



# FINAL DRAFT International Standard

## ISO/FDIS 22174

### 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*

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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 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](http://www.iso.org/directives)).

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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](http://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, 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)” 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” 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 — “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

#### 3.4.14

##### **hot-start polymerase chain reaction**

##### **hot-start PCR**

activation of thermostable deoxyribonucleic acid (DNA) polymerase by an initial heating step to avoid non-specific amplification

### 3.5 Terms related to controls

#### 3.5.1

##### **negative process control**

target free sample which is run through all stages of the analytical process

Note 1 to entry: The process can include sample preparation, enrichment, nucleic acid extraction and target amplification.

#### 3.5.2

##### **positive process control**

sample, spiked with a microorganism, treated in the same way as the *test samples* (3.1.2) to monitor the entire process of the polymerase-chain-reaction-based method