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Microbiology of food and animal feeding stuffs — Specific requirements and guidance for proficiency testing by interlaboratory comparison

Microbiologie des aliments — Exigences spécifiques et lignes directrices pour les essais d'aptitude par comparaison interlaboratoires

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Foreword

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 22117 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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 [9]) and the International Laboratory Accreditation Cooperation (ILAC, see Reference [8]). 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 which a provider (and associated collaborators) of PT schemes shall meet in order to be recognized as competent to provide PT schemes for microbiological analysis. This applies particularly to the specific technical requirements necessary to deal with living microorganisms, such as sample homogeneity and stability, as well as with the interpretation of presence/absence (detection) tests which is not covered by an existing document.

Proficiency testing 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:

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

2) estimation of measurement uncertainty for enumeration methods (see ISO/TS 19036[6]) and limits of detection for presence/absence methods;

3) demonstration of staff competence to perform a specific microbiological examination;

4) evaluation or validation of a given method by the study of trueness and precision;

5) identification of variability between individual laboratories;

6) assignment of a “target” value for an analyte in a material in order to establish a reference material.

However, these aspects are not specifically covered in this Technical Specification.

Proficiency testing schemes are therefore organized to meet certain criteria and the testing programme (frequency, number of samples, number of repeats, etc.) shall meet the requirements of the type of method used and commodity analysed, to achieve the level of control desired by all parties involved.

Microbiology of food and animal feeding stuffs — Specific requirements and guidance for proficiency testing by interlaboratory comparison

1 Scope

This Technical Specification gives requirements and guidance for the organization of proficiency testing schemes for microbiological examinations of:

a) food and beverages;

b) animal feeding stuffs;

c) food production environments and food handling;

d) primary production stages.

This Technical Specification is also potentially 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 Technical Specification relates to the technical organization and the implementation of proficiency testing schemes, as well as the statistical treatment of the results of microbiological examinations.

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

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 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 5725-5, *Accuracy (trueness and precision) of measurement methods and results — Part 5: Alternative methods for the determination of the precision of a standard measurement method*

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

ISO 13528, *Statistical methods for use in proficiency testing by interlaboratory comparisons*

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.

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 datasets 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 Technical Specification cover those EQA activities that meet the definition of proficiency testing.

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

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

3.3
reference strain
microorganism obtained directly from an official culture collection and defined to at least the genus and species level, catalogued and described according to its characteristics and preferably originating from food or water as applicable

[ISO/TS 11133-1:2009[3], 3.3.2]

3.4
recovery percentage
proportion of the assigned value of the target organism recovered by the participant

NOTE 1 The recovery percentage is calculated by multiplying by 100 the number of recovered colony forming units (cfu) per volume or per mass.

NOTE 2 The recovery percentage can be significantly below 100 % when competitive flora 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; in this clause, only areas requiring special consideration for microbiological PT schemes are discussed 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 risk categories 1, 2, and 3, as appropriate (see ISO 7218:2007, 3.2).

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 random Poisson model 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 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 of target microorganisms.

5 Technical requirements and guidance for sample design and content

5.1 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 bacteria 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 aim of examination for pathogens is to prevent illness, and also to detect pathogens at a very low level, before those pathogens can grow to a higher level.

For quantitative (enumeration) methods, the target level shall be appropriate for the levels routinely found in and any statutory specifications applicable to the sample matrices used. The target level should also sometimes be used near 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 dilutions, the expected mean number of organisms in a sample is fewer than 10 colonies per plate.

For qualitative (detection) methods, the target organisms shall generally be required to be at a sufficiently low level to provide a valid challenge to the methodology and to contribute data for validation exercises to establish or verify limits of detection for individual participant laboratories.

5.2 Sources, characterization and traceability of organisms

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

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

Recognized reference strains from international collections should be used where they are most suitable for the scheme purpose; however, laboratory isolates or “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.

In all cases, the organisms used in PT scheme samples should be traceable to the relevant culture collection 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, e.g. for PT schemes for non-cultivable organisms such as human noroviruses. In such circumstances, clinical material can be used to contaminate 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. Target viruses should be fully characterized to strain level by conventional polymerase chain reaction followed by sequencing.

5.3 Background and competitive flora

The total flora of the samples, either naturally or artificially contaminated, shall be chosen to assess the ability of participants to detect and/or enumerate target organisms in the presence of non-target background flora (typical of the sample matrix) and presumptive target organisms which, without appropriate confirmation tests, can lead to false positive results.

Any strains used to simulate background flora shall meet the requirements of 5.2 for characterization and traceability. In naturally contaminated samples, the effects of any background flora on the target organisms shall be determined.

5.4 Matrix selection and effects

All matrices shall be evaluated before use to check for any effects on the target and background floras, e.g. where the matrices reduce the recovery of spiked organisms.

Participants shall be alerted to the nature of the food matrix where such matrices are 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. Suitable and validated preparation procedures shall be included for the information of participants.

Sample matrices used for microbiology PT schemes are often, but not necessarily, sterilized before use. Where natural, unsterilized samples are distributed, the organizers shall determine the effect of the background microflora of the samples.

6 Sample verification by the provider

6.1 General

General requirements for sample verification are given in ISO/IEC 17043 and ISO 13528 (for information, see also Reference [9]); this clause expands the specific requirements and problems 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, the test material shall be assessed for homogeneity, usually prior to but also at the time of testing for unstable fresh materials.

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

It is also necessary to test for homogeneity if materials are to be stored for longer periods of time to ensure criteria are still met before use. 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 required) is suggested.

A test material which 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 heterogeneous 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.

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.

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 (see ISO 13528 and Annex B). However, in general, a material for which the between-unit standard deviation (on the appropriately transformed scale) is $\leqslant 0{,}3\sigma_p$, where σ_p is the target standard deviation used to assess the performance of laboratories, is considered sufficiently homogenous (see ISO 13528).

Any alternative homogeneity test should meet the following criteria (reproduced from Reference [9]):

a) the probability of rejecting a sufficiently homogenous test material should be $\leqslant$ 5 %;

b) the probability of rejecting a test material where between-unit variation is $1{,}5\sigma_p$, in which σ_p is the acceptable between-laboratory variation (expressed as a target standard deviation), is $\geqslant$ 80 %.

An 80 % probability of rejecting a material where between-unit variation is $1{,}5\ \sigma_p$ is based on simulation studies of the duplicate analysis of 10 test units using a method with an analytical standard deviation of $0{,}5\sigma_p$ (i.e. 0,125 log units) and a critical value for T_2 (see Annex B) that meets criterion a) in the previous paragraph. It represents what is achievable with a reasonable amount of analytical effort.

Homogeneity tests are based upon estimations of between-unit variance and analytical (repeatability) variance obtained under repeatability conditions. Suitable methods of testing for such variation are given in Annex B.

The analytical (repeatability) variance should be estimated from replicate analyses of the initial suspensions obtained from test portions (References [15][16]). This analytical variance can also be calculated from the number of counted colonies and the precision of analytical materials in use (Reference [10]).

In microbiology, the between-unit variance shall be estimated under repeatability conditions (in one run). If that is impossible, the between-unit variance includes the within-laboratory reproducibility and can perhaps lead to the false rejection of a satisfactory material.

When the number of counted colonies is sufficiently high (more than 35 to 40 colonies per plate), the analytical standard deviation, σ_{an}, generally satisfies

$$\frac{\sigma_{an}}{\sigma_p} < 0{,}5$$

where σ_p is the target standard deviation, and the test for sufficient homogeneity proposed in Reference [11] should be used (see Annex B). If the number of counted colonies is low (fewer than 35 to 40 colonies per plate), the $T_1 - T_2$ test is recommended (see Annex B).

When replicated test units are provided to participating laboratories, the between-unit variability obtained by participants should be examined by the provider to assess the homogeneity of the material. Although this variability includes within-laboratory reproducibility, the higher number of participating laboratories increases the statistical power of the analysis and can be a good indicator for successive rounds.

When the number of counted colonies is low (say fewer than 20), the analytical (repeatability) variance is high. In that case, the provider should recommend that participating laboratories replicate enumerations of test portions to satisfy the condition (see ISO 13528):

