSI/AIHA/ASSE Z9.5-2012: American National Standard for Laboratory Ventilation.*

Decontamination

Aslanzadeh, J. (2004) *Brief Review: Preventing PCR Amplification Carryover Contamination in a Clinical Laboratory.* Annals of Clinical & Laboratory Science 34(4):389-396.

Bergen, A.W., Qi, Y., Haque, K.A., Welch, R.A., Garcia-Closas, M., Chanock, S.J., Vaught, J., Castle, P.E. (2005) *Effects of Electron-Beam Irradiation on Whole Genome Amplification*. Canc Epi Biom Prev. 14(4):1016-1019.

Braitt, G.R., Rodrigues, E.A., Bueno, C.E., Braitt, A.H. (2013) *Evaluation of active chlorine releasing of sodium hypochlorite during seven days, stored at different temperatures*. Revista Sul-Brasileira de Odontologia 10(2):143-148.

Castle, P.E., Garcia-Closas, M., Franklin, T., Chanock, S., Puri, V., Welch, R., Rothman, N., Vaught, J. (2003) *Effects of Electron-Beam Irradiation on Buccal-Cell DNA.* Am J Hum Genet. 73: 646-651.

Forensic Science Regulator (2015) *The Control and Avoidance of Contamination in Laboratory Activities involving DNA Evidence and Recovery and Analysis*. Available at https://www.gov.uk/government/publications/laboratory-dna-anti-contamination-guidance.

Forensic Science Regulator (2016) *The Control and Avoidance of Contamination in Crime Scene Examination involving DNA Evidence Recovery*. Available at https://www.gov.uk/government/publications/crime-scene-dna-anti-contamination-guidance.

Mifflin, T.E. (2003) *PCR Primer: A Laboratory Manual*. 2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. Chapter 1, Setting Up a PCR Laboratory; p. 5-14.

Qiagen (2006) *Technical Note for the identification, prevention, and elimination of DNA and RNA contaminations*. Available at https://www.qiagen.com/gb/resources/resourcedetail?id=f6c8c89b-5dca-4433-9dea-2d205d016b56&lang=en.

Scilabware (2010) *Technical Information-Plasticware*. Available at http://www.scilabware.com/uploads/PDFs/2010/Technical_information_Plastics.pdf.

Shaw, K., Sesardic, I., Bristol, N., Ames, C., Dagnall, K., Ellis, C., Wittaker, F., Daniel, B. (2008) *Comparison of the effects of sterilization techniques on subsequent DNA profiling*. Int J Legal Med. 122:29-33.

University of Colorado Boulder Department of Environmental Health and Safety (2014) *Common Chemicals Used for Cleaning and Decontamination Guideline*. Available at http://www.colorado.edu/ehs/pdf/commonchem.pdf.

Reagents and Consumables

Archer, E., Allen, H., Hopwood, A., Rowlands, D. (2010) *Validation of a dual cycle ethylene oxide treatment technique to remove DNA from consumables used in forensic laboratories*. Forensic Sci Int: Genet. 4:239-243.

Neureuther, K., Rohmann, E., Hilken, M., Sonntag, M.L., Herdt, S., Koennecke, T., Jacobs, R., Adamski, M., Reisbacher, S., Alfs, K., et al. (2014) *Reduction of PCR-amplifiable DNA by ethylene oxide treatment of forensic consumables*. Forensic Sci Int: Genet. 12:185-191.

Promega Corporation (2014) *Forensic-Grade Products for Human Identification: Preparing for ISO 18385 Requirements.* Available at http://www.promega.com/products/manufacturing-and-custom-capabilities/~/media/files/resources/white%20papers/forensic%20grade%20products%20iso%2018385%20compliance.pdf.

Suggested Reading

Cibulskis, K., McKenna, A., Fennell, T., Banks, E., DePristo, M., Getz, G. (2011) *ContEst: estimating cross-contamination of human samples in next-generation sequencing data*. Bioinformatics 27(18):2601-2602.

Dickens, B., Rebolledo-Jaramillo, B., Shu-Wei Su, M., Paul, I.M., Blankenberg, D., Stoler, N., Makova, K.D., Nekrutenko, A. (2014) *Controlling for contamination in re-sequencing studies with a reproducible web-based phylogenetic approach*. Biotechniques 56(3):134-141.

Gilbert, M.T.P., Hanson, A.J., Willerslev, E., Turner-Walker, G., Collins, M. (2006) *Insights into the Processes behind the Contamination of Degraded Human Teeth and Bone Samples with Exogenous Sources of DNA*. Int. J. Osteoarchaeology 16:156-164.

Gill, P. (2002) *Role of short tandem repeat DNA in forensic casework in the UK: Past, present, and future perspectives*. Biotechniques 32(2):366-385.

Gill, P., Kirkham, A. (2004) *Development of a simulation model to assess the impact of contamination in casework using STRs*. J Forensic Sci. 49(3):485–491.

Gill, P., Whitaker, J., Flaxman, C., Brown, N., Buckleton, J. (2000) *An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA*. Forensic Sci Int. 112(1):17-40.

Jobling, M.A., Gill, P. (2004) *Encoded evidence: DNA in forensic analysis*. Nature Reviews Genetics 5:739-751.

Jun, G., Flickinger, M., Hetrick, K.N., Romm, J.M., Doheny, K.F., Abecasis, G.R., Boehnke, M., Kang, H.M. (2012) *Detecting and Estimating Contamination of Human DNA Samples in Sequencing and Array-Based Genotype Data*. Amer J Hum Genet. 91:839-848.

Kemp, B., Smith, D.G. (2005) *Use of bleach to eliminate contaminating DNA from the surface of bones and teeth*. Forensic Sci Int. 154:53-61.

Korlevic, P., Gerber, T., Gansauge, M.T., Hajdinjak, M., Nagel, S., Aximu-Petri, A., Meyer, M. (2015) *Reducing microbial and human contamination in DNA extractions from ancient bones and teeth*. Biotechniques 58(2):87-93.

Lowe, A., Murray, C., Whitaker, J., Tully, G., Gill, P. (2002) *The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces*. Forensic Sci Int. 129:25-34.

Meakin, G., Jamieson, A. (2013) *DNA transfer: review and implications for casework*. Forensic Sci Int. Genet. 7(4):239-243.

Phipps, M., Petricevic, S. (2007) *The tendency of individuals to transfer DNA to handled items*. Forensic Sci Int. 168:162-168.

Raymond, K., Setlak, J., Stollberg, C. (2013) *Examination of Proposed Manufacturing Standards Using Low Template DNA*. Available at http://www.promega.com/resources/profiles-in-dna/2013/examination-of-proposed-manufacturing-standards-using-low-template-dna/.

Sajantila, A. (2014) *Editors Pick: Contamination has always been the issue!* Investigative Genetics 5:17.

Scherczinger, C.A., Ladd, C., Bourke, M.T., Adamowicz, M.S., Johannes, P.M., Scherczinger, R., Beesley, T., Lee, H.C. (1999) *A systematic analysis of PCR contamination*. J Forensic Sci. 44(5):1042–1045.

Szkuta, B., Harvey, M., Ballantyne, K.M., van Oorschot, R.A.H. (2015) *DNA transfer by examination tools—a risk for forensic casework?* Forensic Sci Int: Genet. 16:246-254.

Technical Working Group on Biological Evidence Preservation: National Institute of Standards and Technology (2013) *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*, NISTIR 7928 available at http://nvlpubs.nist.gov/nistpubs/ir/2013/NIST.IR.7928.pdf.

Appendix 1. Acceptability of genetic data

Once a laboratory has defined a tolerance level, exogenous genetic data generated by a negative or reagent blank control that do not exceed the established tolerance level can be disregarded and the associated sample data can be considered acceptable for reporting purposes.

Some examples of tolerance levels for the negative and/or reagent blank controls are provided in the table below (AT = analytical threshold, ST = stochastic threshold).

Technology	Tolerance Level
Autosomal STRs	Up to two peaks appearing at same or different loci ≥AT but <ST that do not match associated sample(s)
Y-STRs	Single peak ≥AT
MtDNA	Sequence does not match associated sample(s)

The above table is provided strictly for example purposes and does not suggest a specific tolerance level for a negative and/or a reagent blank control. In fact, laboratories can define a tolerance level for a negative control that is different than the tolerance level for a reagent blank control. Additionally, a laboratory's tolerance level definitions may include different acceptability criteria of sample data if/when exogenous data from a negative or reagent blank control matches sample data. Note that a laboratory can also decide that any exogenous genetic data generated by a negative or reagent blank control will cause the associated sample data to be unacceptable for reporting purposes. Laboratories should balance the notion of discarding usable data with tolerating a minimum amount of inconsequential contamination.