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

*Microbiologie des aliments — Réaction de polymérisation en chaîne
(PCR) pour la détection des micro-organismes pathogènes dans les
aliments — Exigences relatives à la préparation des échantillons pour la
détection qualitative*



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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.

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.

ISO 20837 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 detection of food-borne pathogens by PCR is usually performed by means of the following successive (or simultaneous) steps:

- homogenization of the sample;
- (cultural) enrichment of the pathogen under study and sample treatment;
- nucleic acid extraction (optional);
- amplification of nucleic acids from the pathogen under study;
- detection of the amplified DNA from the pathogen under study.

References to International Standards concerning enrichment of bacteria from food matrices are given in Annex A. An example of a specific method for sample preparation is given in Annex B.

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

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

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 criteria and examples for sample preparation in order to obtain PCR-compatible samples or nucleic acids of suitable quality and quantity for PCR.

It provides a description of the general principles involved. References to standards concerning the enrichment of microorganisms are given in Annex A, and a detailed method for DNA extraction is given in Annex B.

This International Standard has been established for food matrices, but could also be applied to feed and agricultural/environmental matrices with some adaptations, if necessary.

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

3 Principle

3.1 General

The objective of the sample preparation methods described is to obtain samples or nucleic acids of suitable quality and quantity for PCR.

NOTE The quality of nucleic acids depends for example on the chemical purity, the average length of the molecules and the structural integrity of the extracted nucleic acid molecules.

Enrichment and sample treatment should allow the detection of low numbers of target microorganisms and the reduction of PCR inhibitory substances. Physical, chemical or biochemical procedures, least destructive to the nucleic acid integrity, should render the sample or the nucleic acid solution compatible with PCR amplification.