ISO/FDIS 22174~~:2024(E)~~

~~Date: 2024-02-27~~

ISO/TC 34/SC 9

Secretariat: AFNOR

Date: 2024-04-10

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection and quantification of microorganisms — General requirements and definitions

Microbiologie de la chaîne alimentaire — Réaction de polymérisation en chaîne (PCR) pour la recherche et la quantification de micro-organismes — Exigences générales et définitions

FDIS stage

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

Published in Switzerland~~.~~

Contents

Foreword v
1 Scope 1
2 Normative references 1
3 Terms and definitions 1
3.1 General terms 2
3.2 Terms related to the extraction and purification of DNA/RNA 4
3.3 Terms related to reverse transcription of RNA to DNA 4
3.4 Terms related to DNA amplification by PCR/RT-PCR 4
3.5 Terms related to controls 6
3.6 Terms related to qPCR 7
3.7 Terms related to dPCR 8
4 Principle 9
4.1 General 9
4.2 Laboratory sample 9
4.3 Sampling, transport and storage 10
4.4 Preparation of test sample 10
5 Microbial enrichment and virus concentration 10
5.1 Microbial enrichment 10
5.2 Virus concentration 10
6 Nucleic acid preparation 10
6.1 General 10
6.2 Prevention of amplification of DNA from dead cells 11
6.3 Nucleic acid extraction, release and purification 11
6.4 Nucleic acid quality and quantity 11
7 PCR amplification 12
8 Detection and confirmation of amplicons 12
9 General environmental laboratory requirements 13
9.1 General 13
9.2 Laboratory setup 13
9.2.1 General 13
9.2.2 Control of flows 14
9.2.3 Cleaning of laboratory 15
9.2.4 Environmental monitoring for nucleic acid contamination 15
10 Reagents and consumables 15
11 Equipment 16
12 Procedure 17
12.1 Enrichment and sample treatment 17
12.2 Amplification 17
12.2.1 General 17
12.2.2 Control reaction 17
12.2.3 Detection of amplicon 19
12.2.4 Data analysis 20
12.3 Evaluation 21
12.3.1 Qualitative evaluation 21
12.3.2 Quantitative evaluation 22
12.4 Test report 23

13 Performance characteristics of PCR-based methods 23

14 Validation and verification of PCR-based methods 23
14.1 General 23
14.2 Validation 23
14.3 Verification 24

Annex A (informative) Fluorescence signals and amplification curve 25
A.1 Fluorescence signals 25
A.2 Amplification curve 25
A.2.1 General 25
A.2.2 Phase 1: Background phase 26
A.2.3 Phase 2: Exponential phase 26
A.2.4 Phase 3: Linear phase 26
A.2.5 Phase 4: Plateau phase 27

Bibliography 28

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~~document 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~~ISO draws attention to the possibility that ~~some of~~ the ~~elements~~implementation of this document may ~~be~~involve the ~~subject~~use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents~~.~~. 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).~~

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 TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces ISO 22174:2005, ISO 20837:2006, ISO 20838:2006 and ISO 22119:2011, which have been technically revised.

The main changes are as follows:

— inclusion of requirements for the implementation of digital PCR;

— inclusion of requirements for laboratory flows monitoring including environmental monitoring for PCR;

— extension of 12.2.2 control reaction with descriptions of the different controls;

— change of 12.3 to include quantitative evaluation;

— inclusion of Clause 14 on validation and verification~~;~~.

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.

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection and quantification of microorganisms — General requirements and definitions

1 Scope

This document specifies the general requirements for the *in vitro* amplification of nucleic acid sequences (DNA or RNA).

This document is applicable to the testing for microorganisms and viruses from the food chain using the polymerase chain reaction (PCR). This document, or parts of it, ~~can be applied~~is applicable to other fields of PCR diagnostics based on a case-by-case evaluation.

The minimum requirements laid down in this document are intended to ensure that comparable and reproducible results are obtained in different laboratories.

This document has been established for microorganisms from the food chain and is applicable to:

— products intended for human consumption;

— products for feeding animals;

— environmental samples in the area of food and feed production and handling;

— samples from the primary production stage.

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 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

ISO 20836, *Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of microorganisms — Thermal performance testing of thermal cyclers*

3 Terms and definitions

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

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

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

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

3.1 General terms

3.1.1
laboratory sample
sample as prepared for sending to the laboratory and intended for inspection or testing

[SOURCE: ISO 7002:1986, A.19]

3.1.2
test sample
sample prepared from the *laboratory sample* (3.1.1) according to the procedure specified in the method of test and from which *test portions* (3.1.3) are taken

3.1.3
test portion
measured (volume or mass) representative sample taken from the *laboratory sample* (3.1.1)

[SOURCE: ISO 6887-1:2017, 3.5, modified — "for use in the preparation of the initial suspension" and Note 1 to entry deleted.]

3.1.4
reference material
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: Reference material producers fulfilling the requirements of ISO 17034 are considered to be competent.

[SOURCE: ISO Guide 30:2015, 2.1.1, modified — Notes to entry deleted and a new Note 1 to entry added.]

3.1.5
matrix
all the components of the sample

[SOURCE: ISO 16140-1:2016, 2.38, modified — "(product)" ~~removed from~~deleted in the term.]

3.1.6
deoxyribonucleic acid
DNA
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

3.1.7
deoxyribonuclease
DNase
enzyme which degrades *deoxyribonucleic acid (DNA)* (3.1.6)

3.1.8
amplicon
deoxyribonucleic acid (DNA) (3.1.6) amplified by *polymerase chain reaction (PCR)* (3.1.17)

3.1.9
ribonucleic acid
RNA
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

3.1.10
ribonuclease
RNase
enzyme which degrades *ribonucleic acid (RNA)* (3.1.9)

3.1.11
nucleic acid
polymer of deoxyribonucleotides or ribonucleotides

3.1.12
target nucleic acid sequence
nucleic acid sequence selected for amplification

3.1.13
endogenous sequence
nucleic acid sequence naturally present in the tested *matrix* (3.1.5)

3.1.14
exogenous sequence
nucleic acid sequence naturally absent in the tested *matrix* (3.1.5)

3.1.15
detection of polymerase chain reaction product
detection of amplicon
process which signals the presence of an *amplicon* (3.1.8)

3.1.16
confirmation of polymerase chain reaction product
confirmation of amplicon
process which demonstrates that the *amplicon* (3.1.8) originates from the *target nucleic acid sequence* (3.1.12)

3.1.17
polymerase chain reaction
PCR
enzymatic procedure that allows in vitro amplification of *deoxyribonucleic acid (DNA)* (3.1.6)

3.1.18
endpoint polymerase chain reaction
endpoint PCR
procedure using PCR amplification followed by separate detection of *amplicons* (3.1.8) after the completion of the PCR

3.1.19
real-time polymerase chain reaction
real-time PCR
procedure which combines PCR amplification with the detection and/or quantification of specific *amplicons* (3.1.8) during the amplification process

3.1.20
multiplex polymerase chain reaction
multiplex PCR
PCR (3.1.17) allowing the detection of multiple targets simultaneously within a single reaction tube, where more primer pairs (and probes) are used within one *master mix* (3.4.4)

3.2 Terms related to the extraction and purification of DNA/RNA

3.2.1
nucleic acid extraction
sample treatment for the release of *nucleic acids* (3.1.11)

3.2.2
nucleic acid purification
method to reduce the amount of *polymerase chain reaction (PCR)* (3.1.17) inhibitors in the eluate

3.3 Terms related to reverse transcription of RNA to DNA

3.3.1
reverse transcriptase
enzyme which catalyses the *reverse transcription* (3.3.2) of *ribonucleic acid (RNA)* (3.1.9) to a complementary single-stranded deoxyribonucleic acid (cDNA)

3.3.2
reverse transcription
RT
synthesis of complementary single-stranded deoxyribonucleic acid (cDNA) from a ribonucleic acid (RNA) template using a *reverse transcriptase* (3.3.1)

3.3.3
reverse transcription-polymerase chain reaction
RT-PCR
method consisting of two reactions, a *reverse transcription (RT)* (3.3.2) of *ribonucleic acid (RNA)* (3.1.9) to single-stranded complementary deoxyribonucleic acid (cDNA), followed by a *PCR* (3.1.17)

Note 1 to entry: One-step RT-PCR is performed in a single tube.

Note 2 to entry: Two-step RT-PCR can either be performed sequentially in a single tube or in two different tubes.

3.4 Terms related to DNA amplification by PCR/RT-PCR

3.4.1
deoxyribonucleic acid polymerase
DNA polymerase
thermostable enzyme which catalyses *DNA* (3.1.6) synthesis

Note 1 to entry: DNA polymerase can also cleave a hybridized nucleic acid molecule using its 5′-3′-exonuclease activity. It is dependent on the type of enzyme and can be present in, for example, Taq-, Tth- and Tfl-polymerase.

3.4.2
deoxyribonucleoside triphosphate
dNTP
solution containing deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP) and/or deoxyuridine triphosphate (dUTP)

3.4.3
thermal cycler
automatic device that performs defined heating and cooling cycles usable for *polymerase chain reaction (PCR)* (3.1.17) or *real-time PCR* (3.1.19) or *digital PCR* (3.7.1)

Note 1 to entry: The thermal cycler can be a block-based or (individual) reaction-chamber-based thermal cycler.

[SOURCE: ISO 20836:2021, 3.2.1, modified — "or digital PCR" ~~has been~~ added.]

3.4.4
master mix
mixture of reagents needed for nucleic acid amplification except for the *target nucleic acid sequence* (3.1.12)

[SOURCE: ISO 17822:2020, 3.27, modified — "~~DNA and the controls" has been removed and replaced by target~~ nucleic acid sequence~~.~~" replaced "DNA and the controls".]

3.4.5
primer
oligonucleotide of defined length and sequence complementary to a segment of the *target nucleic acid sequence* (3.1.12), used to signal the starting point for deoxyribonucleic acid (DNA) polymerase to extend the new DNA strand

3.4.6
fluorescent probe
oligonucleotide of defined sequence coupled with one or more fluorescent molecules

Note 1 to entry: Any system emitting a fluorescence signal after specific hybridization to the *target nucleic acid sequence* (3.1.12) which can be detected by the specific equipment can be used as a fluorescent probe.

3.4.7
background fluorescence
background
intrinsic level of fluorescence resulting from the reagents, consumables and instruments used

3.4.8
molecular beacon
fluorescent probe consisting of three different parts: a central part complementary to the *target nucleic acid sequence* (3.1.12), plus a 5′-part and a 3′-part which are complementary, and where the reporter is attached to one arm of the molecule, while the end of the other arm carries the quencher

3.4.9
hybridization probe
system of two fluorescent probes coupled with one fluorescent molecule each, where one molecule serves as fluorescence resonance energy transfer (FRET) donor and the other serves as FRET acceptor

3.4.10
hydrolysis probe
fluorescent probe coupled with a fluorophore and quencher which are sterically separated by the 5′-3′-exonuclease activity of the enzyme during the amplification process

3.4.11
denaturation
process which results in the separation of the double-stranded nucleic acid into single-stranded nucleic acids

3.4.12
hybridization
specific binding of complementary nucleic acid sequences under suitable reaction conditions

3.4.13
annealing
pairing of complementary single strands of nucleic acids to form a double-stranded molecule