# SLOVENSKI STANDARD
# SIST EN ISO 23691:2026

**01-april-2026**

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**Mikrobiologija v prehranski verigi - Ugotavljanje in uporaba kardinalnih vrednosti (ISO 23691:2026)**

Microbiology of the food chain - Determination and use of cardinal values (ISO 23691:2026)

Mikrobiologie der Lebensmittelkette - Bestimmung und Verwendung von Kardinalwerten (ISO 23691:2026)

Microbiologie de la chaîne alimentaire - Détermination et utilisation des valeurs cardinales (ISO 23691:2026)



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EUROPEAN STANDARD

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EUROPÄISCHE NORM

# EN ISO 23691

February 2026

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English Version

# Microbiology of the food chain - Determination and use of cardinal values (ISO 23691:2026)

Microbiologie de la chaîne alimentaire - Détermination et utilisation des valeurs cardinales (ISO 23691:2026)

Mikrobiologie der Lebensmittelkette - Bestimmung und Verwendung von Kardinalwerten (ISO 23691:2026)

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## European foreword

This document (EN ISO 23691:2026) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 “Microbiology of the food chain” the secretariat of which is held by AFNOR.

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## Endorsement notice

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International Standard

ISO 23691

# Microbiology of the food chain — Determination and use of cardinal values

*Microbiologie de la chaîne alimentaire — Détermination et utilisation des valeurs cardinales*

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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.

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 document 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).

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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).



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

Under the general principles of the Codex Alimentarius on food hygiene, it is the responsibility of the food business operators (FBOs) to control microbiological hazards in foods and to manage microbial risks. Therefore, it is the responsibility of the FBO to implement validated control measures, within the hazard analysis and critical control point (HACCP) system, and conduct studies in order to investigate compliance with the food safety criteria throughout the food chain.

In the framework of microbial risk assessment (MRA), several complementary approaches are developed to estimate risks posed by pathogens or spoilage microorganisms in the food chain. MRA is adopted by regulators under the auspices of the international agency for setting food standards. Predictive microbiology is one of the recognized scientific approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and quality of food, food production processes, food storage conditions and food preparation recommendations dedicated to consumers.

Therefore, this document provides technical rules, procedures and calculations to estimate the cardinal values of a microorganism of concern and use them in combination with challenge test results to simulate and predict its growth in raw materials, intermediate products or end products under reasonably foreseeable food processes, storage and use conditions.

To do so, this document includes the following sections:

— to identify the environmental factor(s) in scope (e.g. temperature, *pH*, $a_w$, organic acids);

— to define the appropriate experimental design;

— to estimate the cardinal values of a microorganism in broth medium;

— to perform a challenge test in the matrix of interest and derive the food correction factor and the maximum microbial population density;

— to use the cardinal values and the food correction factor to predict the growth of the studied microorganism in different conditions of interest (e.g. changes in time and temperature throughout the chill chain, changes in formulation with addition of organic acids or preservatives).



Regulatory authorities can have specific recommendations, and these differences have been included as much as possible in this document. It is, however, possible that additional requirements are needed to get a regulatory approval of the study.

The use of this document involves expertise from the organizing laboratories in relevant fields such as food microbiology, predictive microbiology and statistics. This expertise encompasses an understanding of sampling theory and design of experiments, statistical analysis of microbiological data, and overview of scientifically recognized and available mathematical concepts used in predictive microbiology.

# Microbiology of the food chain — Determination and use of cardinal values

**WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting target microorganism(s) are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.**

## 1 Scope

This document establishes basic principles and specifies requirements and methods to determine the cardinal values of bacteria and yeast strains and use them to predict microbial growth.

The four main steps of the approach are:

a) determination of the cardinal values in culture medium;

b) determination of the correction factor in the target food;

c) validation of the model;

d) simulations.



Four environmental factors are considered: temperature, *pH*, $a_w$ and inhibitors (e.g. organic acids).



NOTE 1 Microbial competition is not considered as an inhibitor in this document and can be addressed by proper modelling approaches.

The determination of cardinal values is performed in a two-step approach:

— the determination of maximum specific growth rates of the studied strain grown in broth under a defined range of values of the studied environmental factor(s);

— the use of recognized predictive microbiology secondary models to fit the obtained experimental data to obtain the cardinal values.

The use of cardinal values in microbial growth simulation is based on predictive microbiology primary and secondary models. The cardinal values are combined with challenge test data to consider the matrix effect. Depending on the goal of the growth simulation, it is important to account for variation of cardinal values between strains within a bacterial or yeast species.

Cardinal values are a good indicator of a strain growth ability for the studied environmental factors. They are therefore used as criteria to select strains, in addition to their origin and virulence, when performing growth challenge tests (see ISO 20976-1) or in methods validation (see ISO 16140 series).

NOTE 2 This document focuses on the determination of cardinal values for one strain. The same methodology can be used to characterize multiple strains independently to cover biological strain variability and include these results in the predictions.

## 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 7218, *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 18787, *Foodstuffs — Determination of water activity*

ISO 20976-1:2019, *Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products — Part 1: Challenge tests to study growth potential, lag time and maximum growth rate*

## 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**
**batch**
group or set of identifiable food obtained through a given process under practically identical circumstances and produced in a given place within one defined production period

Note 1 to entry: The batch is determined by parameters established beforehand by the organization and can be described by other terms (e.g. lot).

[SOURCE: Commission Regulation (EC) No 2073/2005[33], Article 2 (e), modified — "food obtained through" replaced "products obtained from". Note 1 to entry added.]

**3.2**
**binary dilution optical-density-based method**
**binary dilution OD-based method**
method used to stepwise dilute a microbial suspension with a constant dilution factor of two in each step

**3.3**
**independent biological replicate**
experiment performed using a newly prepared culture and a newly prepared medium

**3.4**
**cardinal value**
cardinal parameter
estimated minimum, optimum or maximum values of *extrinsic factors* (3.10) and *intrinsic factors* (3.15) (e.g. temperature, *pH*, $a_w$, inhibitors) that characterize the growth of a given microbial strain

**3.5**
**challenge test**
study of the growth (or inactivation) of microorganism(s) artificially inoculated in food

**3.6**
**coefficient of variation**
$C_V$
ratio of the standard deviation to the mean

**3.7**
**correction factor**
$C_f$
dimensionless value used to link the broth and the food *optimum growth rates* (3.22)

Note 1 to entry: It is the ratio of the optimum growth rate estimated in the studied matrix ( $\dot{\mu}_{Food}$ ) to the optimum growth rate value estimated in broth ( $\dot{\mu}_{Broth}$ ).

**3.8**
**detection time**
$t_d$
time at which the optical density (OD) reaches the pre-defined target during the exponential growth

**3.9**
**exponential growth phase**
phase during which the multiplication of the microbial population is the fastest and when the *maximum specific growth rate* (3.18) is reached

**3.10**
**extrinsic factor**
factor in the surrounding environment of the food or the broth, such as temperature or packaging gaseous composition, which affects the growth kinetics of the microorganism

**3.11**
**gamma concept**
$\gamma$
concept establishing that *intrinsic factors* (3.15) (e.g. *pH*, *water activity* (3.36), inhibitors) and *extrinsic factors* (3.10) (e.g. temperature, packaging gaseous composition) affect the *maximum specific growth rate* (3.18) independently



**3.12**
**gamma function**
$\gamma$ (X)

nonlinear, dimensionless function, normalized between zero (no growth) and one (optimum condition for growth) describing the relative effect of a studied factor (X) on the *maximum specific growth rate* (3.18) (e.g. $\gamma(T)$, $\gamma(pH)$, $\gamma(a_w)$, $\gamma(I)$)

Note 1 to entry: When combined, the effect of the factors is multiplicative.

**3.13**
**growth curve**
graphic representation of the increasing number of living cells of a microbial population in any given intrinsic and extrinsic condition over a period of time

**3.14**
**inoculum**
microbial suspension used to contaminate the studied food or broth at a desired concentration

**3.15**
**intrinsic factor**
factor related to the food matrix itself or the broth, such as nutrients, *water activity* (3.36), organic acids or *pH*, which affects the growth kinetics of the microorganism

**3.16**
**lag phase**
phase, directly after inoculation, during which the microbial population is adapting to the environment, before it enters the *exponential growth phase* (3.9)