

Guidance

The Control and Avoidance of Contamination in Laboratory Activities involving DNA Evidence Recovery and Analysis

FSR-G-208

DRAFT ISSUE

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1. INTRODUCTION

1.1.1 For the purposes of this appendix, contamination is defined as “*the introduction of DNA, or biological material containing DNA, to an exhibit or subsample derived from an exhibit at or after the point when a controlled forensic process starts*”. This is distinct from the adventitious transfer of biological material to an exhibit that can also occur, usually prior to the exhibit or sample being recovered¹ and before investigative agencies have intervened.

1.1.2 From a forensic science perspective, crime investigation activities can be considered as two distinct phases:

- a. the pre-submission phase (scene/victim/suspect), during which investigative agencies are involved in locating, recovering, packaging, storing and transporting exhibits; and
- b. the analytical phase (laboratory) in which the recovered exhibit is processed within a laboratory.

1.1.3 Contamination can occur at any point in these investigation phases. The principal sources of DNA contamination are:

- a. from personnel to the exhibit/DNA sample;
- b. from contaminated consumables (for example, swabs, tubes) to the exhibit/DNA sample; and
- c. from exhibit to exhibit or DNA sample to DNA sample.

1.1.4 Contamination may occur as follows:

- a. directly² (for example, saliva or dandruff from an examiner falling on to an exhibit); or
- b. indirectly³ (for example, biological material present on the outside of exhibit packaging being transferred on to the gloves of an examiner who opens the package and fails to change their gloves before handling the contents, resulting in the indirect transfer of contamination to the exhibit).

1.1.5 Anti-contamination measures fall into two core areas of activity.

¹ Often referred to as background DNA.

² Also described as primary transfer.

³ Also described as secondary transfer.

- a. Prevention of contamination as far as is practicable. Preventative measures entail:
 - i. minimising the chance of contamination occurring by, for example, staff using barrier clothing;
 - ii. restricting access to areas containing exhibits;
 - iii. cleaning laboratory surfaces;
 - iv. rendering consumables human DNA free; and
 - v. ensuring that equipment used at scenes of crime is adequately decontaminated between scenes.

- b. Detection of contamination. This primarily entails:
 - i. comparison of DNA profiles generated from items against a database of reference DNA profiles from personnel from whom there is a significant risk of contamination;
 - ii. cross-checking of profiles within the same batch of samples and from different batches of samples processed within the same laboratory; and
 - iii. investigation of unexpected results.

1.1.6 It is recognised that DNA contamination incidents cannot be eliminated completely, given the prevalence of human DNA within the living and working environment, and the issue is exacerbated by the increasing sensitivity of DNA analytical techniques. Therefore, an effective DNA anti-contamination process requires a combination of approaches both to minimise the risk of occurrence and to maximise the ability to detect contamination when it does occur.

2. SCOPE

2.1.1 This appendix provides requirements, guidance and recommendations primarily on anti-contamination measures for the analytical phase of investigations, namely the control and avoidance of contamination in laboratory activities involving DNA evidence recovery and analysis.

2.1.2 This builds on section 19.2 of the Forensic Science Regulator's (FSR's) Codes, *Regulators Codes of Practice and Conduct for Forensic Science Providers and*

Practitioners in the Criminal Justice System (the Codes) and section 7 of the *DNA Analysis*, FSR C-108 to the Codes (Forensic Science Regulator).

2.1.3 Both prevention and detection of contamination within the laboratory are included in this appendix and should be used in conjunction with FSR-P-302, *The management and use of DNA elimination databases* (Forensic Science Regulator), which provides guidance and requirements for the elimination databases required for the complete criminal investigation process, including laboratory activities.

2.1.4 The interaction of the Forensic Science Regulator’s (FSR’s) guides together with the consumable standards (PAS 377:2012 and ISO18385) is shown in Figure 1.

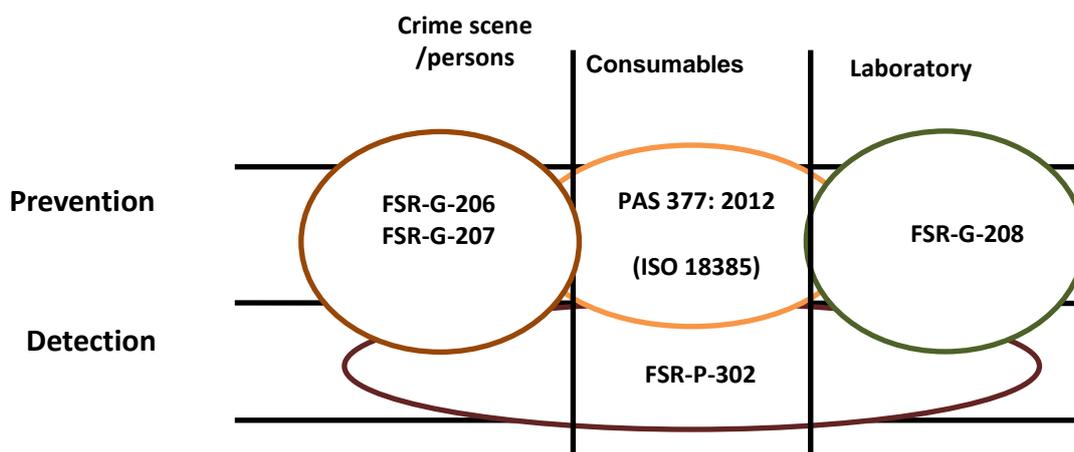


Figure 1: Interaction of anti-contamination guidelines.

2.1.5 Laboratories that use drying cabinets/rooms should comply with the requirements as set out in FSR-G-206 (*The control and avoidance of contamination in crime scene examination involving DNA evidence recovery*) (Forensic Science Regulator).

2.1.6 Forensic laboratories undertaking DNA analysis and recovery are required to demonstrate compliance with the requirements and recommendations within this document, and this will be inspected as part of the ISO/IEC 17025 compliance assessment. Therefore any alternative approaches to the recommendations adopted by laboratories must be demonstrated to be justifiable.

2.1.7 This appendix applies to England and Wales. Scotland and Northern Ireland should also institute parallel arrangements for their jurisdictions.

3. IMPLEMENTATION

3.1.1 This appendix is available for incorporation into a forensic science provider's quality management system from the date of publication. This appendix comes into effect from October 2015.

4. MODIFICATION

4.1.1 This is a consultation draft issue of this document. The finalised document post consultation will form part of the review cycle as determined by the Forensic Science Regulator.

5. TERMS AND DEFINITIONS

5.1.1 The terms and definitions set out in the following documents also apply to this guidance.

- a. Codes of Practice and Conduct *for Forensic Science Providers and Practitioners in the Criminal Justice System* (Forensic Science Regulator).
- b. *DNA Analysis*, FSR-C-108 (Forensic Science Regulator).
- c. *DNA contamination detection: The management and use of staff elimination DNA databases*, FSR-P-302 (Forensic Science Regulator).
- d. *The control and avoidance of contamination in crime scene examination involving DNA evidence recovery*, FSR- G-206 (Forensic Science Regulator).

5.1.2 The word 'shall' has been used in this document where there is a corresponding requirement in ISO/IEC 17025 or the Forensic Science Regulator's Codes; the word 'should' has been used to indicate generally accepted practice and the word 'may' has been used as recommendations.

6. PROCESS DESIGN (ISO/IEC 17025 REF 5.4.3, 5.4.5)

6.1 Overview

6.1.1 Where the method has been designed or determined, whether this is new or a modified process that is intended for introduction into casework, there shall be an assessment to identify any risks or potential risks to the criminal justice system (CJS) related to its use (Forensic Science Regulator, The Codes, ref 20.4.1).

6.1.2 This should ideally be undertaken at the design stage and include identification of components within the overall process that represent significant risk in terms of contamination. Steps shall then be taken to engineer out both the opportunities and mechanisms by which contamination can occur.

6.2 Reducing Risk by Design

Generic

6.2.1 In general terms, segregation between activities is the single most effective measure for the avoidance of contamination. For DNA processes this includes the following:

- a. segregation of the processing of casework and reference DNA samples: separate dedicated laboratories, equipment and personnel (8.2) shall be utilised;
- b. segregation of pre-polymerase chain reaction (PCR) (DNA amplification stage) and post-PCR processing: separate dedicated laboratories, equipment and personnel shall be utilised. staff may only transfer from a post-PCR area to a pre-PCR area in the same day if they shower and change their outer clothing;
- c. segregation between bulk and trace items at any stage of their examination and processing, by utilising separate search and recovery areas as far as possible, and by processing in different batches.

Minimising Manual Processing

6.2.2 The risk of contamination from an operator’s DNA should be minimised by making the best use of robotic handling devices, thereby minimising human contact.

Batch processing (DNA sample to DNA sample contamination)

6.2.3 Minimise the time that samples are held in open receptacles such as unsealed microtitre plates and uncapped microfuge tubes.

6.2.4 Minimise the opportunity for sample to sample transfer by keeping batch sizes manageable.

- a. For processing reference samples, large batches using a full microtitre plate are manageable;
- b. For processing samples for low template analysis the number of samples in a batch should be substantially smaller, and spatial separation between samples in a batch should be maximised by using individual tubes or only a proportion of wells in a microtitre plate.

6.2.5 Design robotic handling so that samples are processed in a linear fashion, ensuring that no sample is moved above another whilst, for example, being transferred in a pipette tip.

6.2.6 Pay careful attention to the detailed programming of sample manipulation steps such as pipetting and centrifugation both in terms of transferring samples and in mixing, to avoid splashing, dripping or the creation of aerosols.

6.2.7 Where samples are stored in microtitre plates, the mechanisms used for sealing plates must ensure that a watertight seal is maintained, and that contamination risks are minimised on re-sampling from individually sealed wells, for example, by including a spin stage to remove any liquid from the internal surface of the seal prior to re-sampling.

Process Flow

6.2.8 Access to DNA clean areas including lobbies shall be controlled and only permitted for individuals who have submitted a DNA sample for inclusion on the laboratory elimination database.

6.2.9 To reduce the migration and introduction of contaminants, DNA clean areas should not be used as a through corridor and the number of people accessing the area should be kept to a minimum.

6.2.10 Samples and associated materials such as carrying racks should ideally be moved from pre-PCR areas to post-PCR areas by means of a service hatch with separate in and out doors. Movement of items in the reverse direction should be minimised and only after thorough decontamination.

6.3 Evaluation of New Processes for Effectiveness of Anti-Contamination Measures

6.3.1 Once a new or modified process intended for introduction into casework has been developed, prior to its implementation it shall be validated in accordance with the Forensic Science Regulator's Codes of Practice and Conduct *for Forensic Science Providers and Practitioners in the Criminal Justice System* (the Codes). This includes an assessment to identify any risks or potential risks to the CJS related to its use that it has not been possible to eliminate by process engineering as detailed above. For any processes that are linked to DNA analysis, this risk assessment shall include the following.

- a. Consideration of contamination, the acceptable levels of which shall be defined in the end user requirements, and the acceptance criteria that must be met in the formal validation of the technique prior to implementation.
- b. Evaluation of the impact of introducing technology that increases the sensitivity of the DNA analysis process compared with existing processes is crucial, as this also inevitably increases the detection of contamination;
- c. Each validation exercise must be designed to test the method or system under consideration against the minimum requirements for that method or system. However, the following examples of elements of validation exercises are useful for evaluating contamination risk:
 - i. testing automated pipetting systems by processing 'chequer-boards': microtitre plates (or equivalent plate or tube layout used by the system) where negative controls and positive samples are arranged

alternately across the plate. the positive samples here should represent the ‘worst case scenario’, that is, the highest sample concentration expected to be encountered using the system;

- ii. evaluations of all negative controls on every batch of samples run throughout the validation exercise, and comparison with existing systems;
- iii. evaluation of all positive samples on each batch of samples run throughout the validation exercise to assess whether minor components could be from contamination, and comparison with existing systems;
- iv. checking for operator contamination throughout the validation exercise;
- v. checking for contamination from consumables throughout the validation exercise; evaluating all new consumables used in the process; and designing suitable quality assurance/control (QA/QC) procedures;
- vi. where contamination is identified in the validation work, the root cause should be investigated and identified, and modifications made to the process to minimise the risk of recurrence.

6.3.2 Once a new system or method has been implemented, a post-implementation review should be carried out after an appropriate interval, and this shall include detailed evaluation of any contamination observed and any resulting requirements for modifications to procedures.

7. LABORATORY DESIGN AND LAYOUT (ISO/IEC 17025 REF 5.3)

7.1 Overview

7.1.1 Areas for examining and extracting DNA, and post-DNA amplification [PCR] analysis areas shall be kept physically separated as the containment of post-DNA amplification product is of great importance. Likewise processing of casework and reference samples shall also be kept physically separated.

7.1.2 In essence a DNA clean area is a room or specified enclosed area or cabinet that can be easily cleaned, and that is kept clean using validated cleaning

procedures. This is facilitated by, for example, avoiding dust-traps. Ideally this should be compliant with ISO 14644 Class 7 standards.

7.1.3 The requirements for a DNA clean area are as follows.

7.2 Structure

7.2.1 DNA clean areas shall have a separate lobby area for gowning up.

7.2.2 Entry into and exit from the DNA clean area is only through the lobby area, and access to the lobby area shall be controlled, for example, by an electronic swipe card system.

7.2.3 Walls shall be of smooth finish, sealed and resistant to degradation from frequent cleaning.⁴

7.2.4 Floors shall be of a readily cleanable laboratory standard material, for example, vinyl, fully sealed and should continue part way up adjoining walls to facilitate cleaning.

7.2.5 The edges between the floors, walls and ceilings should utilise coving that provides a smooth curved join rather than a right angle, to facilitate cleaning by avoiding crevices.

7.2.6 Ceilings shall be of a material resistant to degradation from frequent cleaning, for example, laminated tiles of smooth finish.

7.2.7 Window glazing shall be sealed to prevent draughts and the sills should be sloping and shall have an easily cleanable surface. Where blinds are required these should ideally be on the outside of the window.

7.3 Furniture

7.3.1 Bench surfaces shall be sealed and of laboratory grade, resistant to chemicals such as strong acids, alkalis and solvents, and withstand frequent cleaning.

7.3.2 White benching is recommended, to facilitate cleaning.

⁴ The active agent, corrosive nature and downstream effects from the cleaning materials used need to be understood; surfaces need to be resistant to degradation as a result of frequent contact with the cleaning reagents.

7.3.3 Laboratory chairs shall be height adjustable and covered in a non-porous material such as vinyl, which can withstand frequent cleaning.

7.3.4 Bench workstation drawer units should provide sufficient storage capacity to enable bench surfaces to be kept clear, other than equipment in daily use.

7.4 Lighting

7.4.1 Lighting to ISO 8995 standard is recommended in recessed units finished flush with the ceiling (international (ingress) protection rating IP rated (IP65), in order to provide a smooth waterproof surface for cleaning purposes.

7.4.2 The optimum lighting level should be a minimum of 1,000 lux at bench level, supplemented by variable task lighting at the point of examination, whilst accounting for Health and Safety Executive (HSE) requirements to minimise glare.

7.5 Air Quality and Air Flow

7.5.1 Airborne particulate cleanliness equivalent to ISO 14644-1 Class 7 is recommended.

7.5.2 A minimum airflow of 20 times whole room replacement per hour is recommended. Higher replacement rates may be required to maintain ambient temperature in laboratories utilising equipment such as sequencers with high heat outputs.

7.5.3 Filtration of input air to all DNA clean areas, and output air from post-PCR DNA clean areas by H14 high efficiency particulate air (HEPA) filtration units. This can be achieved, for example, by utilising a HEPA-filtered clean air cabinet as a DNA clean area.

7.5.4 Management of air systems is essential to prevent output of air from post-PCR areas from re-entering pre-PCR areas including search areas.

7.5.5 Minimise adverse draughts or uncontrolled turbulence.

7.5.6 Samples likely to generate particles during their examination shall be examined in a containment hood (see also section **Error! Reference source not found.**).

7.5.7 PCRs should be set up in a clean air hood.

7.6 Air Pressure Regime

7.6.1 An ideal air pressure regime is outlined in Figure 2 below where ‘0’ denotes ambient pressure outside the building, ‘+’ denotes positive air pressure, and ‘++’ is positive air pressure higher than +. This prevents accidental ingress of contaminating material into a pre-PCR DNA clean area and egress of PCR product from a post-PCR area.

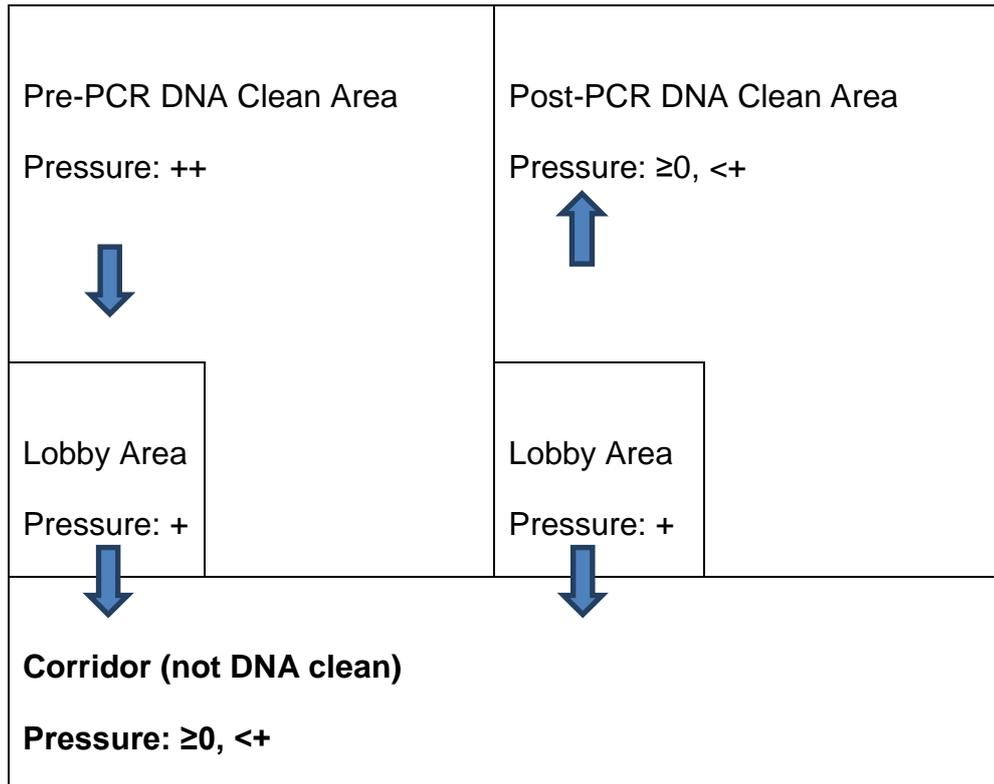


Figure 2: Schematic of relative air pressures for laboratory housing DNA clean rooms/areas.

7.7 Containment of Biohazards

7.7.1 For items that may potentially contain pathogens such as HIV and hepatitis the following shall apply:

- a. items shall be examined in Containment Level 2 or 3 facilities depending on HSE requirements;
- b. items shall be handled in a microbiological safety cabinet of appropriate level as per HSE requirements;

- c. where items are too large for handling within a microbiological safety cabinet, an individual risk assessment shall be undertaken and a decision taken on whether or not to proceed with appropriate safeguards in place.

7.8 Lobby Area

- 7.8.1 The lobby should ideally have interlocking doors to minimise ingress of contaminants, and the door from the outside corridor should open inwards into the lobby.
- 7.8.2 Lobby areas for DNA clean laboratories shall contain a hand-wash sink with hot and cold water, dispensers for soap and disposable towels.
- 7.8.3 Lobby areas for containment laboratories shall use sanitisation gel in preference to hand-wash sinks.
- 7.8.4 Lobby areas shall have coat pegs for hanging up laboratory coats and storage shelves/cupboards for barrier clothing and personal protective equipment (PPE).

7.9 Other

- 7.9.1 Laboratory equipment and stationery are dedicated to each particular DNA clean area, and to each individual workstation within a given area.

8. CONTAMINATION PREVENTION

8.1 Procedures for the Receipt of Exhibits at the Laboratory and Initial Examination – DNA Clean Areas (ISO/IEC 17025 ref 5.8)

- 8.1.1 Exhibits in cases that may require DNA analysis shall only be examined in DNA clean areas and processed in accordance with practices defined in this appendix.
- 8.1.2 Where it is not possible to examine items in a dedicated DNA clean area (for example, for health and safety reasons or bulky items) then the examination area shall be decontaminated as far as possible, background swabs shall be taken from relevant examination areas and surfaces and processed as required to demonstrate the integrity of the DNA results obtained.

- 8.1.3 If there is any issue with the integrity of the item packaging or package seals, the DNA Reporting Officer (RO) or DNA Unit Supervisor/Manager shall be made aware and the case notes annotated accordingly.
- 8.1.4 Consideration should be given to wiping down the outside of the exhibit packaging with, for example, where appropriate Trigene could be used where the packaging is plastic, but not if it is paper.
- 8.1.5 Gloves shall be changed after opening and handling the packaging in order to reduce the risk of secondary transfer. Care shall be taken to ensure that the outer surfaces of the packages do not come into contact with the items therein or with the surface on which the item is being examined.
- 8.1.6 Treatment of split cases:
- a. where DNA analysis is required in addition to other work that is not compatible with DNA clean working practices, sampling for the former shall, wherever possible, be undertaken before the exhibit is taken to any other part of the laboratory;
 - b. where it is impossible for sampling to be undertaken in a DNA clean area, the customer and the RO shall be made aware of the contamination risks and the case notes annotated accordingly.
- 8.1.7 Where an exhibit has been handled in a DNA non-clean area and it is subsequently determined to require DNA analysis, this shall be reported to the appropriate individuals who shall be made aware of the contamination risks and the case notes annotated accordingly. Any profiles generated from the exhibit shall be checked against all staff who handled the unpackaged exhibit and, if necessary, against previous casework undertaken in the DNA non-clean area. Any resulting witness statement shall explicitly refer to the contamination risk and its significance in the context of the case. This is a particular issue when items from historic cases are re-sampled.
- 8.2 Personnel (ISO/IEC 17025 ref 4.1.5, 5.2)**

Staff Training

- 8.2.1 This appendix to the Codes (Forensic Science Regulator) shall be introduced to all new members of staff as part of their induction training.
- 8.2.2 Issues relating to contamination risks and their avoidance in specific processes and methods shall be an integral part of staff training and the relevant issues shall be included within the training plans and manuals.
- 8.2.3 When competencies are being assessed, assessors should ensure that the contamination risks of any process and the means of avoidance are fully understood.

Medical Conditions

- 8.2.4 Where a member of staff has a cold or other medical condition that risks compromising forensic casework, such as persistent coughing or sneezing, consideration should be given to excluding them from DNA clean areas.

Restriction of Staff Movement

- 8.2.5 Staff shall not move from a polymerase chain reaction (PCR) product area to a pre-PCR area in the same day unless they shower and change their outer clothing before entering the pre-PCR area.
- 8.2.6 Staff shall not enter a dedicated casework processing area after working in a reference sample processing area(s) on the same day, unless they shower and change their outer clothing. However, movement from casework to a reference sample processing area is permitted.

8.3 Protective Clothing

- 8.3.1 Outdoor clothing, for example, coats, gloves, scarves, and other personal belongings are not permitted within any DNA clean areas.
- 8.3.2 The following protective clothing shall be worn by all individuals including staff, visitors and service engineers when entering a DNA clean area.

Laboratory Coats

- 8.3.3 Dedicated microbiology-type (Howie type) cloth laboratory coats or disposable laboratory coats of either the microbiology-type or surgical gown type⁵ shall be worn and properly fastened.
- 8.3.4 Dedicated cloth coats shall be laundered at least weekly, ensuring that coats from pre-PCR areas are handled entirely separately from coats from post-PCR areas; disposable laboratory coats shall be discarded at least weekly.
- 8.3.5 Dedicated coats shall be changed before searching items from a different case, individual, location and where other circumstances dictate, for example, after searching a heavily stained exhibit.
- a. It is acceptable not to change laboratory coats when examining different items of clothes that have been worn at the same time by the same individual. However, in this situation the variation from standard practice shall be recorded in the case notes.
 - b. For examination and processing of volume crime samples, it is acceptable to use a lower cost alternative of wearing disposable paper aprons over the laboratory coat and changing the apron between items, rather than the laboratory coat.
- 8.3.6 Dedicated coats shall not be worn outside the DNA clean area to which they have been assigned.

Gloves

- 8.3.7 Disposable gloves shall be worn at all times in a DNA clean area, and removed when leaving the area. Two layers of gloves shall be worn; the inner gloves may be cotton, nitrile or other demonstrable suitable alternative.
- 8.3.8 The wrist of the glove should cover the wrist of the laboratory coat. Where this is not possible, disposable cuffs shall be used to cover the gap.
- 8.3.9 The outer set of gloves shall be either changed or thoroughly cleaned with a validated method for effective removal of DNA whenever they come into contact with a potentially contaminated surface, for example, an opened exhibit package, face, door handle, phone, chair, or retrieval of items from the floor.

⁵ Includes dedicated laboratory wear described as 'scrubs'.

- 8.3.10 Gloves shall be changed between the examination of different items.
- 8.3.11 Gloves do not need to be changed between exhibits whilst these are still in their packaging, for example, when transporting intact exhibit bags to new locations.
- 8.3.12 Staff shall use their judgement to decide whether gloves need to be changed during item examination.
- 8.3.13 Non-powdered gloves shall be worn when using a microscope in a clean room area.

Face Masks

- 8.3.14 When examining exhibits face masks shall be worn that are properly tied and adjusted to cover the nose and mouth.
- 8.3.15 Pinch-nose face masks shall be available to staff who wear glasses.
- 8.3.16 Touching the mask with gloved hand shall be avoided; if it is necessary to adjust the mask then gloves shall be changed.

Hair Cover

- 8.3.17 Disposable mob caps or similar hair cover shall be worn entirely covering the head hair upon entering the DNA clean area.
- 8.3.18 Where necessary, for example, with bearded individuals, additional hair cover (snoods) shall be used to ensure that all facial hair is covered when used in conjunction with the face mask.

Gowning Procedure

- 8.3.19 The gowning/disrobing procedure shall be undertaken in a lobby area or designated area proximal to the entrance/exit of the DNA clean area.
- 8.3.20 Gowning-up shall be undertaken in an appropriate sequence, an example of which is the following:
 - a. on entering the lobby area, immediately put on a mob cap and ensure that all hair is secure within the cap;
 - b. put on a face mask;⁶

⁶ Do not talk at all until the mask is securely fitted.

- c. put on clogs or overshoes;
- d. put on goggles or other eye protection where necessary;
- e. put on gloves;
- f. put on a disposable/laundered laboratory coat; and
- g. change gloves where necessary, for example, when a laboratory coat has been worn previously.

8.3.21 When leaving the DNA clean area:

- a. on re-entering the lobby area, take off the laboratory coat and gloves and wash hands⁷; and
- b. remove goggles, face mask and mob cap.

8.4 Control of Bench Environment

Cleaning

- 8.4.1 In addition to a daily decontamination regime (section 8.6) the search bench shall be decontaminated between items, cleaning top and sides of the bench using a detergent/cleaning solution. The only exception is when clothes are submitted as having been worn by the same individual at the same time, and in the professional judgement of the RO not changing gloves will not impact on the potential interpretation of any evidence. In this situation the variation from standard practice shall be recorded in the case notes.
- 8.4.2 Each item shall be examined on a disposable surface such as a sheet of paper or Benchkote⁸, which is changed between items. The only exception is when clothes are submitted as having been worn by the same individual and at the same time. In this situation the variation from standard practice shall be recorded in the case notes.
- 8.4.3 As far as possible all bench surfaces used for searching examination and processing of exhibits shall be kept clear. This makes it much easier to decontaminate the surface.

⁷ If taking samples/tubes out of the laboratory, clean gloves shall be worn.

⁸ Name for commercially available sheets of paper.

Exhibits Left on Benches

8.4.4 Exhibits, including those that need to dry prior to re-packaging, should not be left uncovered on the bench when not being examined. The exhibits should be covered with disposable paper sheeting, Benchkote or equivalent.

Bench Log

8.4.5 A record by date, time and examiner of all exhibits/cases/batches examined/processed at every workstation or defined area of bench shall be kept. This may be required following a contamination incident, if it is thought necessary to check other cases/batches processed at the same bench or workstation.

Paperwork

8.4.6 Use of paperless case file management systems is strongly recommended. If paper case files are still utilised, these shall not be taken into DNA clean areas; only the relevant pages such as submission forms and examination instructions should be taken in, preferably within a cleaned plastic wallet and wherever possible kept to one side of the work area/station.

8.4.7 Other items required for note taking (pens, pencils, other stationery) shall be provided within the laboratory DNA clean area and not removed.

8.5 Use of DNA Laboratories for Activities other than Casework

8.5.1 At times it may be necessary to use laboratory space for non-casework activities including for training purposes, preparation of body fluid controls for presumptive test reagents, and seeding of items for trials and audits.

Seeding of Items using Body Fluids for Training, Trials or Reference Controls

8.5.2 When clothing, swabs and other items are seeded with significant quantities of liquid body fluids for training or demonstration purposes, the following apply:

- a. all preparations shall be undertaken in an area not being used for DNA casework at the time, and the area shall not be returned to casework use until a deep clean has been undertaken;⁹
- b. all human body fluids shall be provided by donors whose profiles are held on the staff elimination database (SED);
- c. disposable pipettes and containers, or dedicated re-usable pipettes that are not used for any other purpose and are labelled to this effect, shall be used at all times;
- d. a record shall be kept of which samples have been used and where the training items are utilised.

Body Fluid Controls Used For Check of Presumptive Test Reagents

8.5.3 Semen used for acid phosphatase (AP) control papers should be prepared from samples provided by a vasectomised donor wherever possible. If this is not available, the supernatant from a spermic sample can be used. The test papers shall be prepared in a non-DNA casework area.

8.5.4 Blood used should be non-human; typically horse blood is used.

Examination of Seeded Training Items

8.5.5 When (several) people are receiving training with seeded items, a complete laboratory shall be designated for the duration of the training. No casework items shall be subsequently brought into this area until a deep clean has been undertaken and a check has been undertaken via environmental monitoring (Section 8.7).

8.6 Cleaning Processes

8.6.1 Each DNA clean area shall have a cleaning schedule, with the frequency of cleaning dependent on the extent of use of the area and the equipment within it, and a cleaning log shall be maintained to show the daily, weekly or monthly activities undertaken as per the schedule.

Minimum Cleaning Requirements

⁹ For setting up DNA proficiency trials, items shall be seeded with body fluids in a DNA clean room where no casework activities are undertaken.

- 8.6.2 As a minimum the following shall be undertaken using cleaning equipment dedicated solely for use in each DNA clean area and using a cleaning regime validated to provide effective DNA decontamination.
- 8.6.3 Daily or after each use:
- a. bench work surfaces – identify and decontaminate all surfaces that may either directly or indirectly come into contact with the exhibit;
 - b. chairs; and
 - c. centrifuges, inside and out.
- 8.6.4 In addition the following shall be decontaminated in between the examination of different items:
- a. individual pieces of equipment including fibre rollers, pens, rulers, barcode scanners, low power microscopes, and IT equipment (graphic pads and pens, keyboards and all exposed cables, etc.) must be decontaminated before and after use.
- 8.6.5 On a weekly basis:
- a. floors;
 - b. equipment such as centrifuges, microscopes and computers;
 - c. all contact surfaces such as cupboards, door handles and fridges; and
 - d. the insides of drawers.
- 8.6.6 On a monthly basis, whole area deep clean:
- a. lights and vents;
 - b. walls and ceiling; and
 - c. centrifuges decontaminated inside and out.

8.7 Environmental Monitoring

Principle

- 8.7.1 The principle of environmental monitoring is to undertake a programme of testing on a periodic basis to check that particular rooms or areas are DNA clean and to assess whether the decontamination policy for the area in question is both effective and has been carried out properly.

- 8.7.2 Results from such monitoring should be carefully assessed given that, unlike other classes of testing, DNA analysis does not typically include the additional safeguard of processing substrate/search bench controls within batches.
- 8.7.3 Samples are taken by swabbing selected areas and equipment that are in contact with operators and/or the casework items themselves at all stages in the casework supply chain.
- 8.7.4 The sampling regime reflects the risk profile of operation and is proportionate to the risk. For example, drying rooms in which large amounts of biological material are inevitably present should be sampled most frequently. Components typically sampled vary according to the function of the area and examples are given at 8.7.14.

Sampling Schedule

- 8.7.5 All work areas for which assurance of cleanliness is required, that is, DNA clean areas, shall be assessed on a routine basis.
- 8.7.6 The required frequency of sampling should be determined empirically, specified in the sampling plan and evidence provided through use of trend analysis that the risks of contamination for a particular area are being adequately controlled by the cleaning regime. Thus an area that has basic air flow management and where many people regularly work will require more frequent cleaning and monitoring than a low throughput facility with state of the art air flow management.
- 8.7.7 Depending on circumstances, additional non-routine testing may be required, for example:
- a. after a contamination incident has occurred;
 - b. after a laboratory work area has changed function; or
 - c. after maintenance has been completed within the area rendering it 'unclean'.
- 8.7.8 In addition to DNA clean areas, environmental monitoring of specified areas should be undertaken where DNA contamination poses less of a risk, for

example, areas where exhibits are regularly handled but remain sealed within packaging.

8.7.9 Results from monitoring these areas should be treated as an indication of background levels of DNA and used to inform the effectiveness of cleaning regimes within these areas.

Sampling and Analysis

8.7.10 As a minimum, swabs from 20 areas¹⁰ shall be taken from a fully equipped DNA extraction laboratory or an appropriate number from a dedicated work area on each assessment. These shall be taken from a variety of surfaces and items as stipulated in 8.7.14.

8.7.11 Sampling shall be undertaken utilising swabs moistened as appropriate and certified as free from detectable levels of human DNA ('human DNA free').

8.7.12 The swabs shall be extracted, amplified and analysed as per the most sensitive process employed on samples that are processed through the particular laboratory/facility under test including use of positive and negative controls.

- 8.7.13 For each work area a record shall be kept of:
- i. the date that sampling was undertaken;
 - ii. number of areas sampled, and details of what these are;
 - iii. number of failures and items/areas affected;
 - iv. comments on allelic peaks observed (number and peak height, whether the profiles match against the staff elimination database [SED]); and
 - v. corrective actions taken as detailed in section 10.

Areas to be Sampled

8.7.14 Listed below are areas that are vectors for contamination and should be typically sampled. This is not necessarily an exhaustive list.

Barcode scanners	Drawers under benches	Pipettes
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¹⁰ Depending on the size and scope of the laboratory 20 swabs is the minimum number recommended. All relevant areas should be covered within the matrix of the sampling schedule based on risk assessment and trend analysis from monitoring results.

Benches	Fibre optics	Racking in stores
Bench canopies	Forceps	Reagent bottles
Bench screens	Fridge/freezer handles	Safety cabinets/hoods
Centrifuges	Handles to consumables boxes	Shakers
Chairs	Heat sealers	Stationery
Cross-linkers	Incubators	Telephones
Cupboard handles	Instrument knobs	UV lamps
Digital cameras	Lids, tops of containers	Vortex mixers
Door handles	PCs, keyboards, etc.	Water sprays

8.7.15 Areas to swab are assigned different priorities.

- a. Priority 1: These require swabbing in every environmental monitoring exercise, for example, workbench, fridge freezers, keyboards.
- b. Priority 2: A selection of these should be included in each exercise, for example, chair seats, drawer handles.
- c. Priority 3: These should be included occasionally, for example, reagent bottles.

8.7.16 Areas that are not swabbed are chemical waste bins and sharps bins.

Cellular contamination monitoring

8.7.17 The use of Adenosine Triphosphate (ATP) based¹¹ luminometry¹² methods may be used as means of assessing the degree of cellular contamination on a surface in real time, by swabbing the surface and measuring the ATP activity using a hand held device.

8.7.18 Any ATP luminescence methods shall be 'calibrated' for the hand held model used against the absence and low levels of detectable DNA.

¹¹ ATP (Adenosine Triphosphate) is a molecule found in all living cells, including plant, animals and humans as well as bacteria, yeasts etc. The use of ATP luminometry methods has been routinely used in hospitals and the food and beverage processing industry for many years as a means of assessing the degree of cellular contamination on a surface in real time.

¹² Measurement of luminescence

- 8.7.19 The monitoring of ATP activity would not be a direct replacement for all monitoring activities, but can indicate ineffective cleaning and can be used in combination with DNA profiling to allow for efficient and effective monitoring.
- 8.7.20 Extracted DNA, that is, 'naked' DNA (existing outside of the cell) will give no ATP result and therefore ATP-based methods shall not be used as a means of monitoring for cellular contamination in DNA processing laboratories.

Interpretation of Results and Corrective Actions

- 8.7.21 The presence of allelic peaks is considered at all loci, including amelogenin. Results are assessed based on the validated interpretation rules applied by the laboratory undertaking the testing. These give consideration to the approach taken where peaks above background are observed and this monitoring data should be accessible to the DNA RO, together with details of any improvement and corrective actions taken around the period that peaks above background were identified.
- 8.7.22 A mechanism should also be established to enable feedback to the DNA processing unit by the DNA RO where any suspicious (unexpected) or anomalous results have been observed suggesting contamination, so that the DNA unit can investigate and check whether this is the case and undertake decontamination if necessary.

Gross Contamination of An Area

- 8.7.23 In the event of gross contamination or after maintenance staff have entered a DNA clean area, a deep clean shall be undertaken as detailed in 8.6.

Gross Contamination of specific items/areas

- 8.7.24 Where gross contamination of a particular bench surface or item has occurred or is suspected, the affected area/item should be treated using a validated cleaning regime.

Note: Disinfectants such as Trigene are potent inhibitors of PCR, and if they are not removed from areas that come into contact with DNA exhibits, the DNA results can be adversely affected. Great care should therefore be taken to remove all traces of detergent after its use as part of the validated decontamination process. If an inhibition

problem attributable to the cleaning regime persists, consideration should be given taking due account of Health and Safety Executive (HSE) requirements, of replacing the detergent used with Virkon, the latter being a far less potent PCR inhibitor.¹³

Worst Case Scenario

8.7.25 In the event that, due to the time required to process the environmental monitoring swabs, a case is reported that has the same profile as the identified contaminant, the designated manager shall recall the case or take appropriate action as required by the circumstances, and shall inform stakeholders as appropriate (section 10.4).

8.8 Consumables (ISO 17025 ref 6.4.2)

8.8.1 Consumables that are quality assured to be human DNA free shall be utilised in the processing of DNA samples within the laboratory. This also applies to reagents used in processes upstream from DNA processing in joint, split or sequential cases.

8.8.2 Assurance can be provided by the consumables being independently certified as compliant with PAS 377:2012,¹⁴ (ISO 18385 when available) or through quality control (QC) testing of batches of reagents and consumables. If QC testing is relied upon, the most sensitive DNA analysis method for which the consumables are intended shall be used in the QC procedure and the laboratory shall assure itself that sufficient test samples to detect sporadic contamination in a batch of consumables are processed.

9. CONTAMINATION DETECTION

9.1 Positive and Negative Controls

9.1.1 The presence of one or more allelic peaks in a batch negative control or allelic peak(s) additional to those expected in a positive control is indicative of

¹³ Bright JA1, Cockerton S, Harbison S, Russell A, Samson O, Stevenson K (2011); J Forensic Sci. 56(1):181-5. The effect of cleaning agents on the ability to obtain DNA profiles using the Identifiler™ and PowerPlex® Y multiplex kits.

¹⁴ ISO 18385 is currently (as at December 2014) under development and may ultimately replace Annex A in PAS 377:2012.

contamination associated with the DNA laboratory processes. This could have originated from:

- a. samples within the same batch or other batches;
- b. individuals within the DNA laboratory environment;
- c. contaminated consumables; or
- d. other sources through secondary transfer mechanisms.

All such contamination shall be logged internally by the forensic science provider (FSP) in an appropriate system, to enable contamination to be monitored over time.

9.2 Within Batch and Between Batch Comparisons

9.2.1 Potential instances of cross contamination shall be screened, both within and between batches of samples by means of appropriate software comparison programmes, which highlight the degree of similarity (number of shared alleles) between different processed samples. A rolling 6-¹⁵ to 18-month¹⁶ window of between batch comparisons shall be undertaken. Where casework materials are processed in microtitre plates, DNA samples from the same case shall not be positioned in adjacent wells, in order to facilitate detection of potential well to well splash-over.

9.2.2 All instances of cross-contamination shall be logged and monitored by the FSP, with investigation of all incidents and corrective actions being undertaken as required.

9.3 Unexpected Results

9.3.1 DNA Reporting Officers (ROs) should be suspicious of results that do not fit with case circumstances, including a mixture that is not expected from the material processed and that is difficult to explain, based on their organisation's validated interpretation guidelines. In some situations it is appropriate to conduct an investigation into the source of the profile including a full assessment of the

¹⁵ Timescale routinely used as the limit for detecting laboratory staff contamination. It will not be relevant for manufacturing staff contamination checks as it is dependent on the shelf life of the consumables in use.

¹⁶ It has been communicated that contamination has been identified 18 months after individual left.

potential for contamination. Retesting the item may confirm that the profile is unrelated to the case. All opportunities to discover the cause should be taken as described elsewhere in this appendix.

9.4 Comparison Against Elimination Databases

9.4.1 Local elimination databases shall be established and kept up to date comprising all personnel working within the facility and all visitors to the facility who pose a risk of contaminating the DNA samples processed within the organisation and any unexplained/unsourced profiles believed to be as a result of contamination.

9.4.2 Prior to submission to the National DNA Database[®] (NDNAD) or reporting of casework results including those that do not require loading to the NDNAD, all DNA profiles¹⁷ shall be compared against:

- a. the locally held database comprising staff, visitors and unsourced profiles; and
- b. a subset of profiles held centrally within an elimination database comprising profiles of manufacturing staff, police personnel and medical personnel who pose a significant risk of contaminating with their own DNA.

9.4.3 These elimination databases and requirements are detailed in FSR-P-302 (Forensic Science Regulator).

10. MANAGEMENT OF CONTAMINATION INCIDENTS INCLUDING DECONTAMINATION PROCEDURES

10.1.1 If contamination is suspected, the following five-step corrective action process shall be undertaken.

10.2 Identify the Problem

10.2.1 Where contamination is suspected the first stage is to investigate and determine the origin of the contaminant profile and the full extent of the problem. This may

¹⁷ The DNA profile shall as far as possible be single sourced, thereby taking into account the complexity to determine individual contributors from DNA profiles originating from two or more individuals (mixtures).

be achieved by working backwards in a step by step investigative process.

Typically, this may involve comparison of the contaminant profile with:

- a. other samples processed within the same batch;
- b. samples from other batches processed in the same time frame, that is, up to the last six months within the laboratory;
- c. staff who pose a significant risk of contamination throughout the end to end process of location, collection, handling and analysis, see also FSR-P-302 (*ibid.*) and whose profiles are held on local databases (SED) – forensic science provider (FSP) staff, and visitors including maintenance staff and defence examiners – or on central databases (CED) – manufacturers, medical staff, unsourced contaminants and police (PED); and
- d. positive and negative controls.

10.2.2 If the contaminant profile contains too few alleles to allow effective screening against the above, the FSP should aim to rework the sample to produce a more informative result by any appropriate means available such as re-extraction, additional purification steps, dilution of inhibitors, concentrating the extract and/or application of low template methods. The use of these troubleshooting procedures shall be validated by the laboratory prior to their application.

10.3 Determine the Root Cause

10.3.1 The point at which contamination has occurred may be determined by reworking the samples in reverse in a step by step manner – re-electrophoresis, re-polymerase chain reaction (re-PCR), re-extraction. Although alleles under a minimum detection threshold are not reported, these should be considered when performing investigations and corrective actions to assist in determination of the root cause. Typical root causes may be categorised as follows.

- a. Staff-related: These include failure to comply with standard operating procedures (SOPs), or lack of competence that was either not addressed during training or not identified in competency assessment.
- b. System-related: These include a contamination risk not being adequately mitigated during system design and development, which only becomes

apparent after validation and implementation, or that the system has materially changed post-validation.

- c. A combination of factors including staff, systems, and other factors: These include SOPs being insufficiently explicit and therefore open to misinterpretation or omitting information considered to be too obvious to require inclusion, resulting in deviation from the intended procedure by an individual or subset of staff.

10.3.2 Where staff are the root cause, it is essential to determine whether this is limited to the work of just one individual or more than one, and over what timescale. This shall be ascertained by reviewing all potentially affected work, starting at the time of the originally observed contamination incident and working outwards. Dip-testing may be an appropriate means of achieving this in some circumstances. Similarly where there is a systemic issue it is necessary to consider that similar events may have previously gone unnoticed and it may be necessary to review previous work.

10.4 Communication

10.4.1 Case file: In all instances where contamination has been detected or suspected, the facts relating to the incident and any actions agreed either with the customer or internally shall be recorded in the case file of any affected case.

10.4.2 Statement: Any detected contamination shall be disclosed and if it has a significant bearing on a case, this shall be reported in the statement.

10.4.3 Stakeholders: Where the occurrence of contamination has consequences beyond the reporting of an individual case, relevant stakeholders shall be informed including the customer, the Forensic Science Regulator/Regulation Unit, the Crown Prosecution Service (CPS) and the UK Accreditation Service (UKAS) so that additional measures may be considered and taken by the criminal justice system (CJS) as a whole if required.

10.5 Implement Preventative Measures

10.5.1 Actions taken to prevent recurrence depend on the root cause:

- a. where a workspace has been affected by a contamination incident, processing of material within the affected workspace shall cease until it has been subject to the decontamination regimes detailed in 8.6 and demonstrated to have been effective through environmental monitoring detailed in 8.7;
- b. staff-related issues may require the individuals in question not to continue with the work activities under investigation until additional awareness training and competency re-assessment have been successfully completed;
- c. system-related issues may require modification of procedures, these shall be verified as fit for purpose prior to implementation; and
- d. in all instances, a post-implementation review shall be conducted to provide assurance that the preventative measures have been effective.

10.6 Document Event

10.6.1 Full documentation of all contamination events shall be generated and maintained, including the investigation and corrective actions taken in all serious instances. This shall describe:

- a. the deficiency;
- b. the root cause of the deficiency;
- c. the impact on past work;
- d. the remedial action taken; and
- e. evidence from the post-implementation review that the issue has been resolved.

10.6.2 All corrective actions identified shall be logged and managed within an improvement and corrective action process in accordance with the requirements of ISO/IEC 17025.

11. MANAGEMENT OVERSIGHT AND CONTINUOUS IMPROVEMENT (ISO/IEC 17025 REF 4.10, 4.11)

11.1.1 There shall be governance and oversight of contamination avoidance, monitoring and detection by the forensic science provider's (FSP's) senior

management. This shall include a technical authority with responsibility for assessment and review of contamination, including responsibility for escalating contamination issues to senior management where required.

11.1.2 Maintaining a log of contamination events and periodically reviewing these to identify trends and potential for further anti-contamination measures shall be undertaken by the management technical authority as part of an overall continuous improvement process.

11.1.3 There should be good communication with staff and staff ownership of contamination issues. Improvement at the team/unit level should also be encouraged.

11.1.4 Periodic management reviews of contamination shall be undertaken by the FSP on a six-monthly basis as a minimum. These reviews shall monitor all serious contamination events plus provide a summary of lower level contamination¹⁸ observed within DNA processes including:

- a. contamination observed;
- b. testing of consumables where this is undertaken;
- c. contamination rates within batch controls;
- d. batch re-run and re-extraction rates due to contamination; and
- e. environmental monitoring results.

These reviews shall assess contamination trends within the laboratory and be made available to the Forensic Science Regulator (FSR)/Forensic Science Regulation Unit (FSRU), the UK Accreditation Service (UKAS) and the National Assurance Service (NAS¹⁹) to enable overall trends within the industry to be monitored.

11.1.5 After implementation of new methods/systems, post-implementation reviews shall be carried out, which shall include monitoring of any change in contamination rates.

¹⁸ Low level contamination can also include minor components in mixture profiles.

¹⁹ Unit within the National DNA Database®

12. ACKNOWLEDGEMENTS

12.1.1 Acknowledgement is given to the following organisations that provided invaluable input into drafting this Appendix: Forensic Science Northern Ireland, Forensic Science Service Ltd, Key Forensics, LGC Forensics, Orchid Cellmark, Scottish Police Authority and the Forensic Science Regulation Unit.

13. REVIEW

13.1.1 This document is subject to review at regular intervals.

13.1.2 If you have any comments please send them to the address as set out on at: www.gov.uk/government/organisations/forensic-science-regulator, or email: FSREnquiries@homeoffice.gsi.gov.uk

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PAS 377:2012 *Specification for consumables used in the collection, preservation and processing of material for forensic analysis: Requirements for product, manufacturing and forensic kit assembly.*

15. ABBREVIATIONS

Abbreviation	Meaning
AP	acid phosphatase
BS	British Standard

CJS	Criminal Justice System
CPS	Crown Prosecution Service
DNA	deoxyribonucleic acid
EN	European standards
FSP	Forensic Science Provider
FSR	Forensic Science Regulator
FSRU	Forensic Science Regulation Unit
HEPA	High Efficiency Particulate Air
HSE	Health and Safety Executive
IEC	International Electrotechnical Commission
IP	International (Ingress) Protection Rating
ISO	International Organisation for Standardization: A network of the national standards institutes of 157 countries
IT	Information Technology
NAS	National Assurance Service
NDNAD	National DNA Database [®]
PAS	Publicly Available Specification
PC	Personal Computer
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
RO	Reporting Officer
SED	staff elimination database
SOC	scene of crime

SOP	standard operating procedure
UKAS	UK Accreditation Service
UV	Ultra Violet

16. GLOSSARY

Allelic peak: A peak that falls within an allelic window, has well defined allele morphology and a peak height greater than the defined limit of detection of the laboratory.

Casework: Items believed to contain biological material relating to a person of interest (perpetrator) recovered from specific crime events.

Crime sample: An item or sub-item recovered and believed to provide evidence to investigate or prosecute a criminal offence, i.e. crime-related.

DNA clean area: Area in which appropriate DNA contamination prevention measures shall be maintained at all times as defined in section 8.

DNA contamination: The introduction of DNA, or biological material containing DNA, to an exhibit, or subsample derived from an exhibit during or after its recovery from the scene of crime or a person.

Elimination database: Collection of DNA profiles held in a searchable format from staff whose access/role/activities are deemed to be a potential DNA contamination risk. The profiles are used to identify instances of inadvertent contamination.

Forensic science provider: Organisation that undertakes any part of the DNA sample recovery and analytical process on behalf of the police or other Criminal Justice System customers, police evidence recovery laboratories are also included.

Human DNA free: Human DNA is not detectable by the most sensitive DNA profiling techniques currently (December 2014) available.

Laboratory: Any area in which the packaging of an exhibit is opened, or items for DNA analysis are processed, including drying rooms.

Reference: see reference sample.

Reference sample: A biological sample obtained from a known person with the purpose of creating a DNA profile for comparison.

Un sourced contaminant: A DNA profile identified as a contaminant, i.e. following all relevant elimination database checks of which the source has not been identified. No template (negative) controls and quality control batch tests are considered as having originated from the manufacturing supply chain, historically most have been found to come from manufacturing staff.

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