



**International
Standard**

ISO 7218

**Microbiology of the food chain —
General requirements and guidance
for microbiological examinations**

*Microbiologie de la chaîne alimentaire — Exigences générales et
recommandations pour les examens microbiologiques*

**Fourth edition
2024-06**

This document is a preview generated by EMS



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

Published in Switzerland

Contents

| | Page |
|--|-------------|
| Foreword | vii |
| Introduction | viii |
| 1 Scope | 1 |
| 2 Normative references | 1 |
| 3 Terms and definitions | 1 |
| 4 Premises | 5 |
| 4.1 General..... | 5 |
| 4.2 Biosafety considerations..... | 5 |
| 4.3 Laboratory design..... | 5 |
| 4.4 Laboratory areas..... | 5 |
| 4.4.1 General..... | 5 |
| 4.4.2 Areas associated with samples and testing..... | 6 |
| 4.4.3 General areas..... | 6 |
| 4.5 Layout and fittings of the premises..... | 6 |
| 4.5.1 Objectives..... | 6 |
| 4.5.2 Fittings..... | 7 |
| 4.5.3 Other arrangements for laboratory premises..... | 7 |
| 4.5.4 Cleaning and disinfection..... | 8 |
| 5 Personnel | 8 |
| 5.1 General..... | 8 |
| 5.2 Competence..... | 8 |
| 5.3 Verification of ongoing staff competence..... | 9 |
| 5.4 Hygiene..... | 9 |
| 6 Equipment and consumables | 9 |
| 6.1 General..... | 9 |
| 6.2 Sterilization and other heating equipment..... | 10 |
| 6.2.1 General..... | 10 |
| 6.2.2 Autoclave..... | 10 |
| 6.2.3 Culture media preparator..... | 11 |
| 6.2.4 Steamers, including boiling-water baths..... | 12 |
| 6.2.5 Sterilizing oven..... | 12 |
| 6.2.6 Microwave oven..... | 13 |
| 6.2.7 Hotplate, induction cooker and heating mantle..... | 14 |
| 6.2.8 Gas burner or wire incinerator..... | 14 |
| 6.3 Temperature controlled equipment and monitoring devices..... | 15 |
| 6.3.1 General..... | 15 |
| 6.3.2 Incubator..... | 15 |
| 6.3.3 Thermostatically controlled bath..... | 16 |
| 6.3.4 Heating blocks..... | 17 |
| 6.3.5 Refrigerators and cold-storage rooms..... | 18 |
| 6.3.6 Freezer and deep freezer/ultra-low temperature freezer..... | 19 |
| 6.3.7 Temperature-monitoring devices, including automatic recorders..... | 19 |
| 6.3.8 Balances and gravimetric diluters..... | 20 |
| 6.4 Defined volume inoculation equipment..... | 21 |
| 6.4.1 Pipettes and pipettors..... | 21 |
| 6.4.2 Dispensers..... | 22 |
| 6.4.3 Spiral platers..... | 23 |
| 6.4.4 Serial diluters..... | 24 |
| 6.5 Protective cabinets..... | 24 |
| 6.5.1 Description..... | 24 |
| 6.5.2 Use..... | 25 |
| 6.5.3 Cleaning and disinfection..... | 25 |
| 6.5.4 Maintenance and inspection..... | 26 |

| | | |
|-----------|--|-----------|
| 6.6 | Homogenizers, blenders, mixers and shakers | 26 |
| 6.6.1 | Homogenizers and blenders..... | 26 |
| 6.6.2 | Vortex mixers | 27 |
| 6.7 | Stills, deionizers and reverse-osmosis units | 28 |
| 6.7.1 | Description..... | 28 |
| 6.7.2 | Use..... | 28 |
| 6.7.3 | Maintenance..... | 28 |
| 6.7.4 | Verification..... | 28 |
| 6.8 | Separation and concentration equipment..... | 28 |
| 6.8.1 | Immunomagnetic separator (IMS)..... | 28 |
| 6.8.2 | Centrifuge..... | 29 |
| 6.8.3 | Filtration systems..... | 29 |
| 6.9 | Modified atmosphere equipment..... | 29 |
| 6.9.1 | Description..... | 29 |
| 6.9.2 | Use..... | 29 |
| 6.9.3 | Maintenance..... | 30 |
| 6.9.4 | Verification..... | 30 |
| 6.10 | Other equipment..... | 30 |
| 6.10.1 | pH meter..... | 30 |
| 6.10.2 | Colony-counting device..... | 31 |
| 6.10.3 | Timers and timing devices..... | 31 |
| 6.10.4 | Optical microscope..... | 32 |
| 6.10.5 | Glass washers, glassware and other laboratory ware..... | 32 |
| 6.10.6 | Disposable equipment and consumables..... | 33 |
| 6.10.7 | Other equipment and software..... | 34 |
| 7 | Sterilization/decontamination and disposal of laboratory materials..... | 34 |
| 7.1 | Sterilization..... | 34 |
| 7.1.1 | General..... | 34 |
| 7.1.2 | Sterilization by dry heat..... | 34 |
| 7.1.3 | Sterilization by moist heat (steam)..... | 34 |
| 7.2 | Decontamination and disinfection..... | 34 |
| 7.2.1 | Decontamination of glassware and materials before use..... | 34 |
| 7.2.2 | Decontamination of glassware and materials after use..... | 34 |
| 7.3 | Waste management..... | 35 |
| 7.4 | Washing..... | 35 |
| 8 | Preparation and use of culture media and reagents..... | 35 |
| 9 | Laboratory samples..... | 36 |
| 9.1 | Sampling techniques and sampling plans..... | 36 |
| 9.1.1 | General..... | 36 |
| 9.1.2 | Sampling..... | 36 |
| 9.2 | Sample transport..... | 36 |
| 9.3 | Sample receipt..... | 37 |
| 9.4 | Sample handling..... | 37 |
| 9.4.1 | General..... | 37 |
| 9.4.2 | Storage before examination..... | 38 |
| 9.4.3 | Test portions..... | 38 |
| 9.4.4 | Storage of laboratory samples after examination..... | 38 |
| 9.5 | Pre-testing of samples..... | 38 |
| 10 | Examination..... | 39 |
| 10.1 | Hygienic precautions during sample preparation and examination..... | 39 |
| 10.1.1 | General..... | 39 |
| 10.1.2 | Basic precautions..... | 39 |
| 10.1.3 | Sample handling..... | 39 |
| 10.1.4 | Sample handling tools and implements..... | 40 |
| 10.1.5 | Spillages..... | 40 |
| 10.1.6 | Process controls..... | 40 |
| 10.1.7 | Aerosols..... | 40 |

| | | |
|-----------|--|-----------|
| 10.1.8 | Molecular methods..... | 41 |
| 10.2 | Preparation of initial suspension and dilutions..... | 41 |
| 10.2.1 | General..... | 41 |
| 10.2.2 | Concentration..... | 41 |
| 11 | Enumeration (quantitative) methods..... | 41 |
| 11.1 | General..... | 41 |
| 11.2 | Enumeration using a solid medium..... | 42 |
| 11.2.1 | General..... | 42 |
| 11.2.2 | Pour plate technique..... | 42 |
| 11.2.3 | Surface plating techniques..... | 43 |
| 11.2.4 | Enumeration of yeasts and moulds..... | 44 |
| 11.2.5 | Incubation..... | 45 |
| 11.2.6 | Calculation and expression of results obtained with solid culture media..... | 45 |
| 11.2.7 | Calculations for enumeration methods..... | 47 |
| 11.3 | Enumeration using liquid media..... | 54 |
| 11.3.1 | Principle..... | 54 |
| 11.3.2 | General MPN procedure..... | 54 |
| 11.3.3 | Limitations of MPN..... | 54 |
| 11.3.4 | Inoculation procedure..... | 55 |
| 11.3.5 | Choice of MPN configuration..... | 55 |
| 11.3.6 | Incubation..... | 56 |
| 11.3.7 | Interpretation and expression of results..... | 56 |
| 11.3.8 | Determination of MPN values using MPN calculators..... | 56 |
| 11.3.9 | Rarity categories..... | 57 |
| 11.4 | Estimates of uncertainty of test results..... | 57 |
| 12 | Detection (qualitative) methods..... | 58 |
| 12.1 | General..... | 58 |
| 12.2 | Principle..... | 58 |
| 13 | Confirmation and identification methods..... | 58 |
| 13.1 | General..... | 58 |
| 13.2 | Preparation of a pure culture..... | 59 |
| 13.3 | Confirmation methods..... | 59 |
| 13.3.1 | Latex agglutination test..... | 59 |
| 13.3.2 | Nucleic acid hybridization or molecular amplification methods..... | 59 |
| 13.3.3 | Slide agglutination tests..... | 60 |
| 13.4 | Identification methods..... | 60 |
| 13.4.1 | Biochemical galleries..... | 60 |
| 13.4.2 | DNA sequencing..... | 60 |
| 13.4.3 | Mass spectrometry..... | 61 |
| 14 | Selection and characterization of control microorganisms..... | 61 |
| 14.1 | General..... | 61 |
| 14.2 | Characterization of microorganisms..... | 62 |
| 14.2.1 | General..... | 62 |
| 14.2.2 | Phenotypic characterization..... | 62 |
| 14.2.3 | Molecular characterization..... | 62 |
| 14.3 | Selection of control microorganisms..... | 62 |
| 15 | Test report..... | 63 |
| 16 | Laboratory quality control in microbiology..... | 64 |
| 16.1 | General..... | 64 |
| 16.2 | Internal quality control..... | 65 |
| 16.2.1 | General..... | 65 |
| 16.2.2 | Process controls..... | 65 |
| 16.2.3 | Replicate testing..... | 66 |
| 16.2.4 | Spiked samples..... | 66 |
| 16.2.5 | IQC assessment using control charts..... | 66 |
| 16.3 | External quality assessment..... | 66 |

| | | |
|---------------------|--|-----------|
| 17 | Validation and verification of microbiological methods | 67 |
| 17.1 | General..... | 67 |
| 17.2 | Performance characteristics..... | 67 |
| 17.3 | Validation..... | 67 |
| 17.4 | Verification..... | 68 |
| Annex A | (informative) Properties of disinfectants | 69 |
| Annex B | (informative) Confidence intervals for colony count technique | 70 |
| Annex C | (normative) General confirmation tests | 73 |
| Bibliography | | 78 |

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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 ISO/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 fourth edition cancels and replaces the third edition (ISO 7218:2007), which has been technically revised. It also incorporates the Amendment ISO 7218:2007/Amd 1:2013.

The main changes are as follows:

- the calculations section has been simplified and two further calculators have been added;
- the equipment section has been reorganized into groups with similar purposes and requirements;
- cross-references have been added to other general microbiology standards such as those for media, validation and verification, and uncertainty to reduce repetition;
- information on laboratory quality control and characterization of control microorganisms has 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

When conducting microbiological examinations, it is especially important that:

- only those microorganisms present in the samples are detected and/or enumerated;
- these microorganisms do not contaminate the environment.

To achieve this, good laboratory practices are essential, including personal hygiene and aseptic working techniques which exclude extraneous contamination as far as possible.

Only limited information on the precautions to be taken during microbiological examinations is given in this document, so a thorough knowledge of the microbiological techniques and microorganisms involved is essential. It is important that examinations are conducted safely, correctly and as carefully as possible, including monitoring and recording aspects that can affect results, calculating numbers of microorganisms and assessing the uncertainty of test results.

The most common risks and their control in the microbiological laboratory are given in this document. However, work processes in each laboratory can differ and appropriate risk analysis should be considered to ensure good laboratory practices. Periodic evaluation and control of critical points not only maintains safe and hygienic practices but can also improve reliability of test results.

The purpose of this document is to help to ensure the validity of microbiology examinations in the food chain. In particular, to ensure that general techniques for conducting examinations are the same in all laboratories, to achieve consistent results in different laboratories and to contribute to safety of laboratory personnel by preventing risks of infection.

This document includes the main measures necessary for conducting the wide range of microbiological examinations. Additional information is available from the literature listed in the Bibliography (see References [43] to [47]).

In this document, the following verbal forms are used:

- “shall” indicates a requirement;
- “should” indicates a recommendation;
- “may” indicates a permission;
- “can” indicates a possibility or a capability.

In addition, the imperative mood is used to give instructions or where actions are required.

Microbiology of the food chain — General requirements and guidance for microbiological examinations

1 Scope

This document specifies general requirements and gives guidance on microbiological examinations.

It is applicable to:

- the implementation of specific horizontal or vertical International Standards developed by ISO/TC 34/SC 9 or ISO/TC 34/SC 5 for detection or enumeration of microorganisms, named hereafter “specific standards”;
- good laboratory practices for microbiology laboratories testing samples from the food chain;
- guidance for microbiological laboratories testing samples from the food chain on the technical requirements for conforming to ISO/IEC 17025.

The requirements of this general standard supersede corresponding ones in existing specific standards.

Additional instructions for examinations using the polymerase chain reaction (PCR) are specified in ISO 22174.

This document is applicable to examinations for bacteria, yeasts and moulds and can be used, if supplemented with specific guidance, for parasites and viruses. It does not apply to examinations for toxins or other metabolites (e.g. amines) from microorganisms.

This document is applicable to microbiology of the food chain, from primary production stage to food and animal feed products, including the premises where the food or feed production and handling takes place. It is also applicable to the microbiological examination of water where water is used in food production or is regarded as a food in national legislation.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

food chain

sequence of the stages in the production, processing, distribution, storage and handling of a *food* (3.2) and its ingredients, from primary production to consumption

Note 1 to entry: The food chain includes the environment of primary production, food and feed production, and handling.

Note 2 to entry: The food chain also includes the packaging materials intended to come into contact with food, *feed* (3.3) or raw materials.