
**Microbiology of food and animal feeding
stuffs — Polymerase chain reaction
(PCR) for the detection and quantification
of food-borne pathogens — Performance
characteristics**

*Microbiologie des aliments — Réaction de polymérisation en chaîne
(PCR) pour la détection et la quantification des micro-organismes
pathogènes dans les aliments — Caractéristiques de performance*

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Published in Switzerland

Foreword

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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.

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.

ISO 22118 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).

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Introduction

Molecular detection methods have been developed during the last few decades, and are now available for the majority of food-borne pathogens. Some of these methods have the potential for quantitative analysis.

Although until now most methods have been based on the polymerase chain reaction (PCR) and real-time PCR, other molecular detection and quantification principles should be kept under consideration.

To compare molecular methods with conventional methods or with other principles, it is necessary to generate minimum requirements for performance characteristics of the methods to be developed.

This International Standard is part of a series of documents under the general title *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens*:

ISO/TS 20836, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Performance testing for thermal cyclers*

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 22118, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection and quantification of food-borne pathogens — Performance characteristics*

ISO 22119, *Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

The following Technical Specification is in preparation:

ISO/TS 13136, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) belonging to O157, O111, O26, O103 and O145 serogroups — Qualitative real-time polymerase chain reaction (PCR)-based method*

Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection and quantification of food-borne pathogens — Performance characteristics

1 Scope

This International Standard specifies minimum requirements of performance characteristics for the detection of nucleic acid sequences (DNA or RNA) by molecular methods. This International Standard applies to the detection of food-borne pathogens in foodstuffs and isolates obtained from them using molecular detection methods based on the polymerase chain reaction (PCR).

This International Standard is also applicable, for example, to the detection of food-borne pathogens in environmental samples and in animal feeding stuffs.

NOTE Because of the rapid progress in this field, the examples given are those most frequently in use at the time of development of this International Standard.

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2 Normative references (standards.iteh.ai)

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 16140:2003, *Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods*

ISO 22174:2005, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Terms and definitions

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

3.1 analyte

component detected or measured by the method of analysis

NOTE 1 The analyte can be a microorganism or virus, its components or products.

NOTE 2 Adapted from ISO 16140:2003, 3.4.

3.2 qualitative method

method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a certain amount of sample

[ISO 16140:2003, 3.5.]

3.3
quantitative method
method of analysis whose response is the amount of the analyte measured either directly or indirectly in a certain amount of sample

NOTE Adapted from ISO 16140:2003, 3.6.

3.4
robustness testing
<food microbiology> subjecting the proposed method to small procedural changes or environmental factors to determine what influence, if any, they have on method performance

3.5
selectivity
<food microbiology> measure of the inclusivity (detection of the target microorganism or virus) and exclusivity (non-detection of non-target microorganisms or viruses)

3.6
sensitivity
<food microbiology> measure of the lowest number of analyte cells, particles or molecules which can be detected in a single reaction

3.7
specificity
capacity to exclusively recognize the target to be detected, distinguishing it from similar substances and impurities

[ISO 22174:2005, 3.6.4]

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3.8
trueness
closeness of agreement between the expectation of a test result or a measurement result and a true value

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[ISO 3534-2:2006 ^[1], 3.3.3]

3.9
detection limit
limit of detection
LOD
lowest concentration or content of the target organism relative to the defined amount of matrix that can be consistently detected under the experimental conditions specified in the method

NOTE Adapted from ISO 22174:2005, 3.1.8.

3.10
quantification limit
limit of quantification
LOQ
<food microbiology> smallest amount of analyte (that is the lowest actual number of organisms), which can be measured and quantified with defined trueness and precision under the experimental conditions by the method under validation

NOTE Adapted from ISO 16140:2003, 6.2.2.2.3.

3.11
precision
closeness of agreement between independent test/measurement results obtained under stipulated conditions

NOTE 1 Precision depends only on the distribution of random errors and does not relate to the true value or the specified value.

NOTE 2 The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results or measurement results. Less precision is reflected by a larger standard deviation.

NOTE 3 Quantitative measures of precision depend critically on the stipulated conditions. Repeatability conditions and reproducibility conditions are particular sets of extreme stipulated conditions.

[ISO 3534-2:2006 ^[1], 3.3.4]

4 Performance characteristics of qualitative and quantitative detection methods

4.1 General

A molecular detection method shall meet performance characteristics according to this International Standard. Information about the performance characteristics of the molecular detection method shall be made available. This includes specific information on the multi- or single-laboratory trial, including relevant information obtained during pre-validation of the method (e.g. variation of parameters, reagents).

4.2 Scope of the method

The purpose of the method should be indicated. Information regarding the intended use and the limitations of the methods shall be provided. In particular, the method provider shall indicate that the criteria set out in this International Standard are fulfilled.

4.3 Scientific basis

An overview of the principles and references to relevant scientific publications should be provided.

4.4 Selectivity

4.4.1 Inclusivity test

4.4.1.1 General

Empirical results from testing the method with the target microorganism or viruses should be provided. This testing should include all relevant variants or types of the microorganism or viruses according to the scope of the method (4.2).

4.4.1.2 Minimum requirements for specificity

Sequence variants of the target microorganism or viruses should be detected with comparable amplification efficiency, even if sequence differences in the primer and/or probe binding sites are present as indicated by the scope of the method (4.2).

If available, 50 strains of the specific target microorganism or virus should be tested.

4.4.2 Exclusivity test

4.4.2.1 General

Empirical results from testing the method with non-target microorganisms or viruses should be provided. This testing should include both taxonomically closely related and not closely related microorganisms or viruses.

The method should clearly distinguish between target and non-target microorganisms or viruses.

4.4.2.2 Test systems for detecting bacteria

Select a minimum of 30 strains that may cause interference with the target microorganism and food strains naturally present in each food test material. Examples of suitable organisms are listed in Annex A.

Viruses should be included if relevant, e.g. if there are sequence homologies of oligonucleotides to viral nucleic acid sequences.

At least 90 % of the strains should be bacteria. The remainder of the strains should belong to yeasts, moulds or viruses.

A clearly detectable amount of DNA, e.g. representing DNA of 10^6 cells, should be used for the selectivity testing. The suitability of the DNA used for amplification should be confirmed, e.g. by a ribosomal DNA-based consensus PCR system.

4.4.2.3 Test systems for detecting fungi

Select at least 30 strains of non-target microorganisms or viruses.

In the validation of a fungal test system, at least 90 % of the strains should be fungi. The remainder of the strains should belong to bacteria or viruses.

Viruses should be included if relevant. This could be the case if there are sequence homologies of oligonucleotides to viral nucleic acid sequences.

A clearly detectable amount of DNA, e.g. representing DNA of 10^6 cells, should be used for the selectivity testing. The suitability of the DNA used for amplification should be confirmed, e.g. by a ribosomal DNA-based consensus PCR system.

4.4.2.4 Test systems for detecting viruses

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In the validation of a test system for virus detection, at least three non-target virus strains should be tested.

A clearly detectable amount of DNA or RNA, e.g. representing DNA or RNA of 10^6 viral particles or genome equivalents, should be used for the selectivity testing. The suitability of the nucleic acid used for amplification should be confirmed, e.g. by another test system.

4.5 Sensitivity

4.5.1 General

Empirical results from testing the method at different concentrations in order to test the range of use of the method shall be available and shall be described in the validation report.

4.5.2 Minimum requirements for sensitivity for qualitative tests

Food-borne pathogens that require qualitative testing should be detected at levels of 1 cell to 10 cells per assay for bacteria or parasites, and 10 particles to 100 particles or genome equivalents for viruses, in a defined amount of the food matrix under investigation. The concentrations relate to the amount applied before the start of the detection procedure (including enrichment).

NOTE The reaction sensitivity is different from the method sensitivity. The reaction sensitivity can be precisely defined by the amount of the nucleic acid used as template. The method sensitivity is, among other things, dependent on the efficiency of recovery of the extraction per concentration.

The tests shall be carried out on samples containing a microbiological background flora relevant to the food item.

The evaluation of sensitivity should include five different food categories.

4.5.3 Minimum requirements for sensitivity for quantitative tests

The upper and lower limit of the linear range of the method shall be given.

The assessment of these limits and the linear range shall be carried out on samples containing a microbiological background flora relevant to the food item.

The evaluation of sensitivity should include five different food categories.

The user shall ensure that the chosen method covers the given detection range of the target parameter.

4.6 Robustness

4.6.1 General

Results from the empirical testing of the method against small but deliberate variations in method parameters (e.g. variation in concentration of kit components, variation in apparatus, etc.) should be provided, if available.

4.6.2 Robustness determination

Robustness can be determined by performing an interlaboratory study.

The results of the interlaboratory study shall be interpreted as interlaboratory ruggedness. The method is applicable if the results from different laboratories do not vary significantly.

4.7 Analytical controls

Analytical controls in accordance with the requirements of ISO 22174 shall be clearly specified and their interpretation recorded. These shall include positive and negative and internal or external amplification controls, their detailed contents, their usage and the interpretation of the obtained results.

4.8 Trueness and precision

Information on the trueness and precision of the method should be supplied.

4.9 Instruments

The specifications of an instrument can influence the performance of the method. The required equipment for the application of the method should be clearly described with regard to sample preparation and to molecular analysis.

The method provider shall indicate the instrument(s) on which the method was validated.

Laboratories can use instrumentation other than that stated by the method provider if proof is given by experimental verification that comparable results are obtained.

5 Performance characteristics for validation

5.1 General

A method should be validated using the conditions under which it is performed.

The performance characteristics of molecular methods shall be established according to the procedures of ISO 16140, if applicable.