SLOVENSKI STANDARD
oSIST prEN ISO 22174:2023

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Mikrobiologija v prehranski verigi - Polimerazna verižna reakcija (PCR) za ugotavljanje prisotnosti mikroorganizmov - Splošne zahteve in definicije (ISO/DIS 22174:2022)

Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection and quantification of microorganisms - General requirements and definitions (ISO/DIS 22174:2022)

Mikrobiologie der Lebensmittelkette - Polymerase-Kettenreaktion (PCR) zum Nachweis von Mikroorganismen - Allgemeine Anforderungen und Definitionen (ISO/DIS 22174:2022)

Microbiologie de la chaîne alimentaire - Réaction de polymérisation en chaîne (PCR) pour la recherche et la quantification de micro-organismes - Exigences générales et définitions (ISO/DIS 22174:2022)

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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 pathogènes dans les aliments — Exigences générales et définitions

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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).

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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 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 the first edition (ISO 22174:2005), which has been technically revised.

The main changes are as follows:

— inclusion of standards ISO 20837:2006, ISO 20838:2006 and ISO 22119;

— inclusion of requirements for the implementation of digital PCR;

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

— change of the title and inclusion of clause 12.5 to include quantification;

— inclusion of a new clause dealing with validation and verification;

— inclusion of methods from microbiology of the food chain utilizing PCR in the bibliography for information;

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

Because of the large variety of food and feed products, this horizontal method can not be appropriate in every detail for certain products. In this case, different methods which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

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

1 Scope

This document establishes the general requirements for the in vitro amplification of nucleic acid sequences (DNA or RNA). It is applicable to the testing for microorganisms and viruses from the food chain using the polymerase chain reaction (PCR).

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.

Validation of alternative methods, single laboratory validation, interlaboratory validation for non-proprietary or alternative confirmation methods are not covered by this document. These items in the frame of the microbiology of the food chain are covered by the ISO 16140 series.

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 7218, *Microbiology of food and animal feeding stuffs — 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. For definitions concerning validation, see ISO 3534-1, ISO 5725-1 and ISO 16140-1.

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

3.2
background fluorescence
"background"
intrinsic level of fluorescence resulting from the reagents, consumables and instruments used

3.3
confirmation of PCR product
process which demonstrates that the PCR product originates from the target sequence

3.4
denaturation
process which results in the separation of the double-stranded nucleic acid into single-stranded nucleic acid

3.5
deoxyribonucleoside triphosphate
dNTP
solution containing dATP, dCTP, dGTP, dTTP and/or dUTP

3.6
detection
recognition of the presence of the target nucleic acid

3.7
detection of PCR product
process which signals the presence of a PCR product

3.8
digital PCR
dPCR
procedure in which nucleic acid templates are randomly and independently distributed across multiple partitions of nominally equivalent volume, such that some partitions contain template and others do not, followed by PCR amplification of target sequences and detection of specific PCR products, providing a count of the number of partitions with a positive and negative signal for the target template

[SOURCE: ISO 20395:2019, 3.10, modified — Removed Note 1 to entry and Note 2 to entry]

3.9
deoxyribonucleic acid
DNA
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

3.10
DNA polymerase
thermostable enzyme which catalyses DNA 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, for example, in Taq-, Tth- and Tfl-polymerase.

3.11
DNA probe
nucleic acid molecule with a defined sequence used to detect target DNA by hybridization

3.12
target nucleic acid sequence
nucleic acid sequence selected for amplification

3.13
endogeneous sequence
nucleic acid sequence naturally present in the tested matrix

3.14
endpoint PCR
procedure using PCR amplification followed by separate detection of PCR products after the completion of the PCR cycle

3.15
external amplification control
control DNA or RNA added to an aliquot of the extracted nucleic acid in a defined amount or copy number serving as a control for amplification in a separate reaction. This DNA or RNA sequence can be endogenous (naturally present in the tested matrix) or exogeneous (naturally absent in the tested matrix)

3.16
exogenous sequence
nucleic acid sequence naturally absent in the tested matrix

3.17
fluorescent probe
oligonucleotide or oligonucleotide analogue 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 which can be detected by the specific equipment can be used as a fluorescent probe.

3.18
fluorescence resonance energy transfer
FRET
distance-dependent energy transfer from a donor molecule to an acceptor molecule resulting in enhanced fluorescence of the acceptor molecule after excitation with electromagnetic radiation of a defined wavelength

3.19
hot-start PCR
activation of thermostable DNA polymerase by an initial heating step to avoid non-specific amplification

3.20
hybridization
specific binding of complementary nucleic acid sequences under suitable reaction conditions

3.21
hybridization probe
system of two fluorescent probes coupled with one fluorescent molecule each, where one molecule serves as FRET donor and the other serves as FRET acceptor

3.22
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

3.23
internal amplification control
DNA added to each reaction in a defined amount or copy number which serves as an internal control for amplification. This DNA sequence can be endogeneous (naturally present in the tested matrix) or exogeneous (naturally absent in the tested matrix)

3.24
laboratory sample
Sample prepared for sending to the laboratory and intended for inspection or testing

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