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

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

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Foreword

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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 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 TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition International Standard cancels and replaces the first edition Technical Specification (ISO/TS 20836:2005), which has been technically revised. The main changes compared with the previous edition are as follows:

- the Scope has been extended to include both thermal cyclers and real-time thermal cyclers;
- the physical performance testing method has been described in more detail, and the biochemical performance testing method has been taken out;
- information for laboratories regarding ISO/IEC 17025 has 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 document is part of a family of International Standards under the general title *Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food borne pathogens*:

— ISO 22174, *General requirements and definitions*;

— ISO 20837, *Requirements for sample preparation for qualitative detection*;

— ISO 20836, *Thermal performance testing of thermal cyclers*;

— ISO 20838, *Requirements for amplifications and detection for qualitative methods*.

This document 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.

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

1 Scope

This document specifies requirements for the installation, maintenance, temperature calibration and temperature performance testing of standard thermal cyclers and real-time thermal cyclers. It is applicable to the detection of microorganisms as well as any other applications in the food chain using polymerase chain reaction (PCR)-based methods.

This document has been established for food testing, but is also applicable to other domains using thermal cyclers (e.g. environmental, human health, animal health, forensic testing).

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/IEC Guide 98-3:2008, *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:

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

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

3.1 Polymerase chain reaction

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

[SOURCE: ISO 22174:2005, 3.4.1]

3.1.2
PCR method
test method based on the *PCR* (3.1.1) technique

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

3.2 Thermal cycler

3.2.1
thermal cycler
automatic device that performs defined heating and cooling cycles necessary for *PCR* (3.1.1) or real-time PCR

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

[SOURCE: ISO 22174:2005, 3.4.20, modified — “or real-time PCR” and Note 1 to entry have been added.]

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 a *thermal cycler* (3.2.1), which is applied in block-based thermal cyclers onto reaction tubes to prevent condensate of reaction mix to collect inside the cap of the reaction tube or onto the seal, and evaporation from the reaction tube, and which applies pressure onto the tubes to ensure proper thermal contact

3.2.5
PCR temperature protocol
heating and cooling cycles required for *PCR* (3.1.1), typically consisting of denaturation, annealing and extension temperature steps, which are typically repeated 30 to 45 times

Note 1 to entry: In certain *PCR methods* (3.1.2), 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

Note 1 to entry: See Annex D for an example graph of a thermal cycler temperature profile.

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

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

3.3.4
average temperature
T_{avg}

$$T_{avg}(t)=\sum_{i=1}^{N}\frac{T_i(t)}{N}$$

where

$T_{avg}(t)$	is average temperature in °C at time *t*;
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 in s

3.3.5
temperature deviation
T_{dev}
$T_{dev}(t)=T_{avg}(t)-T_{set}$
average temperature (3.3.4) minus *set temperature* (3.3.3) in °C at a specific time in s

3.3.6
minimum temperature
T_{min}
$T_{min}(t)=\min(T_i(t)...T_N(t))$
minimum value of all active temperature sensors in °C at a specific time in s

3.3.7
maximum temperature
T_{max}
$T_{max}(t)=\max(T_i(t)...T_N(t))$
maximum value of all active temperature sensors in °C at a specific time in s

3.3.8
temperature uniformity
$T_{uniformity}$
$T_{uniformity}(t)=T_{max}(t)-T_{min}(t)$
homogeneity of the temperature distribution within the *reaction block* (3.2.2) or chamber, defined as *maximum temperature* (3.3.7) minus *minimum temperature* (3.3.6) in °C at a specific time in s

3.3.9
temperature transition
$T_{transition}$
phase of fast temperature change from one set temperature to another set temperature

3.3.10
ramp rate
heat or cool rate of *thermal cycler* (3.2.1) in °C/s

3.3.11
average ramp rate
V_t

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

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 % temperature of the ramp slope in °C;
$T_{i,90\%}$	is T_i at 90 % temperature of the ramp slope in °C;
t	is time in s.

heat or cool rate of *thermal cycler* (3.2.1) calculated between 10 % and 90 % time of the heating or cooling slope

Note 1 to entry: The heat rate is a positive *ramp rate* (3.3.10). The cool rate is a negative ramp rate.

3.3.12
maximum ramp rate

$V_{t\ \max}$

maximum heat or cool rate during heating or cooling slope in °C/s

3.3.13
maximum temperature overshoot

$T_{i,\text{ovs,max}}$

$$T_{i,\text{ovs,max}} = T_{i,\max}(t)\Big|_{thold=0s}^{thold=15s} - T_i(thold = 30s)$$

maximum temperature (3.3.7) value in °C of all active temperature sensors during temperature overshoot above the *average temperature* (3.3.4) of the *reaction block* (3.2.2) or chamber temperature at hold when heating up

Note 1 to entry: The maximum temperature overshoot is calculated between start and end of the overshoot and is expressed relative to the temperature at 30 s *hold time* (3.3.18).

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* (3.3.1).

3.3.14
minimum temperature undershoot

$T_{i,\text{uns,min}}$

$$T_{i,\text{uns,min}} = T_{i,\min}(t)\Big|_{thold=0s}^{thold=15s} - T_i(thold = 30s)$$

minimum temperature (3.3.6) value in °C of all active temperature sensors during temperature undershoot below the *average temperature* (3.3.4) of *reaction block* (3.2.2) or chamber temperature at hold when cooling down

Note 1 to entry: The maximum temperature undershoot is calculated between start and end of the undershoot and is expressed relative to the temperature at 30 s *hold time* (3.3.18). An undershoot is an overshoot in negative direction.

Note 2 to entry: The undershoot occurs typically between 0 s and 15 s hold time. See Annex D for an example *thermal cycler temperature profile* (3.3.1).

3.3.15
average temperature overshoot

$T_{\text{ovs,avg}}$

$$T_{\text{ovs,avg}} = \sum_{i=1}^{N} \left(\frac{T_{i,\text{ovs,max}}}{N} \right)$$

average value of *maximum temperature overshoots* (3.3.13) of all active block temperature sensors in °C

3.3.16
average temperature undershoot
$T_{uns,avg}$

$$T_{uns,avg} = \sum_{i=1}^{N} \left(\frac{T_{i,uns,min}}{N} \right)$$

average value of *minimum temperature undershoots* (3.3.14) of all active block temperature sensors in °C

3.3.17
overshoot duration
time elapsed between start and end of the overshoot in s

Note 1 to entry: The start of the overshoot is defined as the moment in time where the *average temperature* (3.3.4) exceeds the average hold temperature, calculated at 30 s hold, at the beginning of the overshoot. The end of the overshoot is defined as the moment in time where the average temperature reaches the average hold temperature at the finish of the overshoot.

3.3.18
hold time
time elapsed between start and end of a temperature hold in s

Note 1 to entry: See Annex D for an example to determine start and end of hold.

3.4 Temperature measurement

3.4.1
temperature measurement system
temperature measurement and data logging instrument

3.4.2
sampling frequency
number of samples per second taken from a time-continuous signal to make a time-discrete signal

3.4.3
response time
time required for the *temperature measurement system* (3.4.1), when subjected to a change in temperature, to react to this change

3.4.4
measurement uncertainty
parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the quantity intended to be measured

[SOURCE: ISO/IEC Guide 98-3:2008, B.2.18, modified — "quantity intended to be measured" and replaced "measurand" and the notes to entry have been deleted.]

3.4.5
performance test
test procedure that determines the performance of a *thermal cycler* (3.2.1)

3.4.6
calibration
operation that, under specified conditions, in a first step, establishes a relation between the quantity values with *measurement uncertainties* (3.4.4) provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

Note 1 to entry: Calibration should not be confused with adjustment of a measuring system, nor with auto-check, self-verification test, verification, normalization, installation qualification (IQ), operational qualification (OQ) or performance qualification (PQ).

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified — Note 1 to entry has replaced the original Notes 1, 2 and 3 to entry.]

4 Installation of thermal cyclers

The manufacturer's instructions shall be followed.

The following should be taken into consideration.

a) Thermal cyclers should be installed and operated at suitable environmental conditions that do not invalidate the results or adversely affect the required quality of any test.

b) The environmental conditions that should at minimum be taken into account are room temperature and relative humidity.

See the manual of the thermal cycler for recommended room conditions.

Thermal cyclers shall be located in such a way that free circulation of air is permanently allowed.

See ISO 22174 for guidelines for contamination prevention and separation of incompatible laboratory activities.

5 Maintenance of thermal cyclers

The laboratory shall establish a maintenance programme, where appropriate, and keep records to ensure proper functioning and prevent deterioration of the thermal cyclers.

6 Performance testing of thermal cyclers

6.1 General

If the performance testing method of this document is used as a metrological traceable temperature calibration, as a conformity test or as a reference method, the performance test shall be carried out with a minimum number of sensors that represent at least 12,5 % of wells for reaction blocks or chambers < 96 wells or 12 wells for reaction blocks or chambers > 96 wells (see 6.4.5.1) and metrological traceability (see 6.3) shall be provided up to the level of the thermal cycler. If the performance testing method is used for other purposes, such as supplier's quality control or supplier's after sales service, the number of sensors may be reduced to a minimum number of sensors that represent at least 8 % of wells for reaction blocks or chambers < 96 wells or 8 wells for reaction blocks or chambers > 96 wells and metrological traceability shall be provided up to the level of the temperature measurement system.

In case of individual reaction chambers, each of the individual reaction chambers shall be tested.

The decision chart in Figure 1 can be used to determine if the performance test shall be a metrological traceable calibration or a performance test.

NOTE 1 Calibrations that meet the requirements of the ISO/IEC 17025 are considered to be metrological traceable. ISO/IEC 17025 describes when metrological traceability is required and how metrological traceability is established.

NOTE 2 The chemistry- or biochemistry-based normalization and verification kits available at the time of publication of this document offer no traceability to SI and are associated with high measurement uncertainties, and are therefore inapt as a performance testing method. The normalization kits are developed to optimize the optical detection and related software to enable the collection of fluorescent data in a real-time thermal cycler. These kits are not developed for calibration purposes.

