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Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions

Microbiologie des aliments — Réaction de polymérisation en chaîne (PCR) pour la recherche 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

ISO 22174 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

The polymerase chain reaction (PCR) is a fast, sensitive and specific method for the detection of food-borne pathogens. Although a relatively young technology, the application of PCR-based methods in food analysis is increasing.

In brief, existing protocols can be divided in two main groups, depending on the type of nucleic acid used as target for amplification:

— RNA-based amplification (RT-PCR);

— DNA-based amplification (PCR).

Numerous variations of both methods have been established and can be characterized by their degree of complexity and automation. The level of specificity of the methods varies from screening assays which detect nucleic acid sequences common to a microbiological genus, to specific assays which identify nucleic acid sequences unique to an individual strain- or type-specific nucleic acid sequence.

This International Standard presents a comprehensive list of requirements for PCR-based methods used for the detection of microorganisms in food samples. It contains terms and definitions used in reference to PCR and RT-PCR.

ISO 22174 is part of a series of International Standards and a Technical Specification under the general title *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens*:

— *General requirements and definitions* (ISO 22174);

— *Requirements for sample preparation for qualitative detection* (ISO 20837) [1];

— *Requirements for amplification and detection for qualitative methods* (ISO 20838) [1];

— *Performance testing for thermal cyclers* (ISO/TS 20836) [1].

The International Organization for Standardization (ISO) draws attention to the fact that it is claimed that compliance with this document may involve the use of one or more patents concerning the PCR technology.

ISO takes no position concerning the evidence, validity and scope of these patent rights.

ISO has been informed that Applied Biosystems, Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd. hold patent rights concerning the PCR technology. The companies have assured the ISO that they are willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statements of the holders of these patent rights are registered with ISO. Information may be obtained from:

Licensing Department
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
USA

1) To be published.

and

Roche Molecular Systems, Inc.
Licensing Department
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Alameda, CA 94501
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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights other than those identified above. ISO shall not be held responsible for identifying any or all such patent rights.

Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions

WARNING — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determines the applicability of regulatory limitations prior to use.

1 Scope

This International Standard gives the general requirements for the *in vitro* amplification of nucleic acid sequences (DNA or RNA). It is applicable to the testing of foodstuffs and isolates obtained from foodstuffs for food-borne pathogens using the polymerase chain reaction (PCR).

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

This International Standard has been established for food-borne pathogens in or isolated from food and feed matrices, but is also applicable to other matrices (e.g. environmental samples) and for the detection of non-pathogenic microorganisms.

2 Normative references

The following referenced documents are indispensable for the application 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 3534-1, *Statistics — Vocabulary and symbols — Part 1: Probability and general statistical terms*

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 20837, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for sample preparation for qualitative detection*

ISO 20838, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply. For definitions concerning validation, see ISO 3534-1 and ISO 5725-1.

3.1 General terms

3.1.1
nucleic acid
macromolecule that is the medium for genetic information or acts as an agent in expressing the information

NOTE There are two types of nucleic acid, DNA and RNA.

3.1.2
DNA
deoxyribonucleic acid
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

3.1.3
RNA
ribonucleic acid
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

3.1.4
matrix
products submitted for analysis, which might have differences in chemical composition and physical state

3.1.5
repeatability conditions
conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time

[ISO 3534-1]

3.1.6
reproducibility conditions
conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment

[ISO 3534-1]

3.1.7
detection
recognition of the presence of the target nucleic acid

3.1.8
detection limit
limit of detection
lowest concentration or content of the target organism per defined amount of matrix that can be consistently detected under the experimental conditions specified in the method

3.1.9
identification
process for determining that an isolate belongs to one of the established taxa

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

3.2.1
nucleic acid extraction
sample treatment for the liberation of target nucleic acid

3.2.2
nucleic acid purification
method resulting in a more purified DNA

NOTE In this context, purity refers to the reduction of observable effects of PCR inhibitors on PCR inhibition controls.

3.2.3
PCR quality DNA
DNA template of sufficient length and quantity for PCR

3.2.4
RT-PCR quality RNA
RNA template of sufficient length and quantity suitable for reverse transcription and PCR

3.3 Terms related to reverse transcription (RT) of RNA to DNA

3.3.1
RT
reverse transcription
synthesis of DNA from an RNA template using a reverse transcriptase enzyme combined with an RT-primer in the presence of deoxyribonucleoside triphosphate

3.3.2
reverse transcriptase
enzyme which catalyses the reverse transcription of RNA to DNA using RT-primers

3.3.3
ribonuclease
enzyme which degrades RNA

3.3.4
ribonuclease inhibitor
substance which blocks ribonuclease activity

3.3.5
RT-primer
primer used in reverse transcription

3.3.6
RT mix
mixture of reagents needed for reverse transcription

3.3.7
deoxyribonucleoside triphosphate
dNTP
solution containing dATP, dCTP, dGTP, dTTP and/or dUTP

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

3.4.1
polymerase chain reaction
PCR
enzymatic procedure which allows *in vitro* amplification of DNA

3.4.2
RT-PCR
method consisting of two reactions, a reverse transcription (RT) of RNA to DNA and a subsequent PCR

3.4.3
one-step RT-PCR
method combining reverse transcription (RT) of RNA to DNA and PCR in a single reaction

3.4.4
two-step RT-PCR
method composed of a reverse transcription (RT) and PCR in two separate reactions

NOTE Two-step RT-PCR can be performed sequentially in a single tube or in two different tubes.