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**Microbiology of food and animal feeding  
stuffs — Polymerase chain reaction  
(PCR) for the detection of food-borne  
pathogens — Requirements for  
amplification and detection for qualitative  
methods**

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*Microbiologie des aliments — Réaction de polymérisation en chaîne  
(PCR) pour la détection des micro-organismes pathogènes dans les  
aliments — Exigences relatives à l'amplification et à la détection pour  
les méthodes qualitatives*

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Published in Switzerland

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## 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.

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.

ISO 20838 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).

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## Introduction

The amplification and detection of target nucleic acid sequences is performed to determine whether certain nucleic acid sequences are present or not in the test portion. This determination is relative to appropriate controls and within the detection limits of the analytical method used and test portion analysed.

This International Standard describes the procedure used to detect food-borne microorganisms, including pathogens, by analysing nucleic acids extracted from foodstuffs, feed and environmental samples, or from cultures or cell suspensions prepared from the foodstuff. Appropriate procedures for sample preparation, culturing of microorganisms and extraction of nucleic acids are described in ISO 20837.

The main focus of this International Standard is on PCR-based amplification methods. However, because of the rapid rate of technological change in this area, other amplification technologies and detection methods may be considered.

This International Standard is related to a series of standards and a Technical Specification 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 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.

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# Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods

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

## 1 Scope

This International Standard provides the overall framework for qualitative methods for the detection of food-borne pathogens using the polymerase chain reaction (PCR).

It covers the general requirements for the specific amplification of target nucleic acid sequences and the detection and confirmation of the identity of the amplified nucleic acid sequence.

Guidelines, minimum requirements and performance characteristics described in this International Standard are intended to ensure that comparable and reproducible results are obtained in different laboratories.

This International Standard has been established for food-borne pathogens in or isolated from food and feed matrices, but can also be applied to other matrices, for example environmental samples, or to the detection of other microorganisms under investigation.

## 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 16140, *Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods*

ISO 22174:2005, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

## 3 Terms and definitions

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

## 4 Principle

For the purposes of this International Standard, qualitative analysis consists of screening and/or specific detection of target nucleic acid sequences in the test samples. Specificity can be at genus, species or a lower taxonomic level.

A qualitative result shall clearly demonstrate the presence or absence of the target sequence under study, relative to appropriate controls and within the detection limits of the analytical method used and test portion analysed.

The analysis generally consists of

- a) amplification by PCR of specific target sequences,
- b) detection of the PCR product,
- c) confirmation of the identity of the PCR product, and/or
- d) confirmation by a standardized microbiological cultural method (e.g. an International Standard).

## **5 Reagents**

In all cases, analytically pure reagents suitable for molecular biological applications shall be used. It is generally advisable to take aliquots of the reaction solutions required for a PCR method and to store them under appropriate conditions, for example at  $-20\text{ }^{\circ}\text{C}$ .

### **5.1 DNA polymerase**

A thermostable polymerase (possibly including reverse transcriptase activity) is used for PCR. This may be a purified, native enzyme, or a purified, genetically engineered recombinant form of the enzyme.

It should be used according to the manufacturer's instructions.

Each DNA polymerase may need different experimental conditions, e.g. buffer, temperature.

### **5.2 Reverse transcriptase**

This enzyme is used for transcription of RNA in complementary single-stranded DNA (cDNA) able to be amplified by a subsequent PCR.

It should be used according to the manufacturer's instructions.

### **5.3 Reaction buffer**

The appropriate buffer should be used according to the enzyme manufacturer's instructions. Ready to use reagents are commercially available. Materials used for preparation of a PCR buffer shall be stable with respect to storage and cycling conditions.

It should be used according to the manufacturer's instructions.

### **5.4 Deoxyribonucleoside triphosphates (dNTP), for PCR**

Solutions containing molecular biology grade dATP, dCTP, dGTP, dTTP and/or dUTP, as appropriate, shall be used. They shall be stable during storage and under PCR conditions. They are commercially available.

### **5.5 Primers**

The primers should be selected based on a sequence specifically to detect the DNA of the target microorganism.

### **5.6 Water**

For the amplification reaction water that is DNase- and RNase-free should be used at all times. Suitable ultra pure water is available commercially.



### 5.7 Magnesium chloride (MgCl<sub>2</sub>)

This is supplied either as a component of the reaction buffer or as a separate solution.

### 5.8 Chemicals for detection of PCR products

The chemicals used for the detection system described in a PCR method should be of appropriate quality.

### 5.9 Optional additional reagents

#### 5.9.1 Mineral oil

This is dispensed onto the reaction mix to minimize evaporation during thermal cycling.

#### 5.9.2 Facilitators

These are substances, such as polyethylene glycol or bovine serum albumin, which can be added to the PCR reaction to reduce inhibition by matrix-derived substances [1].

#### 5.9.3 RNase inhibitors

These are added to an RT-PCR to prevent degradation of target RNA by RNase enzymes, which may have contaminated the reagents or plastic ware used in the extraction procedure. RNase inhibitors are commercially available.

#### 5.9.4 Reagents to prevent carry over of PCR products

As a further guard against contamination, a decontamination system (based on psoralene, dUTP and UNG, for example) may be included in the PCR system to minimize the risk of carryover contamination from PCR products produced during previous PCR reactions.

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## 6 Apparatus and equipment

This shall be in accordance with ISO 22174.

In addition to standard laboratory equipment, the following apparatus and equipment shall be used.

**6.1 Thermal cycler apparatus**, capable of reproducibly and accurately performing the temperature and time cycles described in a PCR method.

**6.2 Pipettes**, with filter tips.

At least three sets of pipettes are required, one of each dedicated to

- sample preparation,
- master mix preparation,
- post-amplification steps.

NOTE The use of filter tips is not necessary in the post-amplification steps.

**6.3 Reaction vessels**, suitable for use in thermal cyclers, and which can be repeatedly heated to 100 °C and cooled to 4 °C without damage.