INTERNATIONAL STANDARD

ISO 4307

First edition
2021-10

Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for saliva — Isolated human DNA

Analyse de diagnostic moléculaire in vitro — Spécifications relatives aux processus préanalytiques pour la salive — ADN humain extrait

Reference number
ISO 4307:2021(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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 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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, *in vitro diagnostic medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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.

Introduction

Molecular in vitro diagnostics has enabled a 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 specimen 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.

Genetic examination of DNA is commonly used in clinical practice. This includes, for example, predisposition testing, pharmacogenomics and analysis of genetic disorders with the perspective used in precision medicine. This is a fast-growing field in molecular diagnostics.

Saliva is increasingly used as a non-invasive alternative specimen to blood for the examination of human DNA. Saliva naturally contains microorganisms and extraneous substances (e.g. food debris), which make the composition of saliva more complex and unique among patients/donors. Dedicated measures are therefore needed for informing and preparing patients/donors for the collection and to check compliance with the instructions, in order to reduce the specimen variability. In contrast to invasive specimen collection, saliva collection does not require trained and educated professionals or dedicated facilities. By good instruction and verified collection device safety claims, saliva specimens can be self-collected at home; however, home collection also contributes to high variability in specimen quality. Similarly, medical laboratories/in vitro manufacturers need to be aware of specimen variability when performing design verification and validation.

DNA in saliva can fragment or degrade after collection. In addition, bacteria present in the saliva specimen can continue to grow, thus diluting the human DNA. DNases secreted by these bacteria can also accelerate the DNA degradation. This can impact the sensitivity and reliability of DNA examination.

Standardization of the entire process from specimen collection to the DNA examination is needed to minimize pre-examination impacts such as DNA degradation and fragmentation after saliva collection. This document describes special measures which need to be taken to obtain good quality saliva specimen/samples and isolated DNA therefrom for human DNA examination.

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 saliva — Isolated human DNA

1 Scope

This document specifies requirements and recommendations on the handling, storage, processing and documentation of saliva specimens intended for human DNA examination during the pre-examination phase before a molecular examination is performed.

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

Dedicated measures that need to be taken for saliva collected on absorbing material or by mouth washes are not described in this document. Neither are measures for preserving and handling of native saliva cell-free DNA, pathogens, and other bacterial or whole microbiome DNA in saliva described.

NOTE International, national or regional regulations or requirements can also apply to specific topics covered in 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, *Medical laboratories — Requirements for quality and competence*

ISO 15190, *Medical laboratories — Requirements for safety*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 15189 and the following 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
ambient temperature
unregulated temperature of the surrounding air

[SOURCE: ISO 20184-1:2018, 3.2]

3.2
analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2, modified — The examples were not taken over.]

3.3
examination performance
analytical test performance
analytical performance
accuracy, precision, and sensitivity of a test to measure the *analyte* (3.2) of interest

Note 1 to entry: Other test performance characteristics such as robustness, repeatability can apply as well.

[SOURCE: ISO 20184-1:2018, 3.4]

3.4
DNA stabilizer
compound, solution or mixture that is designed to minimize degradation and fragmentation of *DNA* (3.6)

[SOURCE: ISO 20186-2:2019, 3.5, modified — The term "genomic DNA in blood" has been replaced with "DNA".]

3.5
closed system
non-modifiable system provided by the vendor including all necessary components for the analysis (i.e. hardware, software, procedures and reagents)

[SOURCE: ISO 20186-2:2019, 3.6]

3.6
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.7
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 measurand and include all kinds of parameter testing or chemical manipulation for quantitative or qualitative examination.

[SOURCE: ISO 15189:2012, 3.7, modified — The term and definition is used here without the original notes.]

3.8
examination provider
analytical test provider
entity that provides the specific analytical test

3.9
interfering substance
endogenous or exogenous substance (e.g. stabilization solution) that can be present in specimens and that can alter an examination result

[SOURCE: ISO 20184-1:2018, 3.12]

3.10
microorganism
entity of microscopic size, encompassing bacteria, fungi and protozoa

[SOURCE: ISO 11139:2018, 3.176, modified — The term "viruses" was deleted from the definition.]

3.11
pre-examination process
pre-analytical phase
pre-analytical workflow
process that starts, 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)* (3.12), transportation to and within the analytical laboratory, isolation of *analytes* (3.2), and ends when the analytical examination begins

Note 1 to entry: The pre-examination phase includes preparative processes that influence the outcome of the intended examination.

[SOURCE: ISO 15189:2012, 3.15, modified — An additional term "pre-analytical workflow" was added, and more detail was included.]

3.12
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 — The term and definition is used here without the original notes.]

3.13
proficiency testing
evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons

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

3.14
room temperature
temperature in the range of 18 °C to 25 °C, for the purpose of this document

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

[SOURCE: ISO 20186-2:2019, 3.22]

3.15
saliva
whole saliva
bio-fluid of the mouth composed mainly of secretion originating from the three major salivary glands (parotids, submandibular and sublingual glands) and from salivary glands present in the oral cavity

3.16
saliva collection device
tube or other container in which the *saliva* (3.15) *specimen* (3.12) is collected

3.17
sample
one or more parts taken from a *primary sample* (3.12)

[SOURCE: ISO 15189:2012, 3.24, modified — The examples were not taken over.]

3.18
stability
ability of a *specimen* (3.12)/*sample* (3.17) material, when stored under specified conditions, to maintain a defined property value within specified limits for a specified period of time

Note 1 to entry: The measurand constituent for the purpose of this document is isolated *DNA* (3.6).

[SOURCE: ISO Guide 30:2015, 2.1.15, modified — Note 1 was not taken over. The following words were replaced: “characteristic” by “ability”; “reference material” by “sample material”; “specified” by “defined”.]

3.19
storage
prolonged interruption of the *pre-analytical workflow* (3.11) of a *specimen* (3.12) or *sample* (3.17) or *analyte* (3.2) respectively, or of their derivatives, under appropriate conditions in order to preserve their properties

Note 1 to entry: Long-term storage typically occurs in laboratory archives or in biobanks.

[SOURCE: ISO 20184-1:2018, 3.22, modified — Example in the definition was deleted and "specimen" was added.]

3.20
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 word “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 1 and 3 were not taken over.]

3.21
verification
confirmation, through provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The word “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, modified — Note 1 and Note 2 were not taken over.]

3.22
workflow
structured series of activities necessary to complete a task

[SOURCE: ISO 20184-1:2018, 3.26, modified — The word “structured” was added]

4 General considerations

For general statements on medical laboratory quality management systems and in particular on primary sample collection, reception and handling (including avoidance of cross contaminations) see ISO 15189 or ISO/IEC 17020. The requirements on laboratory equipment, reagents, and consumables according to ISO 15189 shall be followed; ISO 15189 and ISO/IEC 17020 can also apply. For general considerations on specimen collection, transport, receipt, handling, and storage, see ISO/TS 20658. For biobanking, ISO 20387 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 their impact on the stability of biomolecules intended to be examined shall be specified, verified and validated for its intended use. This includes the development of in vitro diagnostic (IVD) medical devices. The stability of the human DNA should be investigated throughout the complete pre-examination process development. The

verification of performance claims as well as the validation of the intended examination shall take into account the variability of the saliva specimen's quality.

During the design and development of a saliva DNA based examination, a risk assessment shall be performed (see also ISO 14971). Mitigation measures for eliminating or reducing identified risks shall be established where required for ensuring the performance of the examination. This shall include the pre-examination workflow steps.

Before or during the design of an examination, it should be investigated and ensured that the human DNA quality parameters such as minimum DNA amount, size and purity required for the examination is/are not compromised in a manner impacting the examination performance.

Safety requirements on specimen collection, transport and handling shall be in accordance with relevant ISO standards such as ISO 15189 and ISO 15190.

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

For all pre-examination steps, the examination manufacturer's instructions shall be followed, if provided.

Where, for justified reasons (e.g. unmet patient needs), a commercial product is not used in accordance with the manufacturer's instructions, responsibility for its verification, validation, use and performance lies with the laboratory.

The manufacturer's material safety data sheet should be considered before first use of any potentially hazardous material (e.g. chemicals in stabilizers).

5 Activities outside the laboratory

5.1 Specimen collection

5.1.1 Information about the specimen donor/patient

The documentation shall include the identity 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 primary sample donor/patient [e.g. healthy, disease type, concomitant disease, demographics (e.g. age and sex)];

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

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

d) the appropriate consent from the specimen donor/patient. See also ISO 15189.

5.1.2 Selection of the saliva collection device by the laboratory

The Saliva DNA examination manufacturer instructions should contain specifications on the saliva collection device(s) to be used. Where the examination manufacturer specifies usage of dedicated saliva collection device(s), these shall be used.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the saliva collection device(s) shall be specified, verified, validated and documented by the laboratory.

The quality and quantity of human DNA can be influenced by inadequate saliva collection procedures, inappropriate storage/shipping conditions as well as DNA isolation procedures[6],[7].

In order to prevent bacterial growth, human DNA degradation and fragmentation for a standardized collection, saliva specimens should be collected in specifically developed saliva collection devices containing appropriate stabilizers.

Alternatively, saliva can be collected in devices without stabilizers, such as cryo-vials or polypropylene tubes, if other appropriate preservation methods according to 5.1.4.3 are going to be used. The use of such tubes is not recommended for self-collection outside the laboratory.

5.1.3 Saliva specimen collection from the donor/patient and stabilization procedures

5.1.3.1 General

A protocol for the saliva specimen collection shall be in place.

For saliva collection a written and visual instruction, for example from the manufacturer, the laboratory or physician, shall be supplied to the donor/patient.

The saliva DNA examination manufacturer instructions should contain specifications and instructions on the saliva specimen collection procedure.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the saliva collection procedure shall be specified, verified and documented by the laboratory.

The instruction for specimen collection shall include:

a) all the requirements necessary for the collection, identification, storage and transport to the laboratory facility;

b) recommendations to follow before the collection, in particular related to nutrition, oral hygiene, such as fasting before specimen collection[8];

c) recommendation for collection to only submit saliva and avoid mucous from coughing/expectoration or nasal secretions (sneezing).

For self-collection, the donor/patient shall be provided with an appropriate saliva collection device, identity tags [e.g. label, Radio Frequency Identification (RFID)], and in general anything needed for the specimen collection, storage and transport.

The donor/patient shall also be provided with an option to confirm conformance with the supplied instruction for the saliva specimen collection, e.g. electronic, paper based.

The identity of the person collecting the specimen, which can be in the form of a code, and the time and date of saliva collection according to ISO 15189 shall be documented.

For the labelling (sample/specimen identification) of the saliva collection tube a routine procedure ISO 15189 or a procedure with additional information (e.g. 2D-barcode) shall be used.

Saliva collection devices shall be used in accordance with the supplied instructions, in particular:

1) to collect the required saliva volume;

2) to mix the saliva with the stabilizers immediately after saliva collection, e.g. by shaking or inverting.

NOTE Unless additives in the saliva collection device are homogenously mixed with the specimen, the saliva DNA quality and quantity can be compromised, which can impact the validity and reliability of the examination results.

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

The donor/patient or the person collecting the saliva specimen from the donor/patient shall confirm conformance with the supplied instruction for the saliva specimen collection.

5.1.3.2 Using saliva collection devices with DNA stabilizers

The examination manufacturer should provide specified and verified instructions for saliva collection (e.g. specific collection device, specimen mixing with stabilizer, etc.) which shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and shall be followed. The saliva collection device manufacturer specifications on storage and transport conditions can serve as a basis/framework for the laboratory's own examination specific verification. These should include instructions for preparation of the donor/patient, such as not to eat, drink, smoke, kiss or chew any substance for at least 30 min before the saliva specimen collection.

NOTE Collection devices with stabilizers usually allow longer transport and storage durations prior to testing.

5.1.3.3 Using saliva collection devices without DNA stabilizers

Where the examination manufacturer allows usage of saliva DNA collection devices without stabilizers, they shall provide specified and verified instructions for the saliva collection which shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and shall be followed. These should include instructions for preparation of the donor/patient, such as not to eat, drink, smoke, kiss or chew any substance for at least 30 min before the saliva specimen collection.

5.1.4 Information on the specimen and storage requirements at saliva collection facility/site

5.1.4.1 General

For specimens intended for extended storage in a biobank, it is usually not known which individual saliva DNA examinations will be performed after the extended storage. Therefore, either devices with DNA stabilizers should be used or, if using devices without DNA stabilizers, a DNA quality and quantity assessment should be performed according to 6.4.

If self-collection is performed, a written and visual instruction for saliva specimen intermediate storage, for example from the manufacturer, laboratory or physician, shall be supplied to the donor/patient.

The donor/patient or the person collecting the saliva specimen from the donor/patient shall confirm conformance with the supplied storage instructions.

The temporary storage in the saliva collection facility contributes to the total duration of storage.

5.1.4.2 Using saliva collection devices with DNA stabilizers

The examination manufacturer should provide specified and verified instructions for the storage and transport of the collected saliva specimen (e.g. duration, temperature) which shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and shall be followed. The saliva collection device manufacturer specifications on storage and transport conditions can serve as a basis/framework for the laboratory's own examination specific verification.

5.1.4.3 Using saliva collection devices without DNA stabilizers

Where the examination manufacturer allows usage of saliva collection devices without stabilizers, they shall provide specified and verified instructions for the storage and transport of the collected saliva specimen (e.g. duration, temperature) which shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and shall be followed. This can require to immediately freeze and store until use at ≤ −20 °C for up to 3 months and at ≤ −80 °C for longer storage duration[10]. The maximum storage duration shall be verified and generally kept to a minimum.

The use of collection devices without DNA stabilizers is not recommended for self-collection outside the laboratory. The storage conditions (i.e. storage duration and temperature) shall be documented.

5.2 Transport requirements

5.2.1 General

The examination manufacturer shall provide specified and verified instructions for transport of the collected saliva specimen (e.g. duration, temperature) which shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and shall be followed.

The required transport conditions shall be documented including any deviations therefrom. Temperature monitoring and recording should be applied in a suitable manner (e.g. temperature logger). If recording is not possible, the ambient temperature should be estimated and documented.

See also ISO 15189.

The transport duration to the laboratory contributes to the total duration for storage. This also includes third-party handling before transporting it to the laboratory.

5.2.2 Using saliva collection devices with DNA stabilizers

Where using saliva collection devices with DNA stabilizers, the device manufacturer's instructions on transport conditions shall be followed (i.e. duration and temperature). Where the examination provider's instructions are more stringent, these shall be followed. The transport conditions (i.e. duration and temperature) shall be documented.

5.2.3 Using saliva collection devices without DNA stabilizers

Where using saliva collection devices without DNA stabilizers, the examination provider's instructions on transport conditions shall be followed. The transport conditions (i.e. duration and temperature) shall be documented.

Where using saliva collection devices without DNA stabilizers and no examination provider's instructions are available, the specimen should be transported at ≤ −20 °C within the specifications given in 5.1.4.3 in order to minimize the degradation and fragmentation of the saliva DNA. The transport conditions (i.e. duration and temperature) shall be documented.

6 Activities inside the laboratory

6.1 Specimen reception

The identity of the person receiving the specimen/sample(s) shall be documented. This can, for example, be done in form of the name or a code.

The saliva specimen reception date, time and collection device type shall be documented. The collection compliance confirmation of the donor/patient or person having collected the saliva specimen from the donor/patient shall be checked.

Non-conformities of labelling, transport conditions and saliva volume differences to specifications, leaking/broken, discoloured devices, not appropriate collection device type or collection compliance confirmation, etc. shall be documented.

Where there are non-conformities in labelling, transport and storage conditions or saliva volume that could affect the validity and reliability of the examination, a new specimen shall be obtained.

6.2 Storage requirements

The examination manufacturer shall provide specified and verified instructions for storage of the collected saliva specimen (e.g. duration, temperature) and these shall be followed.

Where the examination manufacturer does not provide such specifications (e.g. due to former less stringent legal frameworks), the procedure shall be specified, verified and documented by the laboratory. Instructions shall be written accordingly for the user and followed.

The storage temperature and time interval between specimen reception and sample processing for DNA isolation shall be documented. Storage temperature and total storage duration shall not exceed specifications identified in 5.1.4 and 5.2.

The saliva specimen total storage duration shall include the duration for storage at the saliva collection facility (see 5.1.4), for transportation to the laboratory (see 5.2) and for further storage at the laboratory, in a biobank or other institutions. The specified maximum storage duration shall not be exceeded.

6.3 Isolation of the saliva DNA

6.3.1 General

The saliva DNA examination manufacturer shall provide specified and verified instructions for the isolation of DNA and these shall be followed.

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

Where automated DNA isolation procedures are developed, measures shall be in place to avoid cross contamination between specimens/samples.

The 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).

Because of the presence of microorganisms, viruses and food residues in saliva specimens, the isolated human DNA is usually contaminated with microbial DNA and low amounts of DNA originated from food. As the microorganism and virus content in saliva specimens can have a high variability among donors/patients, also the degree of microbial DNA contamination can vary accordingly. This fact can impact the human DNA quantification. The total DNA yield (with no distinction between human and non-human DNA) per ml of saliva collected in specific collection tubes with a stabilizer were reported by some

studies to vary from 0,1 µg to 26 µg[10]. Specimens collected in specific collection tubes containing a stabilizer have a median microbial content lower than those from mouthwash and buccal swab[10].

The reagents and consumables coming in contact with the DNA should be DNase-free. The DNA isolation performance should be tested in a DNA proficiency testing program.

6.3.2 Using a commercial kit

Where the specifications of the examination manufacturer require the use of dedicated commercially available kits for DNA isolation, then these shall be used in accordance with the instructions of the examination manufacturer.

Where there are no examination manufacturer's instructions provided (e.g. due to former less stringent legal frameworks), but the saliva collection device manufacturer has specified a kit for DNA isolation, these can serve as a basis/framework for the laboratory's examination specific verification.

Where no dedicated kits for DNA isolation are specified, the laboratory shall select and use an appropriate DNA isolation kit approved for diagnostic use, specifying saliva as a specimen/sample type, where available. The laboratory shall verify the use of this kit for the intended examination.

6.3.3 Using the laboratory's own protocol

Where no commercially available DNA isolation kit intended for diagnostic use can be successfully verified with the intended examination, the laboratory shall develop its own procedure by either:

— modifying an existing DNA isolation kit for diagnostic use, specifying saliva as a specimen/sample type;

— using a commercially available DNA isolation kit for research use only; or

— developing its own procedure.

The procedure chosen from the list above shall be verified and validated with the intended examination. Instructions for use shall be written accordingly and followed.

NOTE 1 DNA obtained by different DNA isolation procedures can be of different length. In addition, the DNA quantity and quality (e.g. purity) can vary.

NOTE 2 Pretreatment of viscous specimens/samples such as centrifugation, enzyme or chemical pretreatment for liquification can help homogenize the sample and enhance the nucleic acid extraction efficiency.

NOTE 3 Dedicated measures and technologies can be needed in order to avoid carrying over DNA stabilization molecules to the isolated DNA. Stabilization molecules carry over can lead to interferences with the examination.

6.4 Quantity and quality assessment of isolated DNA

The DNA quantity and quality should be checked according to the examination provider's instructions or where provider's instructions are not available, by generally accepted physical, chemical and biochemical procedures prior to performing the examination. These may include one or more of the following, depending on the specific examination:

a) quantification of total amount of nucleic acid by absorbance measurements (A_{260}) or spectrofluorometry.

NOTE Saliva specimens can be rich in microorganisms, viruses and food residues that contribute to nucleic acid amount. These non-discriminative measurements will reflect the total amount of nucleic acids. Human DNA quantification can only be performed with a specific method as described under d).

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

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