INTERNATIONAL STANDARD

ISO 18385

First edition
2016-02-01

Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes — Requirements

Réduire au maximum le risque de contamination de l'ADN dans les produits utilisés pour recueillir et analyser du matériel biologique en criminalistique — Exigences

Reference number
ISO 18385:2016(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword ..... **iv**

Introduction ..... **v**

1 Scope **1**

2 Terms and definitions **2**

3 Abbreviated terms **5**

4 Types of products **5**
4.1 General 5
4.2 Products that come into direct contact with biological stains or material potentially containing human DNA 5
4.3 Chemicals, reagents, solvents, and some disposables involved in the analysis of human DNA 6
4.4 Protective barrier products that are used during the collection and analysis of biological material 6

5 Quality management systems **6**
5.1 General 6
5.2 Documents and records 6
5.3 Authorization 7
5.4 Subcontracting of work and purchase of components 7
5.5 Control of nonconforming product 7
5.6 Corrective and preventive action 8
5.7 Staff contamination detection provision 8

6 Human DNA contamination risk management **8**
6.1 General 8
6.2 Risk assessment 8
6.3 Risk mitigation 10
6.4 Risk control measures 10
6.4.1 Equipment 10
6.4.2 Personnel 10
6.4.3 Cleaning and maintenance 10

7 Environmental human DNA monitoring **11**

8 Requirements for products subject to post-production treatment **11**

9 Requirements for products not subject to post-production treatment **12**
9.1 Product testing 12
9.2 Batch records 12

10 Product packaging, labelling, and documentation **12**

Annex A (normative) **Compliance testing** **13**

Annex B (informative) **Guidance on the effectiveness of post-production treatments currently used in the manufacture of products** **15**

Annex C (informative) **Relevant markers for DNA profiling** **17**

Bibliography **18**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 272, *Forensic sciences*.

Introduction

This International Standard was produced with the aim to create global standards for manufacturers of forensic products used in human DNA analysis. Inadvertent contamination by manufacturers of consumables and reagents, when combined with the improved sensitivity of DNA testing methods, increasingly interferes with forensic analysis.

Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes — Requirements

WARNING — This International Standard calls for the use of procedures that may be a health hazard or cause injury if adequate precautions are not taken.

1 Scope

This International Standard specifies requirements for the production of products used in the collection, storage, and analysis of biological material for forensic DNA purposes, but not those consumables and reagents used in post-amplification analysis.

The consumables and reagents covered by this International Standard include those used for evidence collection (sampling kits), such as swabs, containers, and packaging, and also products used in the analysis of DNA samples, such as tubes and other plasticware, disposable laboratory coats, gloves, and other consumables.

This International Standard applies to the production of consumables and reagents which do not require cleaning for continued use. This International Standard does not cover technical product specifications (i.e. product design).

This International Standard excludes microbiological testing.

This International Standard specifies a requirement for manufacturers to minimize the risk of occurrence of detectable human nuclear DNA contamination in products used by the global forensic community.

An overview of the International Standard is provided in Figure 1.

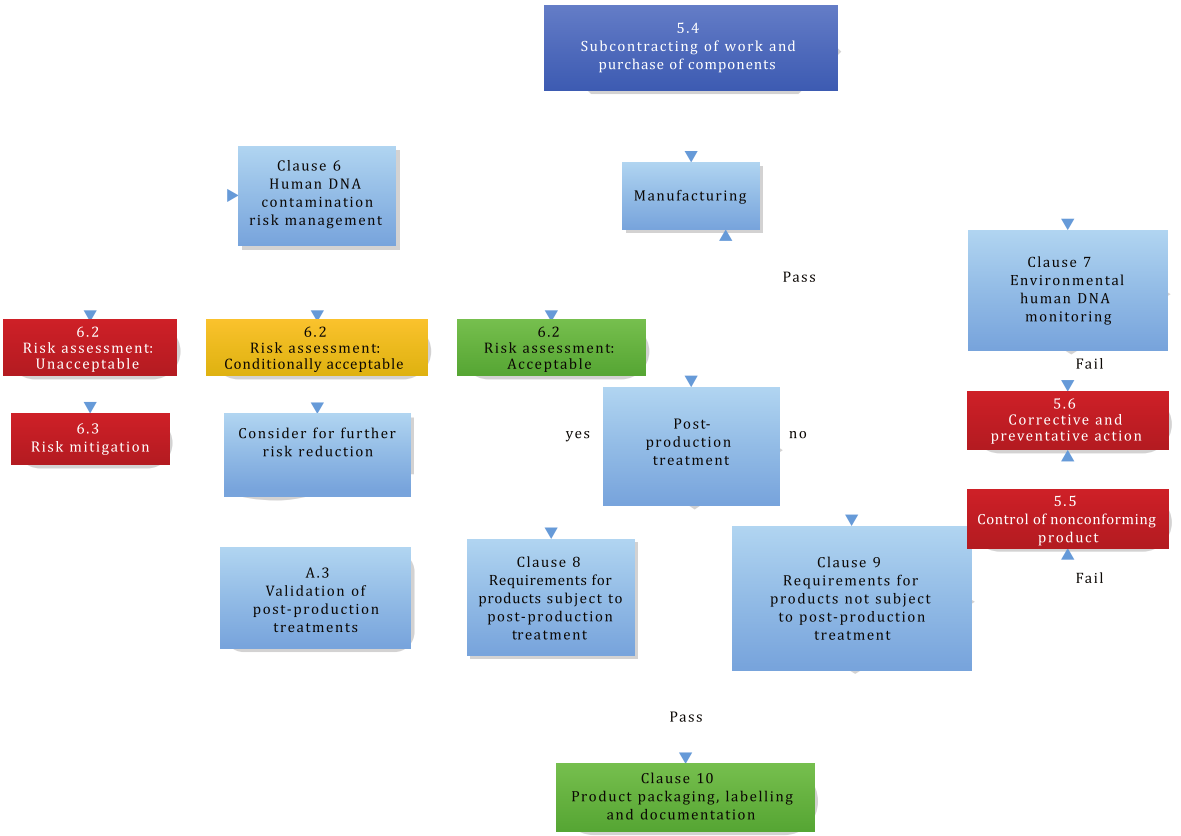

Figure 1 — Overview of the processes covered by this International Standard

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
allele
one of a number of alternatives at a specific location on an individual's DNA

2.2
amplification
process of copying segments of the DNA sequence exponentially

Note 1 to entry: This is also called Polymerase Chain Reaction (PCR).

2.3
amplification negative control
sample with no DNA material used to verify that the *amplification* (2.2) is free of *contamination* (2.7)

2.4
amplification positive control
sample with known DNA material used to verify that the *amplification* (2.2) works

2.5
analytical threshold
relative fluorescence unit value at which a laboratory has determined to call a peak an *allele* (2.1)

2.6
batch release test
test performed by or on behalf of the *manufacturer* (2.15) on a batch of components, which has to be satisfactorily completed before the batch can be released

[SOURCE: ISO/TS 21003-7:2008, 3.1.9]

2.7
contamination
introduction of detectable nuclear DNA or human cellular material during the manufacturing or assembly processes that would compromise the forensic human DNA analysis

2.8
contamination detection limit
value at or above which human DNA is deemed to be 'detected' and below which the compound is deemed to be 'not detected'

2.9
DNA reduction factor
ratio of the DNA quantity of an untreated cell-spiked sample to the DNA quantity of an identical cell-spiked sample that has undergone appropriate post-production treatment

2.10
eluate
solution resulting from the DNA extraction process

2.11
extraction negative control
sample with no cellular or DNA material used to verify that the DNA extraction is free of *contamination* (2.7)

2.12
extraction positive control
sample with known cellular or DNA material used to verify that a DNA extraction works

2.13
ISO 18385 forensic DNA grade
label given to products that have been produced in accordance with this International Standard

2.14
kit
set of consumables and/or chemicals (or reagents), and instructions for use, packaged together and intended for use as specified by the *manufacturer* (2.15)

2.15
manufacturer
organization that produces and/or packages the product

2.16
manufacturing environment
area, room, or space identified for the production and/or packaging of products used to collect and analyze biological materials

2.17
out-of-specification
test results that do not comply with the product specification

2.18
person reference sample
biological material taken from a known source with the purpose of creating a DNA profile for comparison

2.19
post-production treatment
contamination (2.7) reduction treatment conducted after *primary packaging* (2.20) to ensure that any human DNA contaminants above the *contamination detection limit* (2.8) are physically destroyed or not accessible to DNA *amplification* (2.2)

2.20
primary packaging
packaging designed to come into direct contact with the product

[SOURCE: ISO 21067:2007, 2.2.2]

2.21
primary transport container
product designed to transport the sample

EXAMPLE Envelopes, evidence bags, specimen jars, swab boxes, and collection devices with integrated transport mechanisms.

2.22
product
consumables and reagents which do not require cleaning for continued use and are used to collect, store, and analyze biological material for forensic purposes, but not used in post-amplification analysis

2.23
production
process or method for the manufacture of products

2.24
sample
portion of biological material or collected item, on which the test or analysis is carried out

2.25
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The term “validated” is used to designate the corresponding status.

Note 2 to entry: The use conditions for validation can be real or simulated.

[SOURCE: ISO 9000:2015, 3.8.13]

2.26
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The term “verified” is used to designate the corresponding status.

Note 2 to entry: Confirmation can comprise activities such as

— performing alternative calculations,

— comparing a new design specification with a similar proven design specification,

— undertaking tests and demonstrations, and

— reviewing documents prior to issue.

[SOURCE: ISO 9000:2015, 3.8.12]

3 Abbreviated terms

bp	Base pair
DNA	Deoxyribonucleic acid
EO	Ethylene oxide
HEPA	High efficiency particulate air
PCR	Polymerase chain reaction
QA	Quality assurance
qPCR	Quantitative PCR
STR	Short tandem repeat

4 Types of products

4.1 General

For the purposes of this International Standard, the products used in the collection, storage, and analysis of biological material for forensic DNA purposes are as outlined below. These products include, but are not limited to, the examples listed in 4.2, 4.3, and 4.4.

4.2 Products that come into direct contact with biological stains or material potentially containing human DNA

These products are considered to be high risk because the contaminating biological material or DNA is likely to be transferred. As a result, they should undergo post-production treatment provided it does not damage the product.

These products include, but are not limited to, the following examples:

a) gloves;

b) paper substrates onto which samples are deposited from persons;

c) plate seals/covers/strips;

d) plates;

e) primary transport container;

f) sampling kits;

g) spin baskets;

h) swabs;

i) tapes for sample recovery;

j) tips;

k) tubes.

4.3 Chemicals, reagents, solvents, and some disposables involved in the analysis of human DNA

These products are considered high risk because contaminating biological material or DNA is likely to generate a profile. If post-production treatment is not possible and batch release testing is unlikely to detect contamination due to the heterogeneous nature of the material, the stringency of anti-contamination measures adopted during manufacture shall be considered during the risk assessment as outlined in Clauses 6 and 7.

These products include, but are not limited to, the following examples:

a) DNA extraction kits;

b) DNA microconcentrators;

c) PCR amplification kits;

d) other chemicals and reagents used to identify, recover, and analyze biological material.

4.4 Protective barrier products that are used during the collection and analysis of biological material

These products are not specifically designed to come in contact with biological material and are generally protective barriers used to prevent contamination by forensic examiners.

These include, but are not limited to, the following protective clothing:

a) facial hair covers;

b) hairnets;

c) lab coats;

d) masks;

e) scene suits;

f) shoe covers;

g) sleeve guards.

5 Quality management systems

5.1 General

A documented quality management system shall be in place. The quality management system shall comply with a relevant quality-based standard.

NOTE ISO 9001 is an example of a relevant quality-based standard.

5.2 Documents and records

Documented procedures shall be established and maintained for the manufacture of products relevant to this International Standard, and for the creation and monitoring of the manufacturing environments. If applicable, documented procedures shall also define the post-production treatment and testing, and where required, any corrective actions. These documents shall be version controlled and have specific effective dates.

Records of the products shall include details that allow traceability to their component materials and shall include the control numbers that allow the identification of the date of production and the associated manufacturing location.

Documents and records required by this International Standard shall be maintained for a minimum of five years.

NOTE Five years has been nominated as most critical consumables would have expiry dates within this period. Legislation requirements in some countries can require longer periods. Consumers of these products can choose to include details for longer document retention periods in tender documents.

5.3 Authorization

The responsibilities and authority for implementing, performing, and monitoring the procedures and requirements described in this International Standard shall be specified and documented.

5.4 Subcontracting of work and purchase of components

The manufacturer shall evaluate and select suppliers and sub-contractors based on their ability to supply product in accordance with the manufacturers' requirements that assist the manufacturer in meeting this International Standard. Criteria for selection, evaluation, and re-evaluation shall be established. Records of the results of evaluations and any necessary actions arising from the evaluation shall be maintained.

The manufacturer shall retain responsibility for all components of their products.

5.5 Control of nonconforming product

The manufacturer shall ensure that product which does not conform to product requirements is identified and controlled to prevent its unintended use or delivery. A documented procedure shall be established to define the controls for dealing with nonconforming product.

Where applicable, the manufacturer shall deal with nonconforming product by one or more of the following ways:

a) by taking action to eliminate the detected nonconformity;

b) by authorizing its use, release, or acceptance under concession by a relevant authority and, where applicable, by the customer;

c) by taking action to preclude its original intended use or application;

d) by taking action appropriate to the effects, or potential effects, of the nonconformity when nonconforming product is detected after delivery or use has started.

When nonconforming product is corrected, it shall be subject to re-verification to demonstrate conformity to the requirements.

Records of the nature of nonconformities and any subsequent actions taken, including concessions obtained, shall be maintained.

Manufacturers shall establish procedures to notify customers who purchased released products subsequently found to have failed the product specifications or where the quality of the product has been impacted.

5.6 Corrective and preventive action

Documented procedures shall be established to investigate quality issues identified through environmental monitoring (see Clause 7), batch release testing (see Clause 9), or customer complaints relating to product contamination. This shall include recording the following:

— nature and extent of the issue;

— investigation to determine the root cause of the issue;

— corrective and preventive action taken;

— review of the effectiveness of the corrective action.

5.7 Staff contamination detection provision

Manufacturers shall document and implement a policy for the collection of relevant voluntary reference samples from personnel involved in the production of products.

NOTE 1 Refer to legislative or regulatory restrictions or preclusion in your jurisdiction regarding the collection of voluntary reference samples from staff.

A voluntary reference sample should be collected, with written permission, from personnel who have the potential to introduce DNA into the product. Personnel included in the staff contamination detection system should be determined based on risk. If a sample is provided, a relevant DNA profile (see Annex C) shall be generated and recorded for quality assurance purposes. Information on the staff contamination detection system shall be documented.

NOTE 2 Humans are constantly shedding DNA and the inadvertent contamination of product is a constant and unavoidable risk regardless of control measures implemented. When staff contamination of a product occurs, it can lead them to being erroneously linked by police to a crime or a series of crimes. A staff contamination detection system is used to detect the occurrence of contamination and protect against an erroneous linkage.

NOTE 3 The International Commission on Missing Persons provides a secure platform as a repository for DNA profiles of employees of manufacturing companies (www.ic-mp.org).

6 Human DNA contamination risk management

6.1 General

Risk management occurs across the product lifecycle. For the purposes of this International Standard, the scope of risk management is limited to risks that can introduce human DNA contamination into products used to collect, store, and analyze biological material for forensic purposes. The manufacturer shall establish, document, and maintain an on-going process for identifying, estimating, and evaluating the risks (see 6.2), controlling these risks (see 6.3 and 6.4), and monitoring the effectiveness of controls (see Clauses 7 and 9). The manufacturer shall collect and periodically review relevant product and process information (e.g. analysis of data, customer complaints, and corrective and preventive actions) to evaluate if any previously unrecognized risks are present or if a previously identified risk is no longer acceptable. If either of these occurs, the impact on previously implemented risk assessment and control measures shall be evaluated and the risk control measures updated as necessary. The results of the periodic reviews shall be recorded.

6.2 Risk assessment

The manufacturer shall perform and maintain a documented risk assessment evaluating identified risks for the probability of a potential human DNA contamination event occurring and the severity of that event if it were to occur. The risk assessment shall cover all stages of the manufacturing process for products listed in Clause 4.

Underlying assumptions and results of the risk assessment should be supported by corresponding data, quality control history, information, and/or research. When assessing the risk of a contamination event, the decision making process shall take into account the product's intended use and the ability of the manufacturer to detect the contaminant. Other factors influencing the risk event such as existing contamination controls should also be taken into account. These other factors include, but are not limited to, the following:

a) product protection (e.g. handling of open, primary packed or secondary packed product);

b) human product interaction (e.g. manual handling, semi-automated or fully automated process);

NOTE 1 Consideration can be given to the areas where there is a high risk of human DNA transfer or accumulation to the product, for example, a bench top where items are assembled or there is a high level of human-to-product interface.

c) personnel gowning (e.g. clothes, shoes, hairnet, mask, gloves, sleeve guards);

d) design of manufacturing environment (e.g. housing, cabinets, laminar airflow units);

e) design of room (e.g. floor, wall and ceiling surfaces, ventilation, HEPA filtration, positive air pressure, cleanroom);

f) design of workflow (e.g. unidirectional workflow, frequency of product batch production, other products produced in the same environment);

g) secondary contamination risk (e.g. contamination of equipment, secondary contamination of gloves, glove change rules);

h) personnel training (e.g. training in contamination prevention techniques);

i) room access (e.g. limited access to authorized and trained personnel);

j) cleaning regime (e.g. cleaning procedures, targeted surfaces, and frequency);

NOTE 2 Dust can be one of numerous significant contamination sources of human DNA.

k) staff and equipment changes;

l) the type of products (e.g. products that come into direct contact with biological stains or material potentially containing human DNA (see 4.2);

m) post-production treatment (e.g. EO treatment after production step);

n) systematic or sporadic contamination (e.g. bulk material or piece goods);

o) inspection and sampling plan for quality testing, including

— criteria for product acceptance, and

— sensitivity of applied DNA detection method.

NOTE 3 Final product testing cannot provide a high level of assurance of absence of DNA, where contamination is sporadic. If DNA contamination is extensive and homogeneous, product/component testing can identify DNA contamination if sensitive test methods are used.

The manufacturer shall have a documented procedure for determining risk acceptability. Each risk is evaluated for acceptability based on that procedure. Contamination risks are classified as acceptable (negligible risks), conditionally acceptable (minor risks), or unacceptable (significant risks), based on the manufacturer's documented risk acceptability criteria.

6.3 Risk mitigation

Unacceptable contamination risks shall be eliminated or reduced by implementation of additional contamination prevention measures such as those listed in 6.2 a) to o).

Conditionally, acceptable risks should be considered for further risk reduction. If the risk cannot be reduced further, a conditionally acceptable risk is considered acceptable.

If the risk classification is conditionally acceptable or unacceptable, the following risk reduction measures shall be considered, if they are not already in place:

a) personnel gowning including appropriate clothes, shoes, hairnet, mask, gloves, sleeve guards;

b) in areas of open product handling:

   — unidirectional workflow;

   — HEPA filters over air inlets;

   — positive air pressure;

c) laminar airflow units where technically feasible.

6.4 Risk control measures

6.4.1 Equipment

All equipment in direct contact with or that might contaminate the product shall be identified and recorded.

To minimize the risk of contamination, the production processes should be automated where technically and financially feasible.

6.4.2 Personnel

Access to the manufacturing environment shall be restricted. All authorized personnel entering the manufacturing environment shall be provided with appropriate training regarding DNA contamination risk reduction techniques. All training shall be documented.

6.4.3 Cleaning and maintenance

A cleaning schedule shall be established based on risk. The schedule shall take into account the environment and production conducted, and target surfaces and areas for potential human contamination. The cleaning procedure shall be appropriate and effective, documented and shall include a procedure for decontamination of surfaces and areas contaminated with human biological material. Records shall be maintained to demonstrate that appropriate cleaning has been undertaken in accordance with the established schedule.

NOTE As part of the demonstration of appropriateness and effectiveness of a cleaning regime, the manufacturer can use decontaminants and decontamination procedures that have been proven by others to be effective against the substances which would constitute contamination. The initial research data can be included as part of the manufacturer/assembler's documentation.

The documented procedure shall include any requirements for protective clothing to be worn by personnel undertaking maintenance in the manufacturing environment. In addition, any requirements for the cleaning of the equipment or environment following any maintenance shall be documented in the procedure. Records shall be maintained to demonstrate that appropriate cleaning was undertaken in accordance with the documented procedure.

7 Environmental human DNA monitoring

The determination of the need for environmental monitoring and where in the process it is required should be based on risk (see 6.2).

Where required, environmental monitoring shall be performed according to an approved and documented policy and procedure.

The procedure for monitoring shall include locations tested, number of samples per location tested, test method, detection limits, and should also include acceptance levels and testing intervals (in accordance with Annex A). Monitoring shall also be conducted following any clean-up procedure pursuant to any known human DNA contamination events.

All monitoring tests and results shall be documented and the records retained to identify any systemic issues. Data from the monitoring of the environment shall be trended regularly and used as an input into the risk management process. Environmental monitoring warning and action levels shall be determined based on risk and reviewed based on trending data.

A procedure regarding measures that shall be taken with batches of products produced in an environment that has failed the QA monitoring (i.e. DNA contamination is detected) shall be developed and documented.

If the pass parameter is not met, the appropriate action shall then be taken as per documented procedure.

8 Requirements for products subject to post-production treatment

Post-production treatment should be conducted on the products listed under 4.2, so long as the products are not damaged and it does not affect the product specifications. Post-production treatment may be considered for items listed in 4.3 and 4.4. If no post-production treatment is performed or the criteria in Clause 8 cannot be met, then Clause 9 shall apply.

NOTE 1 A method can impact chemical and physical properties of treated products which might interfere with downstream applications (e.g. effects on stability and integrity of purified DNA).

NOTE 2 An example of a product damaged by EO post-production treatment are products containing desiccant.

Safety considerations need to also be addressed when using any post-production treatments, e.g. removal of EO residuals to a safe level.

Post-production methods shall be effective, for guidance see Annex B.

Validation of post-production treatments shall demonstrate that the treatment is effective at reducing amplifiable human DNA contamination using cell spiked samples resulting in a DNA reduction factor of at least 1 000 (see Annex A) and does not introduce interference or negatively impact the performance of products, and that products are safe for human contact.

It is recognized that some components of products may be adversely affected by post-production treatment. Therefore, in these cases, it may only be appropriate to treat individual components of the product.

Manufacturers shall monitor and record compliance with the documented post-production treatment process.

A verification process shall be conducted of the post-production treatment. The frequency shall be determined based on risk. Revalidation should be performed if necessary (e.g. equipment change or material modification).

9 Requirements for products not subject to post-production treatment

9.1 Product testing

Manufacturers shall establish batch release testing that includes testing of the product for the presence of detectable human DNA using current forensic methods (see Annex A). Manufacturers shall develop a batch testing plan and procedure based on risk.

The test method used shall be validated and be sensitive enough to demonstrate conformity to this International Standard (see Annex A).

NOTE ISO/IEC 17025 and ISO 15189 provide guidance in the validation of test methods.

9.2 Batch records

The following information shall be recorded:

a) a lot or batch number;

b) a description of the quality test conducted;

c) limit of detection and/or acceptance criteria for the characteristics tested for;

d) results of the quality tests.

In the case of any out-of-specification result, the nature of the issue, any subsequent actions taken, and justification for what if any additional testing was performed and, if appropriate, how the product was determined to be conforming shall be documented (see 5.5 and 5.6).

10 Product packaging, labelling, and documentation

Products shall be packaged in a manner that maintains their integrity in reference to the requirements within this International Standard.

Manufacturers shall clearly identify when their products have been produced in accordance with this International Standard.

NOTE 1 An example of product identification/labelling can be “ISO 18385 Forensic DNA Grade”.

NOTE 2 The ISO 18385 forensic DNA grade label is given to products that have been produced in accordance with this International Standard.

The manufacturer should also indicate any method of post-production treatment used.

A product that includes multiple components shall be labelled so as to be clear which components adhere to this International Standard.

Annex A
(normative)

Compliance testing

A.1 General

The goal of compliance testing is to show the absence of amplifiable human nuclear DNA fragments that are of sufficient size and/or quantity to interfere with current forensic DNA analysis methods.

Test methods shall include extraction positive and negative controls as well as amplification positive and negative controls where appropriate.

Details of the methods and DNA analysis kits used may be obtained from the relevant forensic laboratories and the manufacturers of the kits.

A.2 Test procedures for environmental monitoring

The areas sampled for environmental monitoring should be swabbed with slightly moistened cotton swabs or equivalent sampling material (e.g. adhesive tape). The size and location of the sampling area of the equipment and/or production surface shall be documented and be appropriate for the item being tested.

It is recommended that each sampling area be 10 cm × 10 cm where possible.

The extraction method used shall be appropriate for samples containing low levels of DNA. The amount of total extracted DNA shall be analyzed using an appropriate real time qPCR test that can detect human DNA or other comparable tests.

The test procedure used shall be validated and be appropriate to demonstrate conformity to this International Standard. All tests shall be conducted by trained and competent personnel and training records maintained.

The results of tests shall be recorded and as a minimum include the following:

a) date testing performed;

b) test sample locations;

c) testing equipment and methods;

d) details to identify the testing laboratory (whether in-house or external);

e) analytical controls;

f) testing results (including pass/fail).

A.3 Validation of post-production treatments

This process shall include samples spiked with the same known quantity of human cellular material, such as cultured cells or white blood cells, in a sufficient quantity to extract human DNA from treated versus untreated samples and quantitate the DNA by qPCR, or a comparable quantitative method of equivalent or better sensitivity, to demonstrate the required DNA reduction-factor of 1 000.

NOTE 1 10 000 cells is a sufficient quantity and is approximately 67 ng of DNA.

Details about types of samples, analysis conditions and interpretation of results shall be recorded and maintained.

NOTE 2 The analysis of samples by qPCR can be established in-house or is available from commercial suppliers such as forensic laboratories and paternity testing laboratories.

A.4 Product testing

A.4.1 Testing of amplification kits

Amplification kits shall be tested to show absence of amplifiable human DNA within the reagents. qPCR quantification kits and STR PCR kits shall be tested using samples containing no template DNA and conducted according to manufacturer recommendations as a minimum.

For qPCR quantification kits, a result above the limit of detection of the assay shall be considered a fail.

For STR PCR kits, a result shall be determined by replicate testing. If 2 of 4 replicates (or equivalent) display 1 or more alleles over the analytical threshold within a read region, it is considered a fail.

A.4.2 Testing of DNA extraction kits

For DNA extraction kits, extraction negative controls shall be tested in accordance with A.4.3.

A.4.3 Testing of other products

Appropriate samples shall be tested. The identified sample (e.g. complete swab tip) shall be used in the DNA extraction method where applicable. The extraction method used should be appropriate for samples containing low levels of DNA. The input volume of eluate should be optimized to maximize sensitivity.

NOTE A suitable volume of eluate is 25 µl to 100 µl.

For the purposes of product testing, one of the following methods shall be used.

a) A DNA analysis kit containing 14 or more relevant STR markers (see Annex C) and conducted according to manufacturer recommendations as a minimum.

   If 2 or more allele/peaks appear above the analytical threshold in the read region, further investigation should be undertaken to determine if it is the result of contamination (e.g. replicate or qPCR testing). A result shall be considered a fail if more than 4 allele/peaks appear over the analytical threshold within the read region.

b) A qPCR system with a limit of detection of at least 0,5 pg/µl, using human inclusive primers, shall be used. A result should be determined by replicate testing. A result equivalent to a concentration of 50 pg or more in an elution volume of 100 µl shall be considered a fail.

c) Or other tests where equivalent or better sensitivity is demonstrated.

Annex B
(informative)

Guidance on the effectiveness of post-production treatments currently used in the manufacture of products

B.1 General

Each manufacturer needs to determine the effectiveness, suitability, and safety for the post-production treatment of their products. All methods can have an impact on chemical and physical properties of treated products which might interfere with downstream applications. Table B.1 provides information on commonly available treatments.

Table B.1 — Suitability for DNA reduction for commonly available post-production treatments

Method	Description	Suitability for DNA reduction	Literature
Electron beam	High-energy electrons from accelerators, products have to pass the electron beam for efficient sterilisation; either direct damaging effect on DNA or produce intracellular radicals that lead to microbial kill	Only the amplification of larger targets are affected, therefore not useful for the treatment of forensic consumables	[30],[32],[37],[39]
Ethylene oxide	Highly reactive cyclic ether; mechanism is alkylation of the amine groups of DNA causing mutations and strand breaks; direct effect on DNA in vitro and in vivo, therefore possible prevention of amplification in a PCR reaction	Effective decrease of the amount of PCR-amplifiable DNA, therefore suitable for the treatment of forensic consumables	[30],[33],[34],[36],[37],[39]
Gamma irradiation	Generated from radioactive decay of a cobalt-60 source; emitted gamma photons have a large penetration capacity, allowing for batch sterilisation; either direct damaging effect on DNA or produce intracellular radicals that lead to microbial kill	Only minor effects on cell-associated DNA, therefore not useful for the treatment of forensic consumables	[30],[31],[37],[39]
Heat/steam sterilization	Denaturation of enzymes and structural proteins, oxidation of cell constituents	Effective decrease of the amount of PCR-amplifiable DNA only with extended autoclaving procedures. Therefore partially suitable for the treatment of forensic consumables	[38]
Hydrogen peroxide gas	Dispersion of hydrogen peroxide solution, oxidizes key cellular components, which inactivate microorganisms.	Effectiveness unknown	
Hydrogen peroxide plasma	Dispersion of hydrogen peroxide solution in vacuum chamber, creating plasma cloud, oxidizes key cellular components, which inactivate microorganisms.	Effectiveness unknown	[35]
UV-light	Optimum wavelength 260 nm; effect on DNA (dimerization of pyrimidines, which prevent elongation during PCR)	Poor penetrability, therefore very limited suitability for the treatment of forensic consumables	[37],[39]

Annex C
(informative)

Relevant markers for DNA profiling

The DNA markers relevant for comparison differ slightly in different regions around the world and between countries. The list below shows examples of the markers generally used in forensic DNA analysis. A relevant DNA profile would consist of 14 of the following markers:

— CSF1PO;

— D1S1656;

— D2S1338;

— D2S441;

— D3S1358;

— D5S818;

— D7S820;

— D8S1179;

— D10S1248;

— D12S391;

— D13S317;

— D16S539;

— D18S51;

— D19S433;

— D21S11;

— D22S1045;

— FGA;

— SE33

— TH01;

— TPOX ;

— vWA.

Bibliography

[1] ISO 3696, *Water for analytical laboratory use — Specification and test methods*

[2] ISO 9001, *Quality management systems — Requirements*

[3] ISO 10993, *Biological evaluation of medical devices*

[4] ISO 10993-7, *Biological evaluation of medical devices — Part 7: Ethylene oxide sterilization residuals*

[5] ISO 11135, *Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices*

[6] ISO 11137,[1)]*Sterilization of health care products — Requirements for validation and routine control — Radiation sterilization*

[7] ISO 11137-2, *Sterilization of health care products — Radiation — Part 2: Establishing the sterilization dose*

[8] ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes*

[9] ISO 14644, *Cleanrooms and associated controlled environments*

[10] ISO 14644-1, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness*

[11] ISO 14644-2, *Cleanrooms and associated controlled environments — Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1*

[12] ISO 14644-4, *Cleanrooms and associated controlled environments — Part 4: Design, construction and start-up*

[13] ISO 14644-5, *Cleanrooms and associated controlled environments — Part 5: Operations*

[14] ISO 14644-7, *Cleanrooms and associated controlled environments — Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments)*

[15] ISO 14644-8, *Cleanrooms and associated controlled environments — Part 8: Classification of air cleanliness by chemical concentration (ACC)*

[16] ISO 14698, *Cleanrooms and associated controlled environments — Biocontamination control*

[17] ISO 14937, *Sterilization of health care products — General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices*

[18] ISO 14971, *Medical devices — Application of risk management to medical devices*

[19] ISO 15189, *Medical laboratories — Requirements for quality and competence*

[20] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

[21] ISO 17665, *Sterilization of health care products — Moist heat*

[22] ISO 31000, *Risk management — Principles and guidelines*

[23] ISO/IEC 31010, *Risk management — Risk assessment techniques*

[24] AAMI TIR 17:2008, *Compatibility of materials subject to sterilization*

1) Replaced by ISO 11137-1, ISO 11137-2 and ISO 11137-3.

[25] AAMI TIR 52, *Environmental monitoring for terminally sterilized healthcare products*

[26] Gill P., Gusmao L., Haned H., Mayr W.R., Morling N., Parson W. Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Sci. Int. Genet*. 2012, **6** pp. 679–688

[27] Puch-Solis R. A dropin peak height model. *Forensic Sci. Int. Genet*. 2014, **11** pp. 80–84

[28] Toothman M.H., Kester K.M., Champagne J., Dawson Cruz T., Scott Street W., Brown B.L. Characterization of human DNA in environmental samples. *Forensic Sci. Int*. 2008, **178** pp. 7–15

[29] UNODC, *Guidelines on Representative Drug Sampling, ISBN 978-92-1-148241-6*

[30] Lambert B.J., Mendelson T.A., Craven M.D. Radiation and ethylene oxide terminal sterilization experiences with drug eluting stent products. *AAPS PharmSciTech*. 2011, **12** pp. 1116–1126

[31] Hoile R., Banos C., Colella M., Walsh S.J., Roux C. Gamma irradiation as a biological decontaminant and its effect on common fingermark detection techniques and DNA profiling. J. *Forensic Sci*. 2010, **55** pp. 171–177

[32] Bergen A.W., Qi Y., Haque K.A., Welch R.A., Garcia-Closas M., Chanock S.J. Effects of electron-beam irradiation on whole genome amplification. *Cancer Epidem Biomark*. 2005, **14** pp. 1016–1019

[33] Nygren J., Cedervall B., Eriksson S., Dusinska M., Kolman A. Induction of DNA strand breaks by ethylene oxide in human diploid fibroblasts. *Environ. Mol. Mutagen*. 1994, **24** pp. 161–167

[34] Bhanot O.S., Singh U.S., Solomon J.J. The role of 3-hydroxyethyldeoxyuridine in mutagenesis by ethylene oxide. *J. Biol. Chem*. 1994, **269** pp. 30056–30064

[35] Miosan M., Barbeau J., Crevier M., Pelletier J., Philip N., Saoudi B. Plasma sterilization. Methods and mechanisms. *Pure Appl. Chem*. 2002, **74** pp. 349–358

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

[37] Shaw K., Sesardic I., Bristol N., Ames C., Dagnall K., Ellis C. Comparison of the effects of sterilisation techniques on subsequent DNA profiling. *Int. J. Legal Med*. 2008, **122** pp. 29–33

[38] Gefrides L.A., Powell M.C., Donley M.A., Kahn R. UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumables. *Forensic Sci. Int. Genet*. 2010, **4** pp. 89–94

[39] Neureuther K., Rohmann E., Hilken M., Sonntag M.L., Herdt S., Koennecke T. Reduction of PCR-amplifiable DNA by ethylene oxide treatment of forensic consumables. *Forensic Sci. Int. Genet*. 2014, **12** pp. 185–191

[40] Taylor D., Bright J.-A., McGoven C., Hefford C., Kalafut T., Buckleton J. Validating multiplexes for use in conjunction with modern interpretation strategies. *Forensic Sci. Int. Genet*. 2016, **20** pp. 6–19

ICS 11.100.99

Price based on 19 pages