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Date: 29 November 2023

Origin: European

Latest date for receipt of comments: 22 January 2024

Project No. 2022/00141

Responsible committee: AW/275 Food analysis - Horizontal methods

Interested committees:

Title: Draft BS EN ISO 11781 Foodstuffs — General guidelines for single-laboratory validation of qualitative real-time PCR methods.

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Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	

DRAFT INTERNATIONAL STANDARD

ISO/DIS 11781

ISO/TC 34/SC 16

Secretariat: ANSI

Voting begins on:
2023-11-29

Voting terminates on:
2024-02-21

Molecular biomarker analysis — General guidelines for single-laboratory validation of qualitative real-time PCR methods

ICS: 67.050

This document is circulated as received from the committee secretariat.

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Reference number
ISO/DIS 11781:2023(E)

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Published in Switzerland

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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 ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

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

Qualitative real-time polymerase chain reaction (PCR) methods currently find broad application for the detection of specific DNA sequences in food, e.g., for the detection and identification of genetically modified organisms and the products derived thereof, for food authentication and speciation and other purposes. It is important that a newly developed food analytical method is fit-for-purpose and meets certain performance characteristics and quality criteria as demonstrated by a particular set of validation experiments.

The data determined by the single laboratory validation are the basis for the decision to apply a method in-house. Furthermore, it helps to decide whether the method in question should be fully validated in the framework of a collaborative study. The statistical model described has been practically applied.^[3] Other models can be applicable, see ISO/TS 16393.

The aim of this document is to provide a protocol for single-laboratory validation of qualitative real-time PCR methods that are applied for food analysis. Procedures for DNA extraction from the food matrix are not included in this document. The procedure described is a recommendation which is underpinned by practical experience in several laboratories. Alternate approaches may be applied if they can be shown to meet the performance criteria set in this document.

Molecular biomarker analysis — General guidelines for single-laboratory validation of qualitative real-time PCR methods

1 Scope

This document describes minimum requirements and minimum performance criteria for conducting a single-laboratory validation study for qualitative (binary) real-time polymerase chain reaction (PCR) methods applied to the detection of specific DNA sequences present in foods.

The document can be applied to any single-laboratory validation of a qualitative real-time PCR method used for the detection of specific DNA sequences in food and food products, e.g. for detection of genetically modified foodstuffs and for species determination, including species known to produce allergenic proteins.

The document does not cover the evaluation of applicability and practicability with respect to the specific scope of the PCR method.

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 16577, *Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following apply.

ISO and IEC maintain terminological 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

probability of detection

POD

probability of a positive analytical outcome of a qualitative method for a given matrix at a given concentration in a single laboratory

Note 1 to entry: For a qualitative real-time PCR method it describes the probability that, for a given number of DNA copies of the target sequence, PCR amplification will take place.

[SOURCE: ISO 16577:2022, 3.9.12, modified — The notes have been removed and a new note 1 to entry was added.]

3.2

PCR efficiency

measured amplification rate for a DNA copy of the target sequence per PCR cycle in relation to the theoretically achievable value of 1

Note 1 to entry: The PCR efficiency is calculated from the slope of a standard curve resulting from the decadic semi-logarithmic plot of quantification cycle (C_q) values over the DNA concentration. The slope from the calculated regression line can be used. The PCR efficiency can either be expressed as absolute number or as percentage.

3.3

limit of detection

LOD_{95 %}

mean number of DNA copies of the target sequence yielding a probability of detection of 0,95

3.4

specificity

property of a method to respond exclusively to the characteristic or analyte under investigation

[SOURCE: ISO 24276, 3.1.4]

4 Principle

Specific primers and also probes, depending on the detection system applied, are designed for specific amplification and detection of a DNA target sequence by a qualitative real-time PCR method. In the next step for single laboratory validation, the method's performance characteristics should be assessed to show that the method complies with the quality criteria stipulated in relevant documents^{[1],[2]}.

For a qualitative real-time PCR method, the main focus of the validation should be the limit of detection (at which the probability of detection is $\geq 95 \%$), the specificity for the DNA target sequence and the robustness to small but deliberate variations in the method parameters.

On the basis of single laboratory validation data, fulfilment of the minimum required performance criteria for a qualitative real-time PCR method can be verified and should comprise the basis for applicability of the method by a single laboratory. A further decision whether to conduct a validation of the method in the framework of a collaborative study can then be taken.

Determination of the reproducibility (inter-laboratory transferability) and how the method performs in different laboratories, in particular the false-positive/false-negative rate obtained with negative/positive test samples, and the probability of detection (POD) across laboratories, can be evaluated by a collaborative study, if the design is appropriate^[3].

5 Single-laboratory validation of the performance characteristics

5.1 General

Guidance for compiling the information required for a complete and detailed description of all components that should be provided with the protocol of qualitative PCR methods, e.g. oligonucleotide sequences, amplicon length, instrument or chemistry specifications, PCR conditions, analytical controls, etc., is described in other relevant documents^{[1],[2]} or ISO 21569.

DNA extraction shall be according to the requirements specified in ISO 21571.

5.2 Limit of detection (LOD_{95 %})

The LOD_{95 %} is expressed as the number of copies of the target sequence and should be determined by means of a dilution series of the target DNA, where in addition to the target DNA each dilution contains a uniform concentration of non-target DNA (background DNA).