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Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood —

Part 2: Isolated genomic DNA

Analyses de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour le sang total veineux —

Partie 2: ADN génomique extrait

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Foreword

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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).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems.*

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

A list of all parts in the ISO 20186 series can be found on the ISO website.

Introduction

Molecular in vitro diagnostics has enabled significant progress in medicine. Further progress is expected by new technologies analysing profiles of nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles of these molecules can change drastically during the pre-examination process, including the specimen collection, transport, storage and processing. Consequently, this makes the outcome from diagnostics or research unreliable or even impossible, because the subsequent examination might not determine the real situation in the patient but an artificial profile generated during the pre-examination processes.

Genomic DNA can fragment or degrade after blood collection. Therefore, special measures need to be taken to secure good quality specimens for genomic DNA examination. This is particularly relevant for examination test procedures requiring high molecular weight DNA (HMW DNA).

Standardization of the entire workflow from specimen collection to the genomic DNA examination is needed due to genomic DNA degradation and fragmentation after blood collection. Studies have been undertaken to determine the important influencing factors. This document draws upon such work to codify and standardize the steps for venous whole blood genomic DNA examination in what is referred to as the pre-examination phase.

In this document, the following verbal forms are used:

— “shall” indicates a requirement;

— “should” indicates a recommendation;

— “may” indicates a permission;

— “can” indicates a possibility or a capability.

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood —

Part 2: Isolated genomic DNA

1 Scope

This document gives guidelines on the handling, storage, processing and documentation of venous whole blood specimens intended for genomic DNA examination during the pre-examination phase before a molecular examination is performed. This document covers specimens collected in venous whole blood collection tubes.

This document is applicable to any molecular in vitro diagnostic examination performed by medical laboratories. It is also intended to be used by laboratory customers, in vitro diagnostics developers and manufacturers, biobanks, institutions and commercial organizations performing biomedical research, and regulatory authorities.

Different dedicated measures are taken for stabilizing blood cell free circulating DNA, which are not described in this document.

NOTE Circulating cell free DNA in blood is covered in ISO 20186-3.

Different dedicated measures are taken for collecting, stabilizing, transporting and storing capillary blood as well as for collecting and storing blood by paper based technologies or other technologies generating dried blood. These are not described in this document.

This document does not cover the isolation of specific blood cells and subsequent isolation of genomic DNA therefrom.

DNA in pathogens present in blood is not covered by this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 15189:2012, *Medical laboratories — Requirements for quality and competence*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2]

3.2
backflow
flow of a liquid opposite to the usual or desired direction

3.3
blood collection set
intravenous device specialized for venepuncture consisting of a stainless steel bevelled needle and tube (tubing) with attached plastic wings and fitting connector

Note 1 to entry: The connector attaches to an additional blood collection device, e.g. a *blood collection tube* (3.4).

3.4
blood collection tube
tube used for blood collection, usually in a vacuum which forces blood from the vein through the needle into the tube

3.5
blood genomic DNA stabilizers
compounds, solutions or mixtures that are designed to minimize degradation and fragmentation of *genomic DNA* (3.12) in blood

3.6
closed system
non-modifiable system provided by the vendor including all necessary components for the examination (i.e. hardware, software, procedures and reagents)

3.7
deoxyribonucleic acid
DNA
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.8
DNase
deoxyribonuclease
enzyme that catalyses the degradation of DNA into smaller components

3.9
examination
analytical test
set of operations having the object of determining the value or characteristics of a property

Note 1 to entry: Processes that start with the isolated *analyte* (3.1) and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2012, 3.7, modified — Term and definition are used here without the original notes; an additional term was added.]

3.10
examination performance
analytical test performance
analytical performance
ability of an examination procedure to measure or detect a particular *analyte* (3.1)

Note 1 to entry: Analytical performance is determined from analytical performance studies used to assess the ability of an in vitro diagnostic examination procedure to measure or detect a particular analyte.

Note 2 to entry: Analytical performance includes such characteristics as analytical sensitivity, detection limit, analytical specificity (interference and cross-reactivity), trueness, precision and linearity.

[SOURCE: ISO/TS 17822-1:2014, 3.2, modified — Two terms have been added.]

3.11
examination provider
analytical test provider
entity that provides the specific analytical test

3.12
genomic DNA
DNA from the nuclear and mitochondrial genomes containing all coding (exon) and non-coding (intron and other) sequences

Note 1 to entry: In this document, reference is only made to genomic DNA present in cells in blood, excluding circulating cell free DNA.

3.13
high molecular weight DNA
HMW DNA
DNA with an average double strand size larger than 50 kb on a pulsed field electrophoresis gel for the purpose of this document

3.14
interfering substances
endogenous or exogenous substances in clinical *specimens* (3.17)/*samples* (3.23) that can alter an examination result

Note 1 to entry: Examples of endogenous substances are blood components and acidic polysaccharides.

Note 2 to entry: Examples of exogenous substances are talc and anticoagulant.

3.15
needle holder
barrel used in routine venepuncture procedures to hold the *blood collection tube* (3.4) in place and to protect the phlebotomist from direct contact with blood

3.16
pre-examination processes
preanalytical phase
preanalytical workflow
processes that start, in chronological order, from the clinician's request and include the examination request, preparation and identification of the patient, collection of the *primary sample(s)* (3.17), transportation to and within the medical laboratory, isolation of analytes, and end when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes, e.g. DNA isolation procedures, which influence the outcome of the intended examination.

[SOURCE: ISO 15189:2012, 3.15, modified — An additional term has been added and more details have been included.]

3.17
primary sample
specimen
discrete portion of a body fluid, breath, hair or tissue taken for examination, study or analysis of one or more quantities or properties assumed to apply for the whole

[SOURCE: ISO 15189:2012, 3.16, modified — Notes to entry have been omitted.]

3.18
primary sample collection device
apparatus specifically intended by an IVD manufacturer to obtain, contain and preserve a body fluid or tissue for in vitro diagnostic examination

[SOURCE: ISO 18113-1:2009, 3.55]

Note 1 to entry: Includes devices intended to store a specimen prior to examination.

Note 2 to entry: Includes both vacuum and non-vacuum specimen collection devices.

3.19
proficiency testing
evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons

[SOURCE: ISO 17043:2010, 3.7, modified — Term and definition are used here without the original notes.]

3.20
RNA
ribonucleic acid
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

3.21
RNase
ribonuclease
enzyme that catalyses the degradation of RNA into smaller components

3.22
room temperature
temperature in the range of 18 °C to 25 °C

Note 1 to entry: Local or national regulations can have different definitions.

3.23
sample
one or more parts taken from a *primary sample* (3.17)

[SOURCE: ISO 15189:2012, 3.24, modified — The example has been omitted.]

3.24
stability
ability of a sample material, when stored under specified conditions, to maintain a stated property value within specified limits for a specified period of time

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — The words “reference material” were replaced by “sample material”.]

3.25
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.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 1 and 3 have been omitted.]

3.26
venous whole blood
blood collected after directly puncturing a vein, usually with a needle and syringe, or other collection device

3.27
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.

[SOURCE: ISO 9000: 2015, 3.8.12, modified — Note 1 and Note 2 have been omitted.]

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.

3.28
workflow
series of activities necessary to complete a task

4 General considerations

For general statements on medical laboratory quality management systems and in particular on specimen collection, reception and handling (including avoidance of cross contaminations) see ISO 15189:2012, 4.2, 5.4.4, 5.4.6 or ISO/IEC 17020:2012, 7.2 and Clause 8. The requirements on laboratory equipment, reagents, and consumables according to ISO 15189:2012, 5.3 shall be followed; ISO 15189:2012, 5.5.1.2 and 5.5.1.3 and ISO/IEC 17020:2012, 6.2 can also apply.

All steps of a diagnostic workflow can influence the final examination result. Thus, the entire workflow, including specimen/sample storage and transport conditions, and its impact on the stability of biomolecules intended to be examined shall be verified and validated. Workflow steps which cannot always be controlled shall be documented and their impact on the examination performance shall be investigated and mitigation measures shall be established to enable the required examination performance. In these cases, risk assessment is recommended.

The stability of the genomic DNA should be investigated throughout the complete pre-examination workflow. Additional post-collection effects can also occur, e.g. genomic DNA fragmentation[8].

Before or during the design of an examination, it should be investigated and ensured that the genomic DNA minimum amount and size required for the examination are not affected by the envisioned entire pre-examination workflow.

Safety procedures for handling and transport shall be in place. Safety regulations on transport and handling shall be considered (see ISO 15189:2012, 5.2.3 and 5.4.5, and ISO 15190).

During the whole pre-examination process, precautions shall be taken to avoid cross contamination between different samples/specimens, e.g. by using single-use material whenever feasible or appropriate cleaning procedures between processing of different specimens/samples.

If a commercial product is not used in accordance with the manufacturer's instructions, responsibility for its validation, verification, use and performance lies with the user.

NOTE International, national or regional regulations or requirements can also apply to specific topics covered in this document.

5 Outside the laboratory

5.1 Specimen collection

5.1.1 Information about the specimen donor/patient

The documentation shall include the ID of the specimen donor/patient, which can be in the form of a code.

The documentation should include, but is not limited to:

a) the relevant health status of the specimen donor or patient [e.g. healthy, disease type, concomitant disease, demographics (e.g. age and gender)];

b) the information about medical treatment and special treatment prior to blood collection (e.g. anaesthetics, medications);

c) the type and the purpose of the proposed examination requested;

d) the appropriate consent from the specimen donor/patient.

See also ISO 15189:2012, 5.4.4.

5.1.2 Selection of the venous whole blood collection tube by the laboratory

The quality of genomic DNA can be influenced (e.g. DNA fragmentation) by inadequate venous whole blood collection procedures, inappropriate storage/shipping conditions as well as DNA isolation procedures[9][10][11][12][13][14][15][16].

Blood should be collected in appropriate venous whole blood collection tubes containing an anticoagulant such as EDTA or acid citrate dextrose (ACD)[17], and their catalogue and lot number should be documented.

NOTE Blood collection tubes containing EDTA as an anticoagulant are preferable for most genomic DNA examination. Blood collection tubes containing heparin as an anticoagulant can impact the purity of the isolated genomic DNA, when using genomic DNA isolation methods not eliminating the heparin. Carrying over of heparin into the genomic DNA eluate can cause inhibitions in examination technologies, such as PCR.

Specifically developed whole blood collection tubes, containing genomic DNA stabilizing reagents, are also available, claiming to standardize blood collection, transport and storage and intended for DNA examinations from whole blood.

5.1.3 Venous whole blood specimen collection from the donor/patient and stabilization procedures

a) The identity of the person collecting the specimen and the time and date of blood collection according to ISO 15189:2012, 5.4.4.3, f) shall be documented.

b) For the labelling (sample/specimen identification) of the blood collection tube a routine procedure [ISO 15189:2012, 5.4.4.3, e)] or a procedure with additional information (e.g. 2D-barcode) shall be used.

c) Standard venepuncture technique can be used. Steps for preventing possible backflow into the donor's/patient's body can be required. The manufacturer's instructions for using the blood collection tubes shall be followed. A blood collection set and needle holder can be required when using blood genomic DNA stabilizer containing tubes. In this case, the instructions of the collection set and needle holder manufacturer shall be followed.

NOTE 1 There is no known specific effect of venous whole blood draw procedure on the genomic DNA. Routine procedures can therefore be used.

d) Blood collection tubes shall be filled in accordance to the manufacturer's instructions and attention should be drawn to the correct positioning of the collection tube during the blood draw as well as the required blood volume.

e) The blood collection tube manufacturer's instructions for mixing or inverting the tube immediately after blood collection shall be followed. Mixing or inverting the blood collection tube shall be done gently.

NOTE 2 Wrong and/or insufficient mixing can be one of the most important pre-examination variables. Unless additives in the blood collection tubes are homogenously mixed with the specimen, the genomic DNA quality can be compromised, which can impact the validity and reliability of the examination results.

f) Any tampering with and/or additions to the specimen shall be documented.

5.1.4 Information about the specimen and storage requirements at the blood collection facility

5.1.4.1 General

As blood genomic DNA can fragment or degrade after blood collection (see Figure A.1) and can thereby affect the validity and reliability of the examination result (see Figure A.2), the documentation regarding the specimen shall include the date and time of blood collection[17].

For specimens dedicated for long-term storage in a biobank, it is usually not known which individual genomic DNA examinations will be performed after the long-term storage, therefore either tubes with genomic DNA stabilizers should be used or, if using tubes without genomic DNA stabilizers, the recommendations for HMW DNA should be followed (see Table 1 and 5.1.4.3.2).

The temporary storage duration in the blood collection facility contributes to the total duration for storage.

5.1.4.2 Using blood collection tubes with stabilizers

For storing the specimens collected in whole blood collection tubes with blood genomic DNA stabilizers, the dedicated whole blood collection tube manufacturer's instructions on storage conditions shall be followed (e.g. temperature, freezing and duration). Where the examination provider's instructions are more stringent, these shall be followed. The storage conditions (temperature and duration, etc.) shall be documented.

5.1.4.3 Using blood collection tubes without stabilizers

5.1.4.3.1 Where using blood collection tubes without blood genomic DNA stabilizers, the examination provider's instructions on storage conditions shall be followed. This can require documentation of storage conditions (temperature and duration, etc.).

5.1.4.3.2 Where using blood collection tubes without blood genomic DNA stabilizers and no requirements on the storage conditions are available from the examination provider, the specimens should be processed as soon as possible.

As blood collection tubes containing EDTA as an anticoagulant are broadly used for genomic DNA examination, the following recommendations (see also Table 1) refer to this blood collection tube type.

For examinations requiring HMW DNA, the specimen should be stored at room temperature for not longer than one day or at 2 °C to 8 °C for not longer than three days (see Figure A.3). For longer storage, the specimen should be kept at −20 °C for not longer than 1 month, or at −70 °C or below for even longer storage.

For the examination of DNA variants not requiring HMW DNA examinations, the specimen should be stored at room temperature for up to 3 days or at 2 °C to 8 °C for up to 7 days (see Figure A.4). For longer storage, the specimen should be kept at −20 °C for up to 3 months or at −70 °C or below for even longer storage.

The storage conditions (duration and temperature, etc.) shall be documented.

5.2 Transport requirements

The required transport conditions shall be documented including any deviations therefrom.

Temperature monitoring should be applied in a suitable manner.

When using blood collection tubes with blood genomic DNA stabilizers, the dedicated tube's manufacturer's instructions on transport conditions shall be followed (duration and temperature, etc.). Where the examination provider's instructions are more stringent, these shall be followed. The transport conditions (duration and temperature, etc.) shall be documented.

When using blood collection tubes without blood genomic DNA stabilizers, the examination provider's instructions on transport conditions shall be followed. This can require the documentation of transport conditions (duration and temperature, etc.).

When using blood collection tubes without blood genomic DNA stabilizers and no examination provider's instructions are available, the specimen should be transported at either room temperature, at 2 °C to 8 °C, or at −20 °C or below within the specifications given in 5.1.4.3.2 and Table 1 in order to minimize the degradation and fragmentation of the genomic DNA[8][17]. The transport conditions (duration and temperature, etc.) shall be documented.

See also ISO 15189:2012, 5.4.5.

The transport duration to the laboratory contributes to the total duration for storage.

6 Inside the laboratory

6.1 Specimen reception

The specimen reception date and time shall be documented as well as the name of the person receiving the specimen. Nonconformities of labelling, transport conditions and blood volume differences to specifications, leaking/broken tubes, etc., shall be documented.

NOTE This includes, for example, a note when specimens shipped at 2 °C to 8 °C or on wet ice are not cool or transportation containers do not contain dry ice as intended by the sender.

Where there are nonconformities in labelling, transport conditions, overall storage and transport duration or blood volume that could affect the validity and reliability of the examination result, a new specimen should be obtained.

The correct identity of the specimen shall be checked. This should include the clinical information (see 5.1.1 and 5.1.3) of the specimen, hospital admission number and/or donor/patient ID, name of the patient, date of birth of the patient.

6.2 Storage requirements

The storage temperature and time interval between specimen receipt and sample processing for genomic DNA isolation shall be documented. Storage temperature and total storage duration shall

not exceed specifications identified in 5.1.4.2, 5.1.4.3.1 and 5.2, and should not exceed specifications identified in 5.1.4.3.2.

The specimen total storage duration shall include the duration for storage at the blood collection facility (see 5.1.4), for transportation to the laboratory (see 5.2) and for further storage at the laboratory or other institutions. Any specified maximum storage duration given by the blood collection tube manufacturer or the provider of the examination shall not be exceeded. If such specifications are not available, the maximum storage duration shall be verified and generally kept to a minimum.

See also Table 1.

Table 1 — Summary of storage conditions for whole blood collection tubes with or without blood genomic DNA stabilizers

Blood collection tube	Blood storage		Examination type
	Duration	Temperature	
With blood genomic DNA stabilizer	According to blood collection tube manufacturer's or examination provider's instructions[a,b]		According to blood collection tube manufacturer's instructions.
Without blood genomic DNA stabilizer	≤1 day[d] Examination provider's instructions[c]	Room temperature[d] Examination provider's instructions[c]	Examinations requiring HMW DNA
	≤3 days[d] Examination provider's instructions[c]	2 °C to 8 °C[d] Examination provider's instructions[c]	Examinations requiring HMW DNA
	≤1 month[d] Examination provider's instructions[c]	−20 °C[d] Examination provider's instructions[c]	Examinations requiring HMW DNA
	≤3 days[d] Examination provider's instructions[c]	Room temperature[d] Examination provider's instructions[c]	Examinations not requiring HMW DNA
	≤7 days[d] Examination provider's instructions[c]	2 °C to 8 °C[d] Examination provider's instructions[c]	Examinations not requiring HMW DNA
	≤3 month[d] Examination provider's instructions[c]	−20 °C[d] Examination provider's instructions[c]	Examinations not requiring HMW DNA
	Longer storage[d] Examination provider's instructions[c] [18]	−70 °C or below[d] Examination provider's instructions[c]	For all examinations

a Requirement according to 5.1.4.2.

b Alternative more stringent requirement according to 5.1.4.2.

c Requirement according to 5.1.4.3.1.

d Recommendation according to 5.1.4.3.2.

6.3 Isolation of the genomic DNA

6.3.1 General

To avoid a cross contamination with amplified material, the isolation of the genomic DNA should not be performed in the same area as the amplification and post-amplification steps of the examination process, unless a closed system is used, which is designed to avoid cross-contamination.

The genomic DNA isolation procedure chosen shall fulfil the requirements and specifications of the intended molecular examination (e.g. DNA quality and quantity, DNA concentration, DNA length). If the examination requires HMW DNA, genomic DNA isolation methods should be verified and validated for this purpose[8].

NOTE Genomic DNAs obtained by different genomic DNA isolation procedures can be of different length. In addition, the genomic DNA quantity and quality (e.g. purity) can vary[8][16].

6.3.2 Examination provider's instructions available

If the specifications of the examination provider require the use of a dedicated commercially available kit, then this shall be used in accordance with the instructions of the examination provider.

NOTE Dedicated procedures for processing frozen specimens/samples can be included in the examination provider's instructions.

6.3.3 Examination provider's instructions not available

6.3.3.1 Using blood collection tubes without DNA stabilizers

6.3.3.1.1 When using blood collection tubes not containing any blood genomic DNA stabilizers, a commercially available kit for the isolation of blood genomic DNA should be used. The kit manufacturer's instructions for isolating the genomic DNA shall be followed.

NOTE Dedicated procedures for processing frozen blood specimens/samples can be included in the DNA isolation kit manufacturer's instructions.

6.3.3.1.2 Alternative genomic DNA isolation procedures can also be used if they are verified for the same requirements and validated for the same intended use. In this case, the instructions for this validated alternative for isolating the genomic DNA shall be followed.

The laboratory's development, verification and validation of this alternative blood genomic DNA isolation procedure, shall specifically include the validation of the entire genomic DNA isolation process including the compatibility of the reagents and substances used.

The reagents and consumables coming in contact with the genomic DNA should be DNase-free.

6.3.3.2 Using blood collection tubes with DNA stabilizers

6.3.3.2.1 When processing blood from tubes containing blood genomic DNA stabilizers, DNA isolation kits specified by the manufacturer of the blood collection tube should be used for the isolation of genomic DNA. The blood collection tube and kit manufacturer's instructions for isolating the genomic DNA shall be followed.

NOTE Dedicated procedures for processing frozen blood specimen/samples can be included in the DNA isolation kit manufacturer's instructions.

6.3.3.2.2 Alternative isolation procedures can be used, if they are verified for the same requirements and validated for the same intended use. In this case, the instructions for the validated alternative for isolating the genomic DNA shall be followed.

NOTE When using alternative isolation procedures, dedicated measures and technologies can be needed in order to avoid carrying over genomic DNA stabilization molecules to the final DNA eluate. Stabilization molecules carry-over can lead to an inhibition of the examination reaction.

The reagents and consumables coming in contact with the genomic DNA should be DNase-free.

6.4 Quantity and quality assessment of isolated genomic DNA

The genomic DNA quantity and quality should be checked according to the examination provider's instructions or according to validated procedures by generally accepted physical, chemical and biochemical procedures[16]. These may include one or more of the following:

a) quantification by absorbance measurements (A_{260}) or spectrofluorometry;

b) test for purity by absorbance measurements (e.g. wavelength scan, A_{260}/A_{280} ratio, A_{260}/A_{230} ratio);

c) test for DNA integrity (by e.g. electrophoresis, capillary electrophoresis, chromatography, molecular methods such as the differential length amplicon ratio);

d) test for presence of interfering substances (using exogenous controls (spiked DNA controls) or inspecting qPCR response curves for anomalies)[23][24] or using an endogenous DNA sequence for a PCR inhibition test by introducing increasing eluate volumes into the examination.

After validation it may be possible to reduce the number of quality control measures.

The genomic DNA isolation performance should be tested in a DNA proficiency test program.

6.5 Storage of isolated genomic DNA

6.5.1 General

For long-term storage, usually the isolated genomic DNA is frozen. However, for genomic DNA preservation other validated methods for archiving can also be used, see for example References [12], [13], [14] and [18].

For long-term storage, aliquots of the isolated genomic DNA should be generated to avoid repeated freezing and thawing or repeated recovery from other archiving systems.

For small DNA amounts, storage vessels with reduced nucleic acid adsorption to the tube wall should be used.

Unintended freeze-drying of the isolated genomic DNA during long-term storage due to water evaporation should be avoided as the DNA can fragment and degrade, which can be critical, e.g. for HMW DNA based examination. Therefore, appropriate storage vessels, such as screw-capped cryogenic vials, avoiding water evaporation during long-term storage, should be used and documented.

Traceability shall be ensured. For long-term storage, a validated system to organize and uniquely mark aliquots in the intended storage temperature making them easily retrievable and identifiable should be in place. Labels suitable for storage temperature with readable 1D- or 2D-barcodes or pre-printed tubes with unique codes provided by manufacturers are recommended to avoid loss or confusion of sample identity.

6.5.2 Genomic DNA isolated with commercially available kits

For storing the isolated genomic DNA before the examination, the DNA isolation kit provider's specific instructions should be followed. Where the examination provider's instructions are most stringent, these shall be followed.

6.5.3 Genomic DNA isolated with the laboratory's own protocols

If the laboratory's own validated genomic DNA isolation procedures are used, the time allowed to store the isolated genomic DNA before examination shall be verified for a reasonable time period.

For long-term storage, the laboratory shall have verified protocols in place on how to store the isolated genomic DNA. DNA can be stored frozen.

For long-term storage, genomic DNA should be eluted in an appropriate buffer and stored at ≤–20 °C[17]. Other validated methods for archiving can also be used[12][13][14][18].

Annex A
(informative)

Impact of pre-examination process steps on venous whole blood genomic DNA quality

A.1 General information on operated experiments

The Standardization and Improvement of Generic Pre–examination Tools and Procedures for in vitro Diagnostics (SPIDIA[1)]) was a European Commission funded, four-and-a-half-year project aiming at the standardization and improvement of pre-examination procedures for in vitro diagnostics[19].

The SPIDIA consortium performed two consecutive pan-European ring trials for identifying and improving critical pre-examination process steps influencing venous whole blood genomic DNA examination[16]. A total number of 197 and 188 European laboratories participated in ring trial 1 and 2, respectively. A proficiency venous whole blood specimen from a single donor was prepared at a central SPIDIA facility and subsequently shipped in polypropylene tubes to the participating laboratories. The sample was shipped in dedicated boxes in order to maintain a constant temperature range of 2 °C to 8 °C.

The participants were asked to isolate the genomic DNA using their routine laboratory procedure and to send the purified DNA sample back at 2 °C to 8 °C to the SPIDIA facility. In order to analyse the impact of pre-examination variables on the genomic DNA, the isolated genomic DNA samples were evaluated for purity, yield, HMW DNA integrity, and presence of PCR interferences at the SPIDIA laboratories.

Results presented below in A.2 to A.5 show important findings.

A.2 Influence of pre-examination variables (blood storage duration and temperature, and DNA isolation methods) on genomic DNA integrity

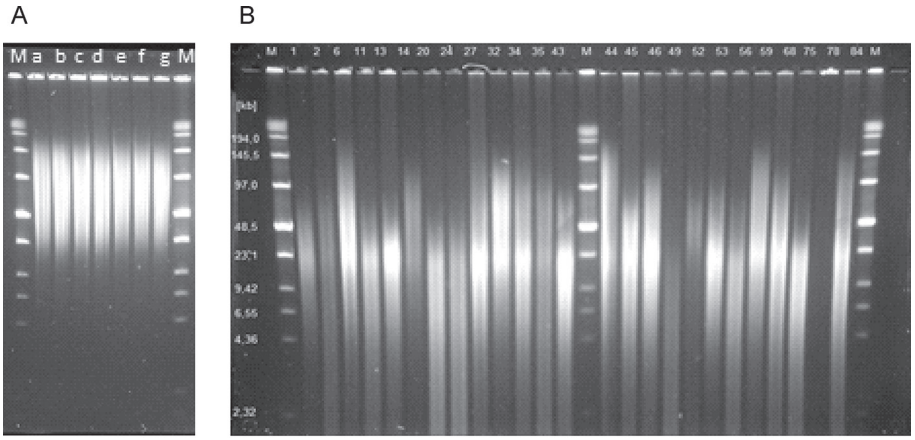
Figure A.1 shows the DNA integrity depending on the routine pre-examination workflow adopted by European laboratories participating in the first ring trial.

Genomic DNA was isolated by the participating laboratories following their own routine procedure (blood storage duration and temperature, and genomic DNA isolation method). Different storage durations (ranging from 5 days to 40 days after blood collection), different storage temperatures (–20 °C, 4 °C, and room temperature), and different genomic DNA isolation procedures (based on columns, magnetic beads, and precipitation procedures) were adopted. Fragmentation of HMW DNA was evaluated by Pulsed Field Gel Electrophoresis (PFGE)[8]. Figure A.1 shows PFGE results in a subset of samples (n = 25).

Figure A.1 A shows the genomic DNA fragment size distribution pattern of samples extracted by a precipitation method immediately after blood collection (T_0).

Figure A.1 B shows the genomic DNA fragment size distribution of samples extracted by participating laboratories.

1) Results incorporated in this document received funding from the European Union's Seventh Framework Programme under grant agreement No 222916. For further information see www.spidia.eu.



Key

A	genomic DNA isolated immediately after blood collection (reference laboratory)	a to g	DNA samples
B	genomic DNA isolated by European laboratories participating in the ring trial	1 to 84	DNA samples
M	ladder marker		

NOTE 1 High molecular weight DNA (HMW DNA) means DNA larger than 50 kb, see 3.13.

NOTE 2 The HMW DNA examination was performed as described in Reference [16].

Figure A.1 — DNA fragment size distribution of blood genomic DNA samples

The genomic DNA samples isolated by participant laboratories showed different degrees of genomic DNA integrity. The genomic DNA integrity was strongly influenced by the different laboratories' internal pre-examination processes (blood storage duration, storage temperature, genomic DNA isolation method).

These results suggest that the implementation of standardized pre-examination processes will be useful in order to obtain more homogeneous genomic DNA quality in different laboratories.

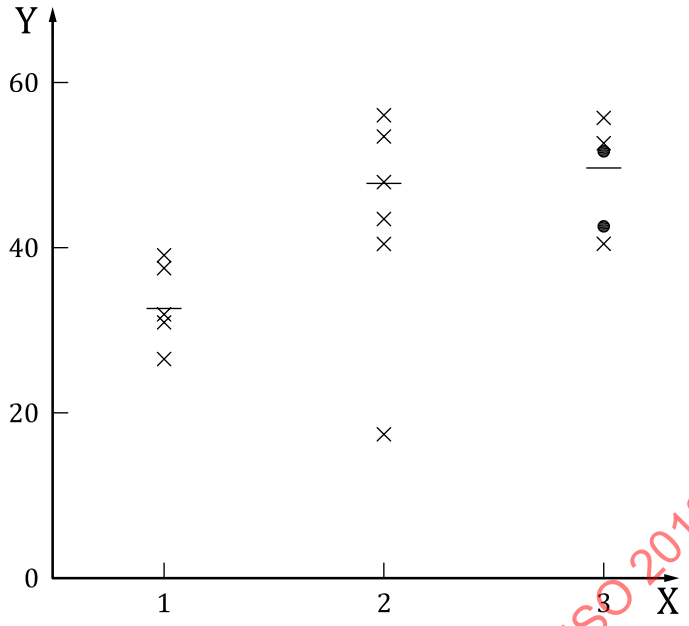
A.3 Influence of genomic DNA integrity on an examination based on long PCR amplicons

Figure A.2 shows the influence of DNA integrity on the results of a diagnostic examination based on the evaluation of the percentage of detected T-cell receptor (TCR) recombinations[20][21].

This examination is used to investigate the function and the kinetics of the immune system (e.g. in patients affected by cancer, infections, diseases)[20][21]. The examination analyses the TCR recombinations by a multiplex-PCR consisting in the 276 PCR reactions covering all potential recombinations. The PCR amplicon lengths are between 250 bp and 4 500 bp. A dedicated and specific software determined percentage of detected TCR recombinations.

A subset of genomic DNA samples (n = 15) from participating laboratories was examined for the capability to detect TCR recombinations[8].

The samples were classified by quality criteria of the TCR recombination examination as “fragmented”, “intermediate” and “intact”.



Key

•	controls	1	fragmented
×	samples	2	intermediate
—	means	3	intact
X	DNA quality classifications	Y	detected TCR recominations (%)

Figure A.2 — Influence of DNA integrity on results of a diagnostic test

The percentage of detected TCR recombinations was different depending on the genomic DNA integrity. The samples classified as "intact" gave the same results as the positive controls. The mean value of samples classified as "intact" or "intermediate" showed similar percentages in TCR recombinations, and higher values compared to the "fragmented" samples.

The integrity of genomic DNA can influence diagnostic test results.

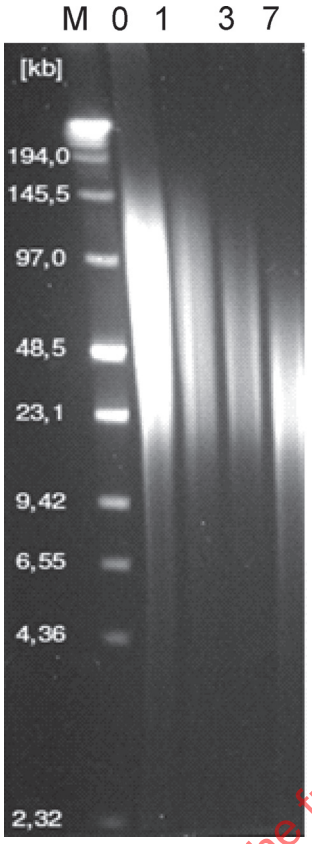
A.4 Influence of blood storage time on the genomic DNA integrity

Figure A.3 shows the DNA integrity depending on blood storage duration.

The venous whole blood specimens were collected in K_2EDTA tubes and stored until DNA isolation. The genomic DNA was isolated immediately after blood collection (0), or after storage at 2 °C to 8 °C for 1 day, 3 days, and 7 days.

A salt-based precipitation method[8] was used for isolating the genomic DNA as this technology allows the isolation of HMW DNA.

The DNA integrity was evaluated by pulsed field gel electrophoresis (PFGE)[8].



Key

M	ladder marker	3	3 days after blood collection
0	immediately after blood collection	7	7 days after blood collection
1	1 day after blood collection		

NOTE 1 The DNA fragment size examination was performed as described in Reference [16].

NOTE 2 High molecular weight DNA (HMW DNA) means DNA larger than 50 kb, see 3.13.

Figure A.3 — DNA integrity in blood genomic DNA samples isolated by a precipitation-based method in relation to the blood storage duration

Although a slight decrease in genomic DNA integrity was observed after 1 day, the genomic DNA was still of high molecular weight. Fragmentation continuously increased until the last time point tested in this experiment (7 days after blood collection).

The genomic DNA integrity can be significantly influenced by the storage duration of specimens. The highest integrity is obtained, when genomic DNA isolation is performed immediately after blood collection.

NOTE 1 Higher blood sample storage temperatures than 2 °C to 8 °C can lead to increased fragmentation of genomic DNA.

NOTE 2 Different genomic DNA isolation methods and protocols can also influence the integrity of the isolated genomic DNA.

A.5 Influence of blood storage conditions on the performance of PCR tests based on short amplicons

Figure A.4 shows the influence of a) the blood storage duration on the genomic DNA integrity and b) on the amplifiability of short amplicons.

