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BSI Standards Publication

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

Part 2: Isolated genomic DNA

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National foreword

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Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus pré-analytiques pour le sang total veineux - Partie 2: ADN génomique extrait

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für venöse Vollblutproben - Teil 2: Isolierte genomische DNS

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European foreword

This document (CEN/TS 16835-2:2015) has been prepared by Technical Committee CEN/TC 140 “In vitro diagnostic medical devices”, the secretariat of which is held by DIN.

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Introduction

Molecular *in vitro* diagnostics has enabled a significant progress in medicine. Further progress is expected by new technologies analysing signatures of nucleic acids, proteins, and metabolites in human tissues and body fluids. However, the profiles of these molecules can change drastically during primary sample collection, transport, storage and processing thus making the outcome from diagnostics or research unreliable or even impossible because the subsequent analytical assay will not determine the situation in the patient but an artificial profile generated during the pre-examination process.

A standardization of the entire process from primary sample collection to genomic DNA analysis is needed due to genomic DNA degradation and fragmentation after blood collection. Studies have been undertaken to determine the important influencing factors. This Technical Specification draws upon such work to codify and standardize the steps for venous whole blood genomic DNA analysis in what is referred to as the preanalytical phase.

1 Scope

This Technical Specification recommends the handling, documentation and processing of venous whole blood specimens intended for genomic DNA analysis during the preanalytical phase before a molecular assay is performed. This Technical Specification covers specimens collected by venous whole blood collection tubes. This Technical Specification is applicable to molecular *in vitro* diagnostic examinations (e.g. *in vitro* diagnostic laboratories, laboratory customers, *in vitro* diagnostics developers and manufacturers, institutions and commercial organizations performing biomedical research, biobanks, and regulatory authorities).

Blood genomic DNA can fragment or degrade after blood collection. Therefore, special measures need to be taken to secure good quality blood samples for genomic DNA analysis. This is particularly relevant for analytical test procedures requiring high molecular weight DNA.

Different dedicated measures need to be taken for preserving blood circulating cell free DNA, which are not described in this Technical Specification. Circulating cell free DNA in blood is covered in CEN/TS 16835-3, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 3: Isolated circulating cell free DNA from plasma*.

Different dedicated measures need to be taken for collecting, stabilizing, transporting and storing capillary blood as well as for blood collected and stored by paper based technologies. These are not described in this Technical Specification.

DNA from pathogens present in blood is not covered by this Technical Specification.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 15189:2012, *Medical laboratories — Requirements for quality and competence (ISO 15189:2012, Corrected version 2014-08-15)*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 15189:2012 and the following apply.

3.1
ambient temperature
unregulated temperature of the surrounding air

3.2
analytical phase
processes that start with the isolated analyte and include all kind of parameter testing or chemical manipulation for quantitative or qualitative analysis

3.3
blood genomic DNA stabilizers
compounds, solutions or mixtures that are made to minimize degradation and fragmentation of genomic DNA in a blood sample

3.4
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: EN ISO 22174:2005, 3.1.2]

3.5
genomic DNA
DNA from the genome containing all coding (exon) and non-coding (intron and other) sequences

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

3.6
high molecular weight DNA
HMW DNA
DNA larger than 50 kb for the purpose of this document

3.7
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), temporary storage, transportation to and within the analytical laboratory, aliquotting, retrieval, isolation of analytes, and end when the analytical examination begins

[SOURCE: EN ISO 15189:2012, 3.15, modified — An additional term was added and more details were included.]

Note 1 to entry: The preanalytical phase may include preparative processes that may influence the outcome of the intended examination.

3.8
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: EN ISO 15189:2012, 3.16, modified — The term and definition is used here without the original notes.]

3.9
room temperature
temperature which is defined as 18 °C to 25 °C for the purpose of this document

3.10
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".]

Note 1 to entry: The measured constituent for the purpose of this document is genomic DNA.

4 General considerations

For general statements on primary sample collection and handling (including avoidance of cross contaminations), see EN ISO 15189:2012, 5.2.6, 5.4.4. Consumables including kits shall be verified before use in examination (see EN ISO 15189:2012, 5.3.2.3); EN ISO 15189:2012, 5.5.1.2 and 5.5.1.3 can also apply.

As all steps of a diagnostic workflow can influence the final analytical performance, the entire workflow, comprising the preanalytical steps, including information on sample stability and storage conditions, and the analytical steps should be verified and validated (see EN ISO 15189).

The stability of the genomic DNA should be investigated throughout the complete pre-analytical workflow.

Before or during the design of the analytical test system it should be investigated and ensured that the genomic DNA minimum amount and size required for the analytical test are not affected by the envisioned entire preanalytical workflow.

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

Safety regulations on facilities, transport and handling shall be considered (EN ISO 15189:2012, 5.2.3 and 5.4.5, and ISO 15190).

5 Outside the laboratory

5.1 Primary venous whole blood collection manual

5.1.1 Information about the primary sample donor

The documentation should include, but is not limited to:

a) the primary donor / patient ID, which can be in the form of a code;

b) the health status and relevant lifestyle factors of the blood donor (e.g. healthy, disease type, gender, age);

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

d) the type and the purpose of the analytical test requested.

See also EN 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 blood collection procedures, inappropriate storage/shipping conditions and DNA isolation procedures, [3], [4], [5], [6], [7], [8], [9], [10].

Blood should be collected in appropriate venous whole blood collection tubes containing an anticoagulant such as EDTA or Acid Citrate Dextrose (ACD) [11].

NOTE Blood collection tubes containing EDTA as an anticoagulant are preferable for most genomic DNA analysis. 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 analytical test technologies, such as PCR.

Specifically developed blood collection tubes, containing genomic DNA stabilizing reagents, are also available aimed to standardize blood collection, transport and storage of venous whole blood.

5.1.3 Primary venous whole blood sample collection from the patient and stabilization procedures

1. The identity of the person collecting the primary sample and the time of blood collection according to EN ISO 15189:2012, 5.4.4.3, f) shall be documented.

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

3. Standard venepuncture technique can be used. Steps for preventing possible backflow may be required. The manufacturers' 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 There is no known specific effect of venous whole blood draw procedure on the genomic DNA. Routine procedures can therefore be used.

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

5. Blood collection tube manufacturers' instructions for mixing or inverting the tube immediately after blood collection shall be followed.

   NOTE Unless additives are homogenously mixed with the blood sample, the genomic DNA quality can be compromised, which can impact the validity and reliability of the analytical test results.

6. Any tampering with and/or additions to the primary sample shall be documented.

5.1.4 Information on the primary blood sample and storage requirements at the blood collection facility

5.1.4.1 General

As blood genomic DNA can fragment or degrade after blood collection (Figure A.1) and can thereby affect the validity and reliability of the analytical test result (Figure A.3), the documentation on the primary blood sample shall include the date and should also include the time of blood collection [11].

For samples dedicated to be archived in a biobank it is usually not known which individual genomic DNA tests will be performed after archiving, 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 primary blood samples collected in blood collection tubes with blood genomic DNA stabilizers, the blood collection tube manufacturers' instructions on storage conditions shall be followed (including the storage duration and temperature). Where the analytical test providers' instructions are more stringent, these shall be followed. The storage conditions (including the storage duration and temperature) shall be documented.

5.1.4.3 Using blood collection tubes without stabilizers

5.1.4.3.1 When using blood collection tubes without blood genomic DNA stabilizers, the analytical test providers' instructions on storage conditions shall be followed. This can require documentation of storage conditions.

5.1.4.3.2 When using blood collection tubes without blood genomic DNA stabilizers and no requirements on the storage conditions are available from the analytical test provider, the primary venous whole blood samples should be processed as soon as possible.

As blood collection tubes containing EDTA as an anticoagulant are most wide spread for genomic DNA analysis the following recommendations (see also Table 1) refer to this blood collection tube type. For analytical tests requiring High Molecular Weight DNA, the blood sample 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. For a longer storage the sample should be kept at −20 °C for not longer than 1 month, or at −70 °C or below for longer storage. For the analysis of DNA variants not requiring HMW DNA analyses, the blood sample should be stored at room temperature for up to 3 days or at 2 °C to 8 °C for up to 7 days (Figure A.4). For a longer storage the sample should be kept at −20 °C for up to 3 months or at −70 °C or below for longer storage.

The storage conditions (including the storage duration and temperature) shall be documented.

5.2 Transport requirements

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

When using blood collection tubes with blood genomic DNA stabilizers, the tubes' manufacturers' instructions on transport conditions shall be followed (including the transport duration and temperature). Where the analytical test providers' instructions are more stringent, these shall be followed.

When using blood collection tubes without genomic DNA stabilizers, the analytical test providers´ instructions on transport conditions shall be followed. This can require the documentation of transport conditions (including the transport duration and temperature).

When using blood collection tubes without genomic DNA stabilizers and no analytical test provider's instructions are available, the primary blood sample 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 blood genomic DNA [11], [12].

See also EN 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 Primary sample reception

The blood sample reception time shall be documented. 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 samples shipped on wet-ice were not cool anymore 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 analytical test result, a new sample should be obtained.

6.2 Storage requirements

The storage temperature and time interval between primary sample 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 primary blood sample total storage duration shall include the duration for storage at the blood collection facility (5.1.4), for transportation to the laboratory (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 analytical test manufacturer shall not be exceeded. If such specifications are not available, the maximum storage duration shall be validated 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		Analytical test type
	Duration	Temperature	
With blood genomic DNA stabilizer	— Blood collection tube manufacturer's instructions[a] — Analytical test providers' instructions[a, b]		According to blood collection tube manufacturer's instructions.
Without blood genomic DNA stabilizer	— ≤ 1 day[d] — Analytical test instructions[c]	— Room temperature[d] — Analytical test instructions[c]	Tests requiring HMW DNA
	— ≤ 3 days[d] — Analytical test instructions[c]	— 2 °C to 8 °C[d] — Analytical test instructions[c]	Tests requiring HMW DNA
	— ≤ 1 month[d] — Analytical test instructions[c]	— –20 °C[d] — Analytical test instructions[c]	Tests requiring HMW DNA
	— ≤ 3 days[d] — Analytical test instructions[c]	— Room temperature[d] — Analytical test instructions[c]	Tests not requiring HMW DNA
	— ≤ 7 days[d] — Analytical test instructions[c]	— 2 °C to 8 °C[d] — Analytical test instructions[c]	Tests not requiring HMW DNA
	— ≤ 3 month[d] — Analytical test instructions[c]	— –20 °C[d] — Analytical test instructions[c]	Tests not requiring HMW DNA
	— Longer storage[d] — Analytical test instructions[c]	— –70 °C or below[d] — Analytical test instructions[c]	For all analytical tests

[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

The laboratory shall validate the entire process comprising sample collection, storage, transportation and isolation of genomic DNA to the final analytical test result according to its internal quality management system (see EN ISO 15189).

To avoid a cross contamination with amplified DNA, the isolation of the blood genomic DNA should not be performed in the same area as the amplification and post-amplification, unless a closed system is used.

The genomic DNA isolation procedure chosen shall fulfil the requirements and specifications of the intended molecular analytical test (e.g. DNA quality and quantity, DNA concentration, DNA length). If the analytical test requires high molecular weight DNA, dedicated genomic DNA isolation methods such as DNA precipitation-based methods should be applied [12]

The reagents and consumables coming in contact with the DNA sample shall be DNase-free.

6.3.2 Using commercial kits

1. When using commercial kits for the isolation of genomic DNA from blood, the manufacturers' instructions for use shall be followed, unless 6.3.2, 4. and / or 6.3.3.1 apply.

2. When processing blood from tubes dedicated for genomic DNA testing and dedicated DNA isolation kits are specified by the manufacturer of the blood collection tube, then these should be used for the isolation of genomic DNA. Alternative extraction procedures can be used, if verified for the same requirements and validated for the same intended use (6.3.3.1, 6.3.3.2).

3. When processing blood from tubes not dedicated for genomic DNA testing, commercially available generic genomic DNA isolation kits can be used.

4. If the specifications of the analytical test provider require the use of a dedicated commercially available kit, then this shall be used in accordance to the analytical test providers' instructions.

NOTE Dedicated procedures for use can be included in the manufacturers' instructions for processing frozen blood samples.

6.3.3 Using the laboratories own protocols

6.3.3.1 If a commercial kit is not used in accordance to its intended use, but is validated fit for purpose as defined by the user, instructions shall be written and followed.

6.3.3.2 If the laboratory uses its own protocol independent from a commercial kit, the validation demonstrating fit for purpose shall be performed and instructions shall be written and followed.

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.

6.4 Quantity and quality assessment of isolated genomic DNA

The genomic DNA quantity and quality should be checked according to diagnostic kit manufacturer's instructions, or according to validated procedures by generally accepted physical, chemical and biochemical procedures [10]. These include:

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

b) test for purity by absorption measurements (wavelength scan, A_{260}/A_{280} ratio);

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

d) test for interference (using exogenous controls (spiked DNA controls) or inspecting qPCR response curves for anomalies).

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

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

6.5 Storage of isolated genomic DNA

For storing and archiving the isolated genomic DNA, the DNA isolation kit providers' specific instructions should be followed.

If there is no information available from the blood genomic DNA isolation kit provider or if laboratories' own validated DNA isolation procedures are used, the time allowed to store the isolated genomic DNA prior to its analysis shall be validated. For long-term storage, the laboratory shall have verified protocols in place how to store the isolated genomic DNA.

For long-term storage, genomic DNA samples should be eluted in an appropriate buffer and stored at −20 °C or below [11].

Appropriate storage vessels, such as cryo-vials, should be used.

For archiving aliquots of the isolated genomic DNA should be generated to avoid repeated freezing and thawing.

Annex A
(informative)

Impact of preanalytical workflow steps on venous whole blood genomic DNA quality

A.1 General information on operated experiments in Annex A

SPIDIA[1)] (Standardization and Improvement of Generic Pre-analytical Tools and Procedures for *in vitro* Diagnostics; www.spidia.eu) was a European Commission funded, four-and-a-half-year project aiming at the standardization and improvement of preanalytical procedures for *in vitro* diagnostics [14].

The SPIDIA consortium performed two consecutive pan-European ring trials for identifying and improving critical preanalytical workflow steps influencing venous whole blood genomic DNA analysis [10]. 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 preanalytical variables on the blood genomic DNA, the isolated genomic DNA samples were evaluated for purity, yield, high molecular weight DNA integrity, and presence of PCR interferences at the SPIDIA laboratories.

Results presented below show important findings.

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

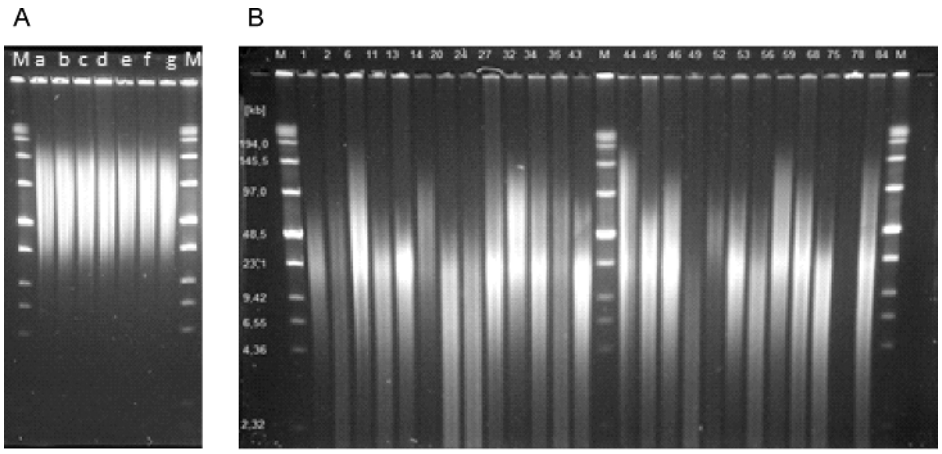
Figure A.1 shows the DNA integrity depending on the routine preanalytical workflow adopted by European laboratories participating in the first ring trial.

Blood genomic DNA was isolated by the participating laboratories following their own routine procedure (blood storage duration and temperature, and DNA isolation method). Different blood storage durations (ranging from 5 days to 40 days after blood collection), different blood storage temperatures (−20 °C, 4 °C, and room temperature), and different 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) [12]. 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 precipitation methods immediately after blood collection (T_0).

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

1) Research funded by the EU FP7 SPIDIA project the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement no 222916. For further information see www.spidia.eu.



Key

A	Blood genomic DNA isolated immediately after blood collection	a to g	DNA samples under A
B	Blood genomic DNA isolated by participating laboratories	1 to 84	DNA samples under B
M	ladder marker		

Figure A.1 —DNA fragment size distribution of blood genomic DNA samples isolated by (A) reference lab and (B) European laboratories participating in the ring trial

NOTE 1 High molecular weight DNA (HMW DNA) means DNA larger than 50 kb, see 3.6.

NOTE 2 The HMW DNA analysis was performed as described in [10].

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 preanalytical workflows (blood storage duration, storage temperature, genomic DNA isolation method).

These results suggest, that implementation of standardized preanalytical workflows will be useful in order to obtain more homogeneous results.

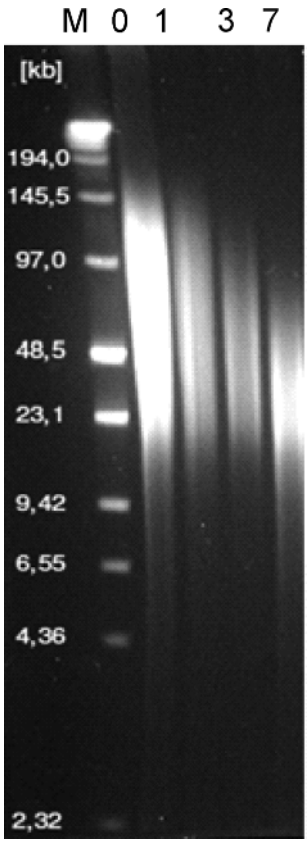
A.3 Influence of blood storage time on the genomic DNA integrity

Figure A.2 shows the DNA integrity depending on blood storage duration.

The blood sample was collected in K_2EDTA tubes. and was 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 [12] 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) [12].



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		

Figure A.2 —DNA integrity in blood genomic DNA samples isolated by a precipitation-based method in relation to the blood storage duration

NOTE 1 The DNA fragment size analysis was performed as described in [10].

NOTE 2 High molecular weight DNA (HMW DNA) means DNA larger than 50 kb, see 3.6.

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 timepoint tested in this experiment (7 days after blood collection).

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

NOTE 3 Higher blood sample storage temperatures than 2 °C to 8 °C can lead to increased fragmentation of genomic DNA.

NOTE 4 Different genomic DNA isolation methods and protocols can also influence the integrity of the isolated genomic DNA.

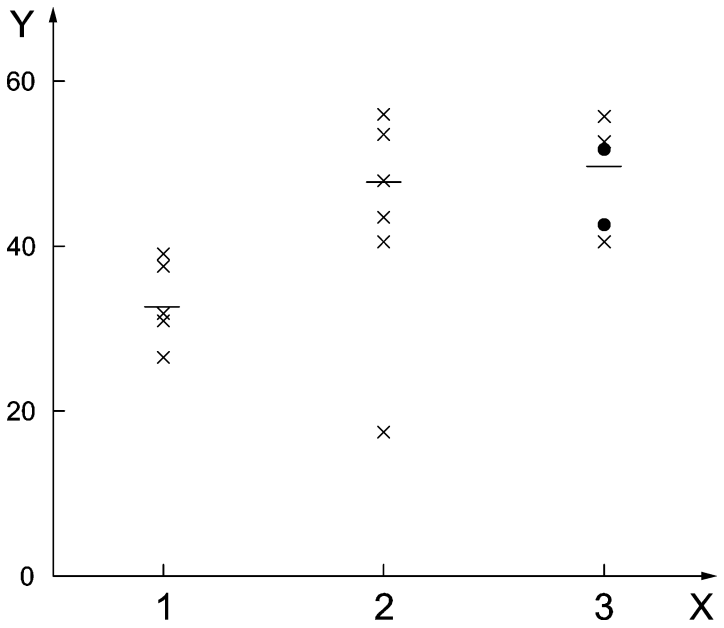
A.4 Influence of genomic DNA integrity on an analytical test based on long PCR amplicons

Figure A.3 shows the influence of DNA integrity on the results of a diagnostic assay based on the evaluation of the percentage of detected T-cell receptor (TCR) recombinations [14], [15].

This assay is used to investigate the function and the kinetics of the immune system (e.g. in patients affected by cancer, infections, diseases) [14], [15]. The assay 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 analysed for the capability to detect TCR recombinations [12].

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



Key

●	controls	1	fragmented
×	samples	2	intermediate
—	mean	3	intact
X	DNA quality classification	Y	detected TCR recombinations (%)

Figure A.3 —DNA integrity influences the 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.

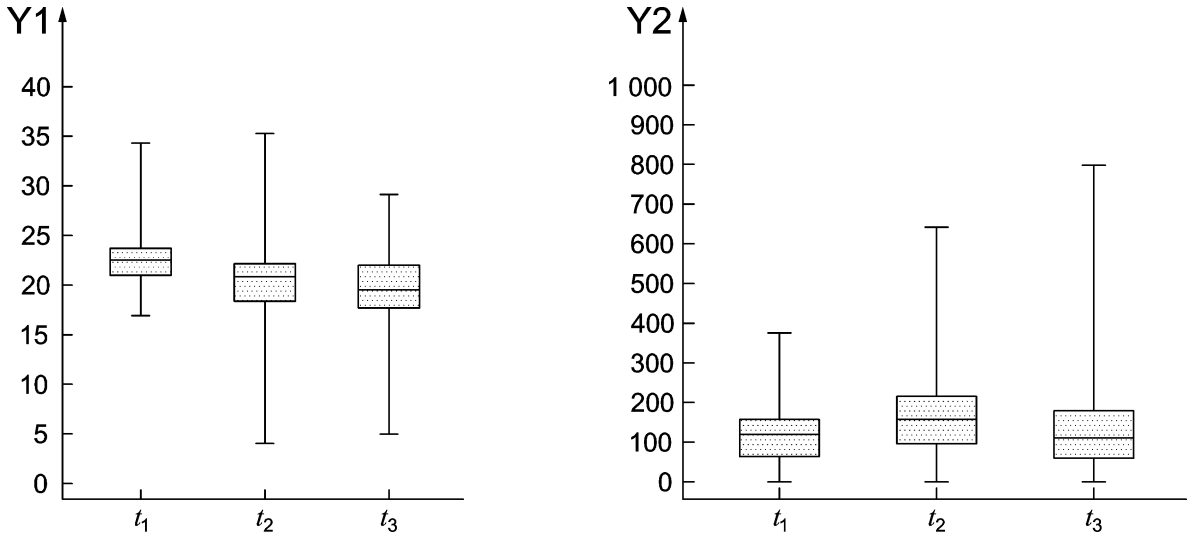
A.5 Influence of blood storage conditions on the performance of PCR tests based on short amplicons

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

The genomic DNA was isolated by laboratories (n) participating in the ring trial following their own routine procedure (blood storage duration and temperature, and genomic DNA isolation methods). Different blood storage durations (ranging from 5 days to 40 days after blood collection), different blood storage temperatures (−20 °C, 4 °C, and room temperature), and different genomic DNA isolation procedures (based on columns, magnetic beads, and precipitation procedures) were used. At SPIDIA laboratories the DNA integrity was analysed by PFGE including a software analysis allowing to quantify the genomic DNA size distribution in the gel [10]. The DNA amplificability was analysed by a qPCR assay [10]. The blood storage duration was classified as: ≤ 6 days, 6 days to 10 days, ≥ 10 days from venous whole blood collection.

Figure A.4, a) shows the distribution of the genomic DNA fragment size peak values depending on the duration of the blood storage [10].

Figure A.4, b) shows the distribution of RNaseP (single copy gene) DNA sequence quantity determined by qPCR depending on the blood storage duration. The RNase P assay represents a “short-PCR amplicon based assay” (amplicon length = 87 bp) [10].



Key

Y1	peak value of DNA fragment size [kb]	
Y2	RNaseP DNA sequence quantity [ng/µl blood]	
t_1	≤ 6 days	(n = 33)
t_2	6 days to 10 days	(n = 55)
t_3	≥ 10 days	(n = 45)

NOTE 1 Boxes show the upper and lower quartile ranges (25^{th} centile to 75^{th} centile). The upper and lower whiskers show the maximum and minimum individual values and the line inside each box shows the median value per data set.

NOTE 2 The genomic DNA integrity analysis was performed as described in [10].

NOTE 3 The statistical analysis was performed by Kruskal-Wallis Test.

Figure A.4 — Influence of blood storage duration on a) HMW DNA integrity and on b) amplificability of a short amplicon

The analysis showed that genomic DNA isolated within 6 days (t_1) had a higher DNA fragment size peak value (p-value = 0,019) in comparison to genomic DNA isolated after 6 days t_2 and t_3 (Figure A.4, a)), whereas the result of the RNaseP short amplicon qPCR test was not influenced by the different blood storage durations. However, this might be different for other qPCR tests depending on e.g. the target sequence and the blood sample quality.

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