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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

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Microbiologie de la chaîne alimentaire — Exigences et lignes directrices pour la réalisation des tests d'épreuve microbiologique — Partie 1: Tests de croissance pour étudier le potentiel de croissance, le temps de latence et le taux de croissance maximal

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Foreword

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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).

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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.

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Introduction

Under the general principles of the Codex Alimentarius on food hygiene, it is the responsibility of food business operators (FBOs) to control microbiological hazards in foods and to manage microbial risks. Therefore, FBOs implement validated control measures[11] 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. Challenge testing is one of the recognized 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 for consumers.

This document provides technical rules, calculations and approaches to investigate the ability of inoculated microorganism(s) of concern to grow, survive or be inactivated in raw materials and intermediate or end products under reasonably foreseeable food processes, storage and use conditions. The objective and the scope of the document are to determine the experimental design and the selection of the study conditions. Regulatory authorities can have different recommendations, and these differences have been included as much as possible. It is, however, possible that specific requirements should be incorporated to get regulatory approval of the challenge test.

As growth and inactivation kinetics are clearly different, the ISO 20976 series consists of two parts, under the general title, *Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products*:

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— *Part 1: Challenge tests to study growth potential, lag time and maximum growth rate*

— *Part 2: Challenge tests to study inactivation potential and kinetics parameters* (to be developed)

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The use of the ISO 20976 series involves expertise in relevant areas, such as food microbiology, food science, food processing and statistics. The statistical 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 modelling. Even though many mathematical models are available to describe and predict bacterial growth, the gamma-concept (γ-concept)[22] is particularly useful for further simulations using the data generated from the challenge test, e.g. to assess the growth at storage temperatures other than the one(s) tested, or in helping to design better food formulations and storage conditions, and thus improving the microbial quality and/or safety of the food under consideration.

For practical reasons, the term “food” includes feed.

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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

1 Scope

This document specifies protocols for conducting microbiological challenge tests for growth studies on vegetative and spore-forming bacteria in raw materials and intermediate or end products.

The use of this document can be extended to yeasts that do not form mycelium.

2 Normative references

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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.

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ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

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

ISO 18787:2017, *Foodstuffs — Determination of water activity*

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
bacterial spore
resistant form of bacteria that is dormant until the *germination* (3.9) step

3.2
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 may be described by other terms, e.g. lot.

[SOURCE: Commission Regulation (EC) No 2073/2005]

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3.3
cardinal value
estimated minimal, optimal and maximal values of physico-chemical factors (e.g. temperature, pH, a_w) that characterize the growth of a given microbial strain

3.4
control unit
unit of food identical to the *test unit* (3.24) but not artificially contaminated (used as a blank)

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

3.6
experimental datapoint
result of analysis of a *test unit* (3.24) per unit weight ($\log_{10}$ cfu/g), per unit volume ($\log_{10}$ cfu/ml), or per unit area ($\log_{10}$ cfu/cm^2)

Note 1 to entry: For specific cases, the enumeration results may be expressed in $\log_{10}$ MPN.

3.7
exponential growth phase
phase in which the microbial population is exponentially multiplying as rapidly as possible; growth is dependent on the growth medium and environment (temperature, humidity, etc.)

Note 1 to entry: Figure 1 describes the three phases of microbial growth kinetics.

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3.8
generation time
Tg
time it takes for the microorganisms to increase by a factor 2, also known as doubling time

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3.9
germination
mechanism in which a *bacterial spore* (3.1) starts becoming a *vegetative cell* (3.25)

3.10
growth potential
Δ
difference between the decimal logarithm of the highest concentration of the target microbial population ($\log_{max}$) and the decimal logarithm of the initial concentration of this microbial population ($\log_i$)

Note 1 to entry: The $\log_{max}$ and $\log_i$ refer to concentrations and are expressed in $\log_{10}$ cfu/g or $\log_{10}$ cfu/ml or $\log_{10}$ cfu/cm^2

3.11
maximum growth rate
kinetics parameter to characterize the *exponential growth phase* (3.7), represented by the slope of the curve showing the evolution of the natural logarithm (μ_{max}) or decimal logarithm (V_{max}) of the population as a function of time, under constant growth conditions

3.12
inoculum
microbial suspension at the desired concentration used to contaminate *test units* (3.24)

3.13
lag phase
phase, directly after inoculation, during which the microbial population is adapting to the environment, before it enters the *exponential growth phase* (3.7)

Note 1 to entry: Figure 1 describes the three phases of microbial growth kinetics.

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3.14
lag time
λ
kinetics parameter in time unit to characterize the *lag phase* (3.13)

3.15
pH value
measure of the concentration of acidity or alkalinity of a material in an aqueous solution

[SOURCE: ISO 5127:2017, 3.12.2.29, modified — Notes 1 and 2 to entry have been removed.]

3.16
primary model
mathematical model describing the changes of microbial counts as a function of time

3.17
organizing laboratory
laboratory with responsibility for managing the *challenge tests* (3.5)

3.18
sampling
selection of one or more units or portions of food such that the units or portions selected are representative of that food

3.19
sampling point
time at which the *test units* (3.24) are analysed and which are represented as *experimental datapoints* (3.6) on the kinetics graph

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3.20
secondary model
mathematical model describing the effects of the environmental factors (e.g. temperature, pH, a_w) on the parameters of the *primary model* (3.16) (e.g. growth rate)

3.21
sporulation
mechanism by which *vegetative cell* (3.25) forms spore

3.22
stationary phase
phase in which the microbial population is at its maximum level

Note 1 to entry: Figure 1 describes the three phases of microbial growth kinetics.

3.23
test portion
measured (volume or mass) representative sample taken from the *test unit* (3.24) for use in the analysis

[SOURCE: ISO 6887-1:2017, 3.5, modified — The end of the definition has been changed from “taken from the laboratory sample for use in the preparation of the initial suspension” and the Note 1 to entry has been removed.]

3.24
test unit
measured (volume or mass) amount of the food used for inoculation

3.25
vegetative cell
state of microbial form that is capable of growing under favourable environmental conditions

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3.26
water activity
a_w
ratio of the water-vapour pressure in the foodstuff to the vapour pressure of pure water at the same temperature

[SOURCE: ISO 18787:2017, 3.1, modified — The definition has been condensed and the formula and Notes 1 and 2 to entry have been removed.]

4 Principle

4.1 General

The aim of the study shall be clearly defined (e.g. assessment/validation of the food shelf-life as a control measure, assessment of microbial stability). The experimental design shall be in accordance with that purpose and shall take into account the steps of the food chain for which microbial growth is assessed. The decision criteria shall be clearly defined (see 7.2).

Knowledge from the FBO on its products (e.g. characteristics or production process) shall be combined with expertise in food microbiology and analytical sciences to ensure the robustness of the study. The organizing laboratory shall have knowledge and skills in food microbiology, food science and technology, and statistics to design and conduct the studies, interpret the results and draw the conclusions. The analyses shall be conducted under a quality assurance system (e.g. in accordance with ISO/IEC 17025).

Challenge tests aim at studying the growth potential or growth kinetics (lag time and maximum growth rate) in order to assess, for example, the food shelf-life as a control measure or the microbial stability of a food.

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Growth potential studies are most appropriate to:

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— validate the microbiological shelf-life of a food under reasonably foreseeable conditions of use and storage between production and consumption, ensuring relevant microbiological criteria are met throughout the product shelf-life;

— assess if a product, tested under specific conditions, supports the growth of the inoculated microorganism.

Such challenge tests will only validate the specific food characteristics and conditions applied for the study. When microbiological criteria are not fulfilled or conditions (e.g. food formulation, physico-chemical characteristics, type and/or concentration of preservatives added, packaging, storage temperature) are changed, a new growth potential study needs to be carried out in order to validate the new conditions.

Growth kinetics studies are most appropriate for:

— assessing the effect(s) of intrinsic (e.g. pH, a_w, preservatives) and extrinsic characteristics (e.g. gas composition, temperature) that have a significant impact on the behaviour of the target microorganism;

— providing data for developed models to simulate the effect of such factors on microbial behaviour in the studied food under reasonably foreseeable storage conditions (time and temperature);

— comparing the simulation results to ensure that relevant microbiological criteria are met throughout the food shelf-life.

Growth kinetics studies are used to estimate and validate the microbiological shelf-life of a food. They are particularly suitable in the last steps of the food development, including reformulation, new packaging, and alternative processing conditions.

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A growth kinetics study can be more informative than a growth potential study. However, growth kinetics studies are more complex in terms of study design, execution, results interpretation and exploitation, particularly in cases where various factors are included.

The behaviour of a microbial population in a food, i.e. microbial growth kinetics, is dependent on the characteristics of the food (e.g. a_w, pH, preservatives concentrations), the food storage conditions (temperature, packaging format and gas composition), the food processes, the physiological state of the microorganism and interactions with the natural background microorganisms.

Microbial growth kinetics are defined by three major phases (see Figure 1).

a) Lag phase: This phase is characterized by the lag time (λ), which corresponds to the intersection between the exponential growth phase line (plotted in semi-log coordinates) and the horizontal line crossing through the initial cell concentration[15][18]. For spore-forming microorganisms, lag time includes spore germination and outgrowth.

   Lag time is dependent on the food characteristics (e.g. physico-chemical and microbiological), inoculation levels and storage conditions (e.g. temperature, relative humidity, gas composition). Lag time is also dependent on the physiological state of the microorganism contaminating the food and any stress experienced by these cells or spores.

b) Exponential growth phase: This phase is characterized by the growth rate (μ_{max} or V_{max}), which corresponds to the maximum increase in natural or decimal logarithm of cell number per unit of time.

   The growth rate corresponds to the slope of the curve showing the evolution of the natural logarithm (μ_{max}) or decimal logarithm (V_{max}) of the population over time during the exponential phase. The food characteristics (e.g. physico-chemical and microbiological) and storage conditions (e.g. temperature, relative humidity, gas composition) can significantly influence microbial growth rates. The growth rate of a microbial population is unaffected by its initial concentration and physiological states.

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   The relationship between the generation time (Tg) and μ_{max} is given by Formula (1):

$$\mu_{max} = \ln(2) / Tg \quad (1)$$

   The slope of the curve plotting the $\log_{10}$ of the microbial population against time, V_{max}, and its relationship to maximum growth rate is given by Formula (2):

$$\mu_{max} = V_{max} \cdot \ln(10) \quad (2)$$

c) Stationary phase: In this phase, the microbial population is at its maximum level.

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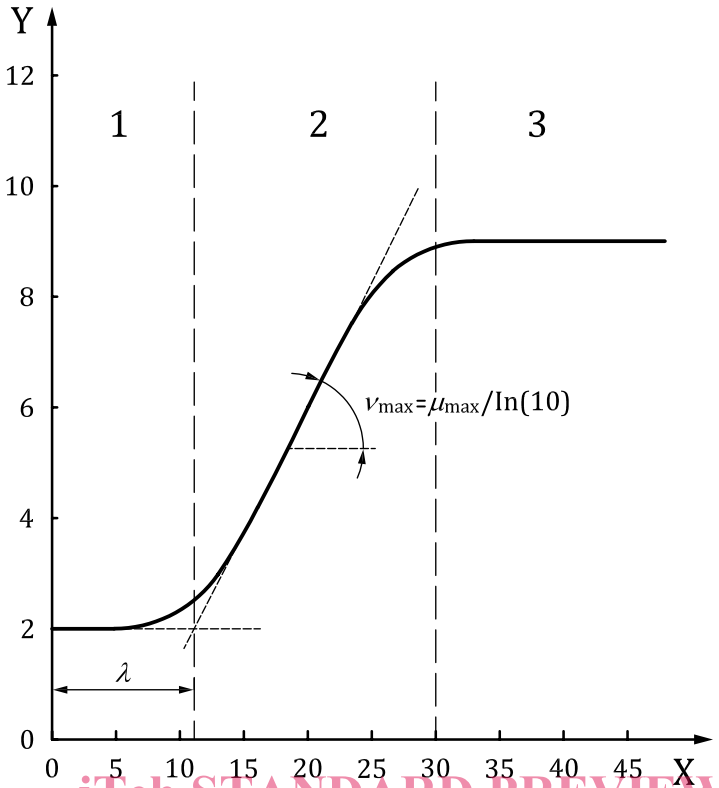

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Key

Y $\log_{10}$ (cfu/g)
X time (days)
1 lag phase
2 exponential growth phase
3 stationary phase

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Figure 1 — Microbial growth kinetics with three major phases

4.2 Estimation of the growth potential

The food characteristics (e.g. physico-chemical and microbiological) and storage conditions (e.g. temperature, relative humidity, gas composition) can significantly influence the microbial growth potential.

The inoculum shall be adapted to conditions that mimic the microbial cell or spore injury induced by food handling/processing or any phenomena that can trigger subsequent adaptive responses to growth conditions, in order to mimic natural microbial behaviour in the food.

This type of test is designed to estimate the changes in concentration of the microbial population during the challenge test. These tests can be used to determine whether there is significant microbial growth in a foodstuff and to quantify the increase in the microbial population under a given set of storage conditions.

It is important to have a minimum of five sampling points that are evenly distributed across the entire shelf-life, to get an accurate estimation of the growth potential (see 14.2).

Growth potential does not provide information on the length of the lag phase, growth rate value or maximum stationary-phase level. This makes the growth potential unsuited for extrapolating the results to other conditions.

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