SLOVENSKI STANDARD
oSIST prEN ISO 20836:2020

01-marec-2020

Mikrobiologija živil in krme - Polimerazna verižna reakcija (PCR) za ugotavljanje prisotnosti povzročiteljev zastrupitev s hrano - Preskus izvedbe preiskave s pomnoževalnikom (ISO/DIS 20836:2020)

Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Thermal performance testing of thermal cyclers (ISO/DIS 20836:2020)

Mikrobiologie von Lebensmitteln und Futtermitteln - Polymerase-Kettenreaktion (PCR) zum Nachweis von pathogenen Mikroorganismen in Lebensmitteln - Leistungsprüfung für PCR-Geräte (ISO/DIS 20836:2020)

Microbiologie de la chaîne alimentaire - Réaction de polymérisation en chaîne (PCR) pour la recherche de micro-organismes pathogènes dans les aliments - Essais de performance des thermocycleurs (ISO/DIS 20836:2020)

Ta slovenski standard je istoveten z: **prEN ISO 20836**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 20836:2020 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 20836

ISO/TC **34**/SC **9**

Secretariat: **AFNOR**

Voting begins on:
2020-01-09

Voting terminates on:
2020-04-02

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Thermal performance testing of thermal cyclers

Microbiologie de la chaîne alimentaire — Réaction de polymérisation en chaîne (PCR) pour la recherche de micro-organismes pathogènes dans les aliments — Essais de performance des thermocycleurs

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 20836:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee CEN/TC 275, *Food analysis*, Working Group 6, *Microbiology*, in collaboration with ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 20836:2005), which has been technically revised.

The main changes compared to the previous edition are as follows:

— the scope has been extended to include both thermal cyclers and real-time thermal cyclers;

— the document type has been changed from ISO/TS to an ISO-Standard;

— the physical performance testing method has been described in more detail, the biochemical performance testing method has been taken out;

— information for laboratories regarding ISO/IEC 17025 have been included;

— the performance testing method has been aligned with ISO/IEC 17025;

— compliancy testing has been added;

— in Annex C two procedures to set PCR method based specifications have been added.

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

This Standard is part of a series of International Standards under the general title *Microbiology of the food chain – Polymerase chain reaction (PCR) for the detection of food borne pathogens*:

— General requirements and definitions (ISO 22174);

— Requirements for sample preparation for qualitative detection (ISO 20837);

— Performance testing for thermal cyclers (ISO 20836);

— Requirements for amplifications and detection for qualitative methods (ISO 20838).

This Standard describes a method for performance testing for standard thermal cyclers and real-time thermal cyclers that allows laboratories to evaluate if the thermal cycler used is suitable for the intended use and meets the specifications set by the laboratory.

The described method is based on a physical method that measures directly in the thermal cycler block in block based thermal cyclers and in tubes in heated chamber based thermal cyclers. The described method provides a measurement uncertainty that is sufficiently low to allow meaningful comparison to specifications.

Furthermore, the method does meet the criteria of a metrological traceable calibration method in case it is used by ISO/IEC 17025 compliant laboratories.

Attention is drawn to the possibility that some methods described in this document may be subject to patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Thermal performance testing of thermal cyclers

1 Scope

This International Standard provides requirements for the installation, maintenance, temperature calibration and temperature performance testing of standard thermal cyclers and real-time thermal cyclers and is applicable to the detection of food-borne pathogens as well as any other applications in food and feeding stuffs using polymerase chain reaction (PCR) based methods.

This standard has been established for food testing, but can also be applied in other domains using thermal cyclers (e.g. environmental, human health, animal health and forensic testing). There can be other requirements in specific documents.

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 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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 https://www.iso.org/obp

3.1 PCR

3.1.1
polymerase chain reaction
PCR
enzymatic procedure which allows *in vitro* amplification of DNA

[SOURCE: ISO 22174:2005 3.4.1]

3.1.2
PCR method
test method based on the PCR technique

Note 1 to entry: Examples include, but are not limited to, PCR, quantitative real-time PCR (qPCR), reverse transcriptase PCR (RT PCR) and reverse transcriptase quantitative real-time PCR (RT qPCR).

3.2 Thermal cycler

3.2.1
thermal cycler
automatic device which performs defined heating and cooling cycles necessary for PCR or real-time PCR

[SOURCE: Adapted from ISO 22174:2005 3.4.20]

Note 1 to entry: The thermal cycler can be a block based or (individual) reaction chamber based thermal cycler.

3.2.2
reaction block
heated and cooled metal block in which PCR reaction vials, containing the PCR reaction mix, can be inserted

Note 1 to entry: The block can be heated and cooled by a number of technologies, among which Peltier heating and cooling is the most abundantly used.

3.2.3
reaction chamber
heated and cooled chamber in which PCR reaction vials, containing the PCR reaction mix, can be inserted directly or in a rotor

Note 1 to entry: The chamber can be heated and cooled by a number of technologies, among which air heating and cooling is the most abundantly used.

3.2.4
heated lid
heated cover of thermal cycler which is applied in block based thermal cyclers onto reaction tubes to prevent condensation of reaction mix to cap of reaction tube and evaporation from reaction tube and applies pressure onto the tubes to ensure proper thermal contact

3.2.5
PCR temperature protocol
heating and cooling cycles required for PCR, typically consisting of denaturation, annealing and extension temperature steps which are repeated typically 30-45 times

Note 1 to entry: In certain PCR methods a two-step temperature protocol is used in which annealing and extension are combined to one step.

3.3 Temperature characteristics

3.3.1
thermal cycler temperature profile
graph of the course of the temperature by performing measurements at defined intervals (see Annex D for an example graph of thermal cycler temperature profile)

3.3.2
$t_{(i)}time$
temperature in °C of sensor *i* at time stamp *time* in s

3.3.3
set temperature
t_{set}
target temperature programmed to be reached in °C

3.3.4
average temperature

$$t_{avg}(time) = \sum_{i=1}^{N} \frac{t_i(time)}{N}$$

where

$t_{avg}(time)$	is average temperature in °C at time stamp *time*;
i	is sensor i of N
N	is total number of sensors.

average of measured values of all active temperature sensors in °C at a specific time stamp in s

3.3.5
temperature deviation

$$t_{dev}(time) = t_{avg}(time) - t_{set}$$

average temperature minus set temperature in °C at a specific time stamp in s

3.3.6
minimum temperature

$$t_{min}(time) = min(t_i(time) \ldots t_N(time))$$

minimum value of all active temperature sensors in °C at a specific time stamp in s

3.3.7
maximum temperature

$$t_{max}(time) = max(t_i(time) \ldots t_N(time))$$

maximum value of all active temperature sensors in °C at a specific time stamp in s

3.3.8
temperature uniformity

$$t_{uniformity}(time) = t_{max}(time) - t_{min}(time)$$

homogeneity of the temperature distribution within the reaction block or chamber, defined as maximum temperature minus minimum temperature in °C at a specific time stamp in s

3.3.9
average ramp rate

$$V_t = \sum_{i=1}^{N} = \left(\frac{t_{i,90\%} - t_{i,10\%}}{time_{i,90\%} - time_{i,10\%}} \right)$$

where

V_t	is ramp rate in °C/s
i	is sensor i of N
N	is total number of sensors.
$t_{i,10\%}$	is t_i at 10 % time of the ramp slope in °C

$t_{i,90\%}$ is t_i at 90 % time of the ramp slope in °C

time is time in s

heat or cool rate of thermal cycler calculated between 10 % and 90 % time of the heating or cooling slope.

Note 1 to entry: The heat rate is a positive ramp rate. The cool rate is a negative ramp rate.

3.3.10
maximum ramp rate
V_{tmax}
maximum heat or cool rate during heating or cooling slope in °C/s

3.3.11
maximum temperature overshoot
$$t_{i,ovs,max} = t_{i,max}(time)\Big|_{timmehold=endovershoot}^{timehold=beginovershoot} - t_i(timehold = 30s)$$
maximum temperature value in °C of all active temperature sensors during temperature overshoot above the average temperature of the reaction block or chamber temperature at hold when heating up

Note 1 to entry: The maximum temperature overshoot is calculated between begin and end of the overshoot and is expressed relative to the temperature at 30 s hold time.

Note 2 to entry: The overshoot occurs typically between 0 s and 15 s hold time. See Annex D for an example thermal cycler temperature profile.

3.3.12
minimum temperature undershoot
$$t_{i,uns,min} = t_{i,min}(time)\Big|_{timmehold=end\ undershoot}^{timehold=begin\ undershoot} - t_i(timehold = 30s)$$
minimum temperature value in °C of all active temperature sensors during temperature undershoot below the average temperature of reaction block or chamber temperature at hold when cooling down.

Note 1 to entry: The maximum temperature undershoot is calculated between begin and end of the undershoot and is expressed relative to the temperature at 30 s hold time. An undershoot is an overshoot in negative direction.

Note 2 to entry: to entry The undershoot occurs typically between 0 s and 15 s hold time. See Annex D for an example thermal cycler temperature profile

3.3.13
average temperature overshoot
$$t_{ovs,avg} = \sum_{i=1}^{N}\left(\frac{t_{i,ovs,max}}{N}\right)$$
average value of maximum temperature overshoots of all active block temperature sensors in °C

3.3.14
average temperature undershoot
$$t_{uns,avg} = \sum_{i=1}^{N}\left(\frac{t_{i,uns,min}}{N}\right)$$
average value of maximum temperature undershoot of all active block temperature sensors in °C