BS EN ISO 22118:2011



BSI Standards Publication

Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection and quantification of food-borne pathogens — Performance characteristics (ISO 22118:2011)



National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 60372 3

ICS

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2011.

Amendments issued since publication

Date Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

July 2011

EN ISO 22118

ICS 07.100.30

English Version

Microbiology of food and animal feeding stuffs - Polymerase chain reaction (PCR) for the detection and quantification of foodborne pathogens - Performance characteristics (ISO 22118:2011)

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 (ISO 22118:2011)

Mikrobiologie von Lebensmitteln und Futtermitteln -Polymerase-Kettenreaktion (PCR) zum Nachweis und zur quantitativen Bestimmung von pathogenen Mikroorganismen in Lebensmitteln - Leistungsmerkmale (ISO 22118:2011)

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Foreword

This document (EN ISO 22118:2011) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2012, and conflicting national standards shall be withdrawn at the latest by January 2012.

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Foreword

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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.

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 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.]

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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]

3.8

trueness

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

[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 10⁶ 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 10⁶ 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

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 10⁶ 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.

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If this is not applicable, the method performance characteristics shall be determined for a specific method application, i.e. a specific analytical procedure for a well-defined scope of the method (4.2).

As a minimum requirement for in-house validation, the test shall be carried out by at least two different persons. The tests shall be carried out on samples with a contamination level at the minimum requirements for the sensitivity tests.

Laboratory tests in the validation studies, starting from nucleic acid extraction, should be run at least in duplicate per sample.

5.2 Determination of the limit of detection, limit of quantification, and range of use for molecular detection methods

5.2.1 Qualitative methods

5.2.1.1 **General**

A qualitative method shall be validated in the same way as it is intended to be used.

The method, including any prior enrichments or concentration steps, should have a sensitivity of 1 cfu to 10 cfu or viral genome equivalents in a defined amount of the food matrix under investigation. It should not give rise to a significant number of false-positives.

A concept of using false-positive and false-negative rates to describe the trueness and precision of a qualitative assay has been developed for microbiological assays (Reference [5]).

A critical issue in the validation of this type of method is the use of naturally contaminated test materials. If not available, artificially contaminated samples can be used , e.g. with the inoculation levels:

0 cfu (blank); 1 cfu to 10 cfu;

10 cfu to 100 cfu;

in a defined amount of the food matrix under investigation.

Usually, two strains relevant for the matrix are selected.

Qualitative tests result in yes or no answers.

False-negative results indicate the absence of a given analyte when in fact the analyte is present in the sample; false-positive results indicate the presence of an analyte that is not present in the sample. An increase in the number of false-negative results is observed when the amount of analyte approaches the limit of detection (LOD) of the method.

The LOD for a qualitative method can be expressed as the concentration of an analyte that gives a positive result with a probability of 0,95.

This implies a rate of false-negative results of 0,05 or less. During validation of a qualitative molecular assay, it is also important to determine the number of false-positive results.

Both false-positive and false-negative results can be expressed as rates.

5.2.1.2 False-positive rate

This is the probability that a known negative sample has been classified as positive by the method. The false-positive rate is the number of misclassified known negatives divided by the total number of negative

samples (misclassified negative plus the number of correctly classified known negatives) obtained with the method.

For convenience, the false-positive rate, $p_{\rm f+}$, can be expressed as a percentage:

$$p_{f+} = \frac{n_{f+}}{n_{r-} + n_{f+}} \times 100 \%$$

where

 n_{f+} is the number of misclassified known negative samples;

 $n_{\rm r-}$ is the number of real negative test results.

5.2.1.3 False-negative rate

This is the probability that a known positive sample has been classified as negative by the method. The false-negative rate is the number of misclassified known positives divided by the total number of positive samples (misclassified positives plus the number of correctly classified known positives) obtained with the method.

For convenience, the false-negative rate, p_{f_-} , can be expressed as a percentage:

$$p_{f-} = \frac{n_{f-}}{n_{r+} + n_{f-}} \times 100 \%$$

where

 n_{f-} is the number of misclassified known positive samples;

 n_{r+} is the number of real positive test results.

5.2.2 Quantitative methods

The validation of methods is described in ISO 5725-1 ^[2], ISO 5725-2 ^[3], and Reference [4]. The determination of a LOD or a limit of quantification (LOQ) is not necessary to establish the validity of a method for a given application. For example, if the method is to be used for determinations ranging from 1 000 cfu/g to 100 000 cfu/g, it is not necessary to determine the LOD to be 1 cfu/g. It is necessary to determine the range of use of the method in the validation study. The method should be used only in that range.

The LOD for a quantitative method can be expressed as the concentration of an analyte that gives a positive result with a probability of 0,95.

This implies a rate of false-negative results of 0,05 or less.

The LOQ corresponds to the lowest concentration of the range of use.

6 Validation report

6.1 General

The validation report shall contain at least the following information:

a) name of the laboratory;

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- b) dates of validation;
- c) number of samples used;
- d) number and types of matrices used;
- e) results of the selectivity tests:
 - number and names of target strains used, including the amount of DNA or RNA used in the reaction for specificity tests;
 - 2) number and names of non-target microorganisms or viruses, including the amount of DNA or RNA used in the reaction for specificity tests;
 - 3) results of the tests of the ability to amplify the DNA or RNA used for the selectivity test;
- f) results of the sensitivity tests;
- g) information about the robustness of the method;
- h) information about the analytical controls used;
- i) information about the instruments used;
- j) information about precision and trueness;
- k) any other relevant observations made during the validation study.

6.2 Qualitative methods

The validation report for qualitative methods should consider information about LOD (or the sensitivity) and the selectivity. The false-positive rate, $p_{\rm f+}$, can be used to calculate a value for selectivity, expressed as a percentage, of $100-p_{\rm f+}$, and the false-negative rate, $p_{\rm f-}$, can be used to calculate a value for sensitivity, expressed as a percentage, of $100-p_{\rm f-}$.

6.3 Quantitative methods

The validation report of quantitative methods should consider information about accuracy (trueness and precision), range of use and if necessary the LOD and/or LOQ.

Annex A

(informative)

Minimum list of microorganisms for use in the exclusivity tests for detection bacteria

—	Bacillus cereus
_	Brochothrix thermosphacta
_	Campylobacter jejuni
—	Citrobacter spp.
_	Clostridium perfringens
—	Enterobacter spp.
	Escherichia coli
_	Lactobacillus spp.
—	Listeria monocytogenes
_	Pseudomonas spp.
—	Salmonella enterica
_	Staphylococcus aureus
_	Yersinia enterocolitica
—	Aspergillus spp.
_	Saccharomyces spp.

The list includes:

Bibliography

- [1] ISO 3534-2 :2006, Statistics Vocabulary and symbols Part 2: Applied statistics
- [2] ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results Part 1: General principles and definitions
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