



BSI Standards Publication

**Molecular biomarker analysis —
Requirements and guidance for single-
laboratory validation of qualitative real-time
polymerase chain reaction (PCR) methods**

National foreword

This British Standard is the UK implementation of EN ISO 11781:2025. It is identical to ISO 11781:2025. It supersedes PD CEN/TS 17329-1:2021, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

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

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English Version

**Molecular biomarker analysis - Requirements and
guidance for single-laboratory validation of qualitative
real-time polymerase chain reaction (PCR) methods (ISO
11781:2025)**

Analyse de biomarqueurs moléculaires - Exigences et
recommandations pour la validation intralaboratoire
des méthodes de PCR qualitative en temps réel (ISO
11781:2025)

Untersuchung auf molekulare Biomarker -
Anforderungen und Leitfaden für die
Einzellaborvalidierung qualitativer Real-time-PCR-
Verfahren (ISO 11781:2025)

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European foreword

This document (EN ISO 11781:2025) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by October 2025, and conflicting national standards shall be withdrawn at the latest by October 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 17329-1:2021.

Any feedback and questions on this document should be directed to the users' national standards body/national committee. A complete listing of these bodies can be found on the CEN website.

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Endorsement notice

The text of ISO 11781:2025 has been approved by CEN as EN ISO 11781:2025 without any modification.

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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).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis - Horizontal methods*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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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 for 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, e.g. some parts of the ISO/TS 21569 series^{[1][2]} and ISO/TS 20224 series^[3]. Other models can be applicable, see ISO/TS 16393^[4].

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 — Requirements and guidance for single-laboratory validation of qualitative real-time polymerase chain reaction (PCR) methods

1 Scope

This document specifies 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 is applicable 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 the detection of genetically modified foodstuffs and for species determination, including species known to produce allergenic proteins).

The document does not apply to single laboratory validation of qualitative microbiological real-time PCR methods.

The document does not apply to 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 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 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 — Note 1 to entry replaced Notes 1 and 2 to entry.]

3.2**polymerase chain reaction efficiency****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* (3.1) of 0,95

3.4**specificity**

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

[SOURCE: ISO 24276:2006, 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 shall be assessed to show that the method complies with the quality criteria stipulated in relevant documents.^{[5][6]}

For a qualitative real-time PCR method, the main focus of the validation shall be the limit of detection (LOD_{95 %}) (at which the probability of detection (POD) 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 (interlaboratory 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 POD across laboratories, can be evaluated by a collaborative study, if the design is appropriate.^[6]

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) is described in other relevant documents, see References ^[5] and ^[6] and ISO 21569^[8].

DNA extraction shall be in accordance with the requirements specified in ISO 21571.

5.2 Limit of detection

The LOD_{95 %} is expressed as the number of copies of the target sequence and shall 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).