TECHNICAL SPECIFICATION

ISO/TS 20836

First edition
2005-08-01

Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Performance testing for thermal cyclers

Microbiologie des aliments — Réaction de polymérisation en chaîne (PCR) pour la recherche des micro-organismes pathogènes dans les aliments — Critères de performance pour les thermal cyclers

Reference number
ISO/TS 20836:2005(E)

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Published in Switzerland

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

ISO/TS 20836 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

This Technical Specification is part of a series of International Standards under the general title *Microbiology of food and animal feeding stuffs — 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/TS 20836);

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

The International Organization for Standardization (ISO) draws attention to the fact that it is claimed that compliance with this document may involve the use of one or more patents concerning the PCR technology.

ISO takes no position concerning the evidence, validity and scope of these patent rights.

ISO has been informed that Applied Biosystems, Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd. hold patent rights concerning the PCR technology. The companies have assured the ISO that they are willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statements of the holders of these patent rights are registered with ISO. Information may be obtained from:

Licensing Department
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
USA

and

Roche Molecular Systems, Inc.
Licensing Department
1145 Atlantic Avenue
Alameda, CA 94501
USA

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

Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Performance testing of thermal cyclers

WARNING — The use of this Technical Specification may involve hazardous materials, operations and equipment. This Technical Specification does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this Technical Specification to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This Technical Specification provides basic requirements for the installation, performance and maintenance of thermal cyclers. Although thermal cyclers are robust technical equipment, they do require regular maintenance. Their cooling/heating elements, either Peltier or other technology, have a limited lifetime. Proper functioning of the cooling/heating element depends both on the quality of the cooling/heating devices and proper use and care.

In addition to outlining the requirement for a defined maintenance programme, procedures are described for the determination of thermal cycler performance by biochemical or physical methods (see Annexes A and B).

2 Normative references

The following referenced documents are indispensable for the application 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 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22174 and the following apply.

3.1
heated lid
optional feature of a thermal cycler used to prevent evaporation from the reaction tube

3.2
temperature uniformity
homogeneity of the temperature within the thermally controlled unit (e.g. heating block)

3.3
biochemical performance test
test procedure which determines the performance of a thermal cycler by biochemical means (e.g. a temperature-sensitive PCR)

3.4
physical performance test
test procedure which determines the performance of a thermal cycler by physical means

3.5
non-robust PCR system
PCR system in which minor deviations from the prescribed PCR chemistry or PCR temperature protocol result in a less efficient amplification

3.6
critical position
position or area where a deviation of the sample temperature from the displayed temperature is more likely to occur than in other areas of the thermal cycler sample holder

4 Installation of thermal cyclers

The manufacturer's instructions should be followed.

The following should be taken into consideration:

a) thermal cyclers should be installed and run at an appropriate temperature and humidity;

b) thermal cyclers should be located

- so they can be inspected visually,
- in such a way as to allow a steady exchange of heat with the environment and free circulation of air, and
- in a constant environment.

5 Maintenance of thermal cyclers

The laboratory should establish and employ a defined maintenance programme for each thermal cycler machine, based on the documented hours of use or number of runs.

6 Performance tests

6.1 General

The performance testing of each thermal cycler should be carried out and documented, either indirectly using a biochemical performance test or directly using a physical performance test.

6.2 Biochemical performance test

A biochemical procedure for thermal cycler performance testing should be carried out, which may be a temperature-sensitive non-robust PCR method.

An example of a suitable method is described in Annex A. Any other PCR methods that can be shown to fulfil the requirements may also be used.

The performance test frequency should be based on the documented hours of use or the number of runs of each thermal cycler machine.

6.3 Physical performance test

A physical test procedure should be carried out to measure the actual sample temperature within each well during the temperature cycle, and to compare it to the displayed temperature.

An example of a suitable method is described in Annex B. A physical performance test should be undertaken if a biochemical performance test is not performed, or if the biochemical performance test indicates inefficient amplification.

The performance test frequency should be based on the documented hours of use or the number of runs of each thermal cycler machine.

7 Test report and documentation of irregularities

The test report shall conform to ISO/IEC 17025 and should contain at least the following:

— identification number of the thermal cycler;

— a reference to this Technical Specification and the Annex used for the test;

— the date of testing;

— person responsible for the analysis;

— test results;

— any particular points observed during testing;

— any deviation, additions to or exclusions from the test specification;

— any other information relevant to the specific test.

The laboratory should have documented procedures in place to identify and address any irregularities concerning thermal cycler performance (see also ISO/IEC 17025).

Annex A
(informative)

Biochemical performance test — PCR method for testing the temperature accuracy

A.1 General

This method verifies the accuracy of the control of the annealing temperature of thermal cyclers. The test is sensitive to an increase in the real annealing temperature relative to the displayed temperature during a defined temperature programme cycle.

A.2 Principle

The accuracy of the programmed/displayed temperature is tested using a specifically designed PCR. The PCR samples are highly sensitive to an overshoot of the annealing temperature during the temperature cycles.

A 362 base pair (bp) DNA sequence, which contains the target site for the PCR, is generated by amplifying the flanking region of the cloning site of the pGEM vector with a standard M13 sequencing primer pair. The cloned sequence is shown in Figure A.1.

CAGGAAACAG CTATGACCAT GATTACGCCA AGCTATTTAG GTGACACTAT AGAATACTCA AGCTATGCAT
CCAACGCGTT GGGAGCTCTC CCATATGGTC GACCTGCAGG CGGCCGCGAC AGTGATTAGC AACCTCGGTA
CCATATACTA ACTCGATACA GAAACATCGG TTGGTGATCG ATCGAGGTTT TTAAAAACCC CCTCTAGCTA
GCTAGCTAGC GATTGCTTCA CCAAGAAGAG CTCCAACAGC CTGATGGCAT CAAGTTACAC AATCCCGCGG
CCATGGCGGC CGGGAGCATG CGACGTCGGG CCCAATTCGC CCTATAGTGA GTCGTATTAC AATTCACTGG
CCGTCGTTTT AC

Figure A.1 — Template and primer sequences used in a thermal cycler performance test

The sequence of the PCR product was derived by using the M13-primers ('double'-underlined) in the PCR amplification of the target sequence from the modified pGEM plasmid (pSC17[1)]). The primers used in the test system for the thermal cycler validation are highlighted and shown separately (VAL1 and VAL2, see A.3.5.3 and A.3.5.4). The sequence of the PCR product generated in this thermal cycler performance test is underlined. A mismatch is shown in bold.

The primers VAL1 and VAL2 produce a 116 bp PCR product, which is detected by agarose gel electrophoresis. Due to the mismatch at the 3'-end of primer VAL1, the PCR is sensitive to an overshooting of the in-sample annealing temperature compared to the defined temperature. An overshooting from 63 °C to 66 °C leads to less efficient amplification and to a non-detectable PCR product of 116 bp.

The samples shall be placed at positions that are representative of the thermally controlled area, including critical positions. When the critical positions are not known, the samples should cover central and corner positions [1].

An example of a 96-well thermal cycler is given in Figure A.2.

1) This is an example of a suitable product available commercially. This information is given for the convenience of the user of this Technical Specification and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

A.3 Reagents

Use only analytically pure reagents suitable for molecular biological detection methods.

A.3.1 Water.

For the amplification reaction, use water that is DNase- and RNase-free at all times. Suitable ultrapure water is available commercially.

A.3.2 PCR-buffer (without $MgCl_2$), 10× (150 mmol/l TRIS, pH 8; 500 mmol/l KCl).

A.3.3 $MgCl_2$ solution, $c(MgCl_2)$ = 25 mmol/l.

A.3.4 dNTP solution, c(dNTPs) = 10 mmol/l (each).

A.3.5 Oligonucleotides.

Oligonucleotides require purification after synthesis.

A.3.5.1 Forward primer for the construction of the 362 bp target sequence

M13mp8 phage cloning vector (GenBank accession No. M77826.1).

Primer M13(-26): 5'-CAg gAA ACA gCT ATg AC-3'.

A.3.5.2 Reverse primer for the construction of the 362 bp target sequence

M13mp8 phage cloning vector (GenBank accession No. M77826.1).

Primer M13(-20): 5'-gTA AAA CgA Cgg CCA gT-3'.

A.3.5.3 Forward primer for testing the annealing temperature of thermal cyclers

Synthetic construct:

Primer VAL1: 5'-gAT ACA gAA ACA TCg gTT ggC-3'.

A.3.5.4 Reverse primer for testing the annealing temperature of thermal cyclers

Synthetic construct:

Primer VAL2: 5'-gTg TAA CTT gAT gCC ATC Agg-3'.

A.3.6 Plasmid pSC17[1)].

A.3.7 Thermostable DNA-polymerase (for hot-start PCR), 5 U/µl.

A.3.8 Agarose, suitable for DNA electrophoresis and for the intended size separation.

A.3.9 Boric acid (H_3BO_3), for the TBE buffer system only.

A.3.10 Bromophenol blue ($C_{19}H_9Br_4O_5SNa$) and/or **xylene cyanole FF** ($C_{25}H_{27}N_2O_6S_2Na$).

A.3.11 Glacial acetic acid (CH_3COOH), for the TAE buffer system only.

A.3.12 Ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA) ($C_{10}H_{14}N_2O_8Na_2$).

A.3.13 Ethidium bromide (EtBr) ($C_{21}H_{20}N_3Br$).