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Foodstuffs - General guidelines for the validation of qualitative real-time PCR methods - Part 1: Single-laboratory validation

Denrées alimentaires - Lignes directrices générales pour la validation des méthodes de PCR qualitative en temps réel - Partie 1 : Validation intralaboratoire

Lebensmittel - Allgemeine Anleitung für die Validierung qualitativer Realtime-PCR-Verfahren - Teil 1: Einzellaborvalidierung

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European foreword

This document (CEN/TS 17329-1:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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 consists of two parts:

— Part 1: Single-laboratory validation

— Part 2: Collaborative study

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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 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 aim of this document is to provide a protocol for single-laboratory validation of qualitative real-time PCR methods which are applied for food analysis. The procedure described is a recommendation that is underpinned by practical experience in several laboratories. It is possible to apply alternative approaches for which it can be shown that the performance criteria mentioned in the present document are achieved.

1 Scope

This document describes the performance characteristics and minimum performance criteria which should be taken into account when conducting a single-laboratory validation study for qualitative (binary) real-time polymerase chain reaction (PCR) methods applied for the detection of specific DNA sequences present in foods.

The protocol was developed for qualitative real-time PCR methods for the detection of DNA sequences derived from genetically modified foodstuffs. It is applicable also for single-laboratory validation of qualitative PCR methods used for analysis of other food materials, e.g. for species detection and identification.

The document does not cover the evaluation of the applicability and the 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.

EN ISO 21571:2005, *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction (ISO 21571:2005)*

EN ISO 24276, *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions (ISO 24276)*

ISO 16577, *Molecular biomarker analysis — Terms and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and EN ISO 24276 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
probability of detection
POD
probability of a positive analytical outcome of a qualitative method for a given matrix at a given concentration

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.

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 (Cq) 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.