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Microbiology of the food chain — Specific requirements and guidance for proficiency testing by interlaboratory comparison

Microbiologie de la chaîne alimentaire — Exigences spécifiques et recommandations relatives aux essais d'aptitude par comparaison interlaboratoires

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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.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition cancels and replaces ISO/TS 22117:2010, which has been technically revised. The following changes have been made:

— updates have been made to align the document with ISO 13528:2015.

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

General requirements for organization of proficiency testing (PT) schemes of all types are given through ISO/CASCO (Committee on Conformity Assessment) in ISO/IEC 17043. Additionally, general guidance is available from the International Union of Pure and Applied Chemistry (IUPAC), see Reference [12]. However, these recommendations may not be directly applicable to all cases and should be interpreted specifically for different laboratory sectors where PT schemes are organized. For this reason, a document is needed to establish the criteria for a provider (and associated collaborators) of PT schemes for microbiological examinations to meet and be recognized as competent. This applies particularly to the specific technical requirements necessary to deal with microorganisms, such as sample homogeneity and stability, as well as with the interpretation of detection tests which is not covered by an existing document.

PT schemes for microbiology laboratories are mainly used to evaluate performance, particularly trueness (bias) and in some cases precision, of food microbiological examinations in specific laboratories.

Additionally, data from such PT schemes can be used:

a) to provide information to the organizations responsible for laboratory acceptance within an official control framework and to allow continuous monitoring;

b) to aid laboratory accreditation in a general framework of quality management;

c) to inform those responsible for quality in the participating laboratories as part of the educative elements of external quality assessment of trueness (bias).

Information from PT schemes may also be used for:

— identification of the possible sources of errors, particularly the bias component of uncertainty, to improve performance;

— estimation of uncertainty of test results, in conjunction with routine results, for quantitative (enumeration) methods (see ISO/TS 19036) and levels of detection for qualitative (detection) methods;

— demonstration of staff competence to perform a specific microbiological examination;

— evaluation or validation of a given method by the study of trueness, precision and robustness;

— identification of variability in test results between individual laboratories;

— assignment of a “target” value for a microorganism in a material in order to establish a reference material (see ISO 17034).

However, these aspects are not specifically covered in this document.

PT schemes are therefore designed to meet certain criteria and the testing programme (frequency, number of samples, number of repeats, etc.) to meet the requirements of the type of method used and commodity tested, to achieve the level of control required by all parties.

Microbiology of the food chain — Specific requirements and guidance for proficiency testing by interlaboratory comparison

1 Scope

This document specifies requirements and gives guidelines for the organization of proficiency testing (PT) schemes for microbiological examinations of

a) foods and beverages,

b) feeding animals,

c) environmental samples from food and feed production and handling, and

d) primary production stages.

This document is also applicable to the microbiological examination of water where water is either used in food production or is regarded as a food in national legislation.

This document relates to the technical organization and implementation of PT schemes, as well as the statistical treatment of results of microbiological examinations.

This document is designed for use with ISO/IEC 17043 and ISO 13528, and deals only with areas where specific or additional details are necessary for PT schemes dealing with microbiological examinations for the areas specified in the first paragraph.

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 3534-1, *Statistics — Vocabulary and symbols — Part 1: General statistical terms and terms used in probability*

ISO 3534-2, *Statistics — Vocabulary and symbols — Part 2: Applied statistics*

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 13528:2015, *Statistical methods for use in proficiency testing by interlaboratory comparison*

ISO/IEC 17043:2010, *Conformity assessment — General requirements for proficiency testing*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 3534-1, ISO 3534-2, ISO 5725-1, ISO 13528, ISO/IEC 17043 and the following 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/

NOTE 1 Some terms used in the text have different meanings in microbiology and statistics, e.g. homogeneity, heterogeneity, test, sample, distribution. The context clarifies whether the terms refer to microbiological test samples or data sets used for statistical analysis.

NOTE 2 Some providers of proficiency testing use the term “external quality assessment” (EQA) to indicate schemes with broader application to all areas of operation of a laboratory and a particular educational remit. The requirements of this document cover those EQA activities that meet the definition of proficiency testing.

3.1
target organism
microorganism that is the designated analyte for a proficiency testing sample

3.2
background flora
microorganisms included in a proficiency testing sample that are naturally present or can be introduced to compete with or mimic the target microorganism

3.3
matrix
all the components of the sample

[SOURCE: ISO 16140-1:2016, 2.38, modified — In the term, “(product)” has been removed.]

3.4
reference strain
microorganism obtained directly from an official culture collection or reference laboratory and defined to at least the genus and species level, catalogued and described according to its characteristics and preferably originating from food, food production areas, primary production stages, animals or water, as applicable

[SOURCE: ISO 11133:2014, 3.4.2, modified — In the term, “an official culture collection or reference laboratory” has replaced “a reference culture collection, i.e. a culture collection, which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections’ Organisation (ECCO)”, and “food production areas, primary production stages, animals" has replaced “animal feed, the food or feed production environment”.]

3.5
recovery percentage
proportion of the assigned value of the *target organism* (3.1) recovered by the participant

Note 1 to entry: The recovery percentage is calculated by multiplying by 100 the number of recovered colony-forming units (cfu) per volume or per mass and dividing by the assigned value.

Note 2 to entry: The recovery percentage can be significantly below 100 % when competitive microflora and matrix effects are present in a proficiency testing sample.

4 Scheme design and purpose

4.1 General

General requirements for designing PT schemes are given in ISO/IEC 17043. This clause discusses areas requiring special consideration for microbiological PT schemes in the context of these general principles.

4.2 Scheme objectives

The primary objective of any PT scheme is to provide information to enable laboratories to have confidence in the reliability of their results.

The detailed requirements for a documented plan of a PT scheme are covered in ISO/IEC 17043:2010, 4.4.1.3, and the plan should also include reference to any relevant legislation. An example of a plan for a typical microbiology food examination scheme is given in Annex A.

The studies required to establish a new PT scheme are extensive and shall be clearly defined in the scheme objectives. These should include, as a minimum, the requirements listed in Clause 5. Requirements for checking individual rounds of testing, including homogeneity and stability testing, should also be established in the scheme design and be appropriate for the scheme objectives.

4.3 Laboratory requirements for schemes

General requirements for appropriate laboratory facilities to handle all aspects of PT schemes are given in ISO/IEC 17043:2010, 4.3.1, and safety requirements are covered in ISO/IEC 17043:2010, 4.6.2.4.

For microbiology schemes, providers shall have a documented policy to bring hazards to the attention of participants and ensure that relevant safety advice is given (see Clause 7). For example, food microbiology laboratories shall have facilities for dealing with microorganisms of biosafety levels 1 and 2, as appropriate (see ISO 7218).

4.4 Choice of test matrices

General requirements to document test matrices in the scheme plan are given in ISO/IEC 17043:2010, 4.4.1.3, and choice of the matrices to reflect routine sample types in ISO/IEC 17043:2010, 4.4.2.3.

The reasons for the choice of matrix type should be stated (e.g. to provide levels of sample stability and homogeneity that are fit for the intended purpose of the scheme).

The description of the test items shall specify the sample matrix (natural or simulated); whether artificially or naturally contaminated; the source and country of origin to comply with international transport regulations; and any method of preservation used (e.g. freeze-dried, air-dried).

4.5 Information on test methods used by the PT provider

The general requirements for methods to be used by the PT provider are given in ISO/IEC 17043:2010, 4.4.1.3.

If the scheme is targeted at one or more tests specified in or required by legislation, the routine quality control tests on the scheme samples (e.g. homogeneity and stability) shall be undertaken in accordance with the methods stipulated in that legislation and this shall be stated (ISO/IEC 17043:2010, 4.5.1).

Participants shall be encouraged to use their routine methods but, where they are undertaking tests in accordance with legislation, some degree of guidance shall be given, e.g. reference to ISO methods, legislative texts, or peer-reviewed publications (ISO/IEC 17043:2010, 4.5.1).

4.6 Statistical design

General requirements for statistical design are given in ISO/IEC 17043:2010, 4.4.4.

An outline of the statistical design for PT schemes for microbiology shall indicate that the statistical tests to be used are influenced by the level of homogeneity of the test material which, in turn, is influenced by the random variation in distribution of the microorganisms.

Except for low numbers, a log-normal distribution is usually expected in quantitative testing data and suitable statistical analysis methods shall be used for such data [ISO/IEC 17043:2010, B.3.1.4 d)]. Where low numbers are required in quantitative test items (e.g. water or beverage examination), a Poisson distribution is more applicable, as the variation in numbers of organisms between different units of material becomes relatively large and can mask variations in performance.

Sample homogeneity shall normally be sufficient, such that it does not significantly influence the observed variation between laboratories.

Semi-quantitative enumeration tests and qualitative detection tests require different statistical methods to analyse data and these are discussed further in 8.3 and 8.4.

The scheme plan shall clarify distinctions between performance testing for methods for detection and those for enumeration (or quantification for viruses) of target microorganisms.

5 Technical requirements and guidance for sample design and content

5.1 Sources, characterization and traceability of organisms

The characteristics of the target organisms shall be established before use to assess performance reliably, especially in schemes where participants may use different methodologies.

Target viruses shall give expected results when tested by reference methods. Target parasites can be identified by microscopy or molecular methods depending on their size and/or other characteristics.

Both typical and atypical strains of target bacteria should be considered and included in the scheme programme to challenge laboratory performance.

Recognized reference strains from international collections or reference laboratories should be used where they are most suitable for the scheme purpose; however, laboratory isolates or so-called "wild" strains isolated from the matrices used by PT schemes are useful to reflect routine situations more closely. Where these are used, they should be sufficiently characterized according to the appropriate International Standard reference methods, to ensure that any atypical reactions are apparent to the organizers before use.

NOTE Strains, particularly wild isolates, can adapt to culture media and environment unless the number of passages is kept to a minimum.

Spore suspensions can be used to inoculate samples intended to enumerate moulds as these help to improve stability and homogeneity. A method to prepare these is given in Annex B.

In all cases, the organisms used in PT scheme samples should be traceable to the relevant reference source or to valid characterization data held by the organizers.

Under certain circumstances, it is not possible to use reference cultures or materials from internationally recognized collections or cultured laboratory strains, for example, PT schemes for non-cultivable organisms such as human noroviruses or parasites. Naturally contaminated samples may be used, if available, or clinical material can be used to contaminate ("spike") a test matrix artificially, either through immersion, spraying or, in the case of bivalve shellfish, through bioaccumulation. The method of artificial contamination should be as close to the "natural" route of contamination as possible. Extreme care should be used when manipulating human clinical material, faecal or vomitus samples and these should be screened for additional pathogens before use.

For distributions to be used with serological or molecular methods, it may not be necessary to distribute live, or even whole, microorganisms. Use of inactivated microorganisms, target antigens or nucleic acid sequences will often be safer and these may be more stable. Stability of such materials should be determined by the scheme provider and, in all cases, the targets should give expected results in reference methods.

5.2 Target organisms level

The target organisms shall be provided at levels suitable to show that examination methods are fit-for-purpose and to reflect levels likely to be found in the sample matrices being tested (ISO/IEC 17043:2010, 4.4.2.3). Where pathogenic microorganisms are the target, the levels should also take account of and

reflect the levels likely to cause hazard to human health and, if appropriate, any limits specified in microbiological criteria.

NOTE The level causing hazard to human health is not always known with accuracy and depends on the susceptibility of individuals. The main aim of examination for all pathogenic organisms (bacteria, viruses, parasites) is to prevent illness, but also to detect pathogenic bacteria at a very low level, before they can grow to a higher level.

For quantitative (enumeration) methods, the target level shall be appropriate for the levels routinely found in, and any specifications applicable to, the sample matrices used. The target level should also sometimes be near to the limit of quantification of routine methods to challenge the performance of the participants across the applicable range of the method. However, samples should not be dispatched with organism levels so low that, when using routine methods and dilutions, the expected mean number of organisms in a sample is fewer than 10 colonies per plate or less than 1 MPN/g (< 100 MPN/g for bivalve molluscs).

For traditional qualitative (detection) methods, target bacteria shall be at a sufficiently low level to provide a valid challenge to the methods and contribute validation data for performance criteria or verify levels of detection for individual participant laboratories.

5.3 Non-target organisms and interferences

The total microflora of PT samples, either naturally or artificially contaminated, is usually chosen to assess the ability of participants to detect and/or enumerate target bacteria in the presence of background flora. This background flora can include non-target strains typical of the sample matrix and presumptive target organisms which, without appropriate confirmation tests, can lead to false-positive results. However, basic schemes intended for specific purposes may provide samples containing only the target bacteria.

Any strains added to matrices to simulate background flora shall meet the requirements of 5.1 for characterization and traceability.

Determine any adverse effects of the background flora of artificially contaminated samples on the target bacteria (e.g. inhibition or other interference) before such samples are used.

5.4 Matrix selection and effects

All matrices shall be evaluated before use to check for any effects on spiked target and background flora, for example, whether a matrix reduces the recovery percentage of the spiked organisms. It may be useful to include information for participants on food matrices known to affect recovery of microorganisms adversely (e.g. those which bind and retain cells, such as fatty materials) or have bactericidal or bacteriostatic properties.

Include suitable and validated (or verified) preparation procedures for the proficiency samples in the information for participants.

Sample matrices used for microbiology PT schemes are often, but not necessarily, sterilized before use. Alternatively, the absence of the target is checked by other means (e.g. use of special sources of matrices).

Where natural, unsterilized sample matrices are distributed, the organizers shall determine the effect of any background microflora on the target organisms before use. Also, absence of target organisms in natural samples is usually required if these are to be artificially spiked. For example, in a PT scheme to detect parasitic *Anisakidae* larvae in fish fillets, use only freshwater fish fillets as the larvae are found only in sea fish.

6 Sample verification by the provider

6.1 General

General requirements for sample verification are given in ISO/IEC 17043 and ISO 13528. This clause expands the specific requirements and any particular issues for homogeneity and stability testing in materials containing living microorganisms.

6.2 Sample homogeneity testing — General considerations

(See also ISO/IEC 17043:2010, 4.4.3 and B.5.)

Proficiency tests may involve the preparation of a bulk test material, which is then subdivided into individual portions, as similar as possible to each other, for distribution to participants. Alternatively, test portions may be individually inoculated for distribution.

Whatever preparation method is used, assess the test material for homogeneity, usually before but also at the time of testing for less stable fresh materials.

Perform a homogeneity test, based on relevant statistical principles (ISO/IEC 17043:2010, 4.4.3.2 and B.5), on each batch of samples. Such tests are given in ISO 13528 or, as an alternative, Annex C.

The number of samples to be tested from each batch should also be sufficient to obtain ongoing information on the homogeneity of the batch; 10 samples (tested in duplicate if appropriate) is suggested.

A test material that is less than sufficiently homogenous may still be used in a proficiency test round (ISO/IEC 17043:2010, 4.4.3.1, Note 3) provided suitable statistical principles are used to take account of the greater variance between samples (see ISO 13528). A statistical plan for such materials, including replicate analysis of several samples (see ISO 5725-5), should be used to minimize the effects of lack of homogeneity on the evaluation of participant performance.

For parasite detection, each test material is generally spiked with a known number of organisms and homogeneity checked by microscopy counts of each sample, by at least two operators, since homogeneous distribution of target parasites in bulk material is difficult to achieve.

For virus methods, the bacteriological terminology used in 6.3 and 6.4 may not be applicable, but similar principles apply to ensure sufficient homogeneity in distributed PT samples.

6.3 Homogeneity testing for quantitative (enumeration) samples

General requirements and procedures for testing homogeneity of quantitative proficiency test materials are given in ISO/IEC 17043:2010, 4.4.3 and B.5, and ISO 13528, but alternative procedures may sometimes be required for microbiological materials.

Materials that show between-unit variation large enough to affect the assessment of laboratory performance significantly should not be used in interlaboratory studies, unless special requirements and methods of data analysis apply (e.g. low numbers of microorganisms in drinking water and other samples).

The criterion for "sufficiently homogenous" is defined by the requirements of the interlaboratory comparison. However, in general, a material is considered sufficiently homogenous if the between-unit standard deviation (on the appropriately transformed scale) is $\leq 0{,}3\sigma_{pt}$, where σ_{pt} is the target standard deviation used to assess the performance of laboratories (see ISO 13528).

Any alternative homogeneity test should meet the following criteria:

a) the probability of rejecting a sufficiently homogenous test material should be ≤ 5 %;

