International Standard

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

Analyse de biomarqueurs moléculaires — Exigences et recommandations pour la validation intralaboratoire des méthodes de PCR qualitative en temps réel

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Foreword

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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.

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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.]

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