
**Microbiology of the food chain —
Method validation —**

**Part 1:
Vocabulary**

*Microbiologie de la chaîne alimentaire — Validation des méthodes —
Partie 1: Vocabulaire*

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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 16140-1, together with ISO 16140-2, cancels and replaces ISO 16140:2003, which has been technically revised. It also incorporates the Amendment ISO 16140:2003:Amd.1:2011.

ISO 16140 consists of the following parts, under the general title *Microbiology of the food chain — Method validation*:

- *Part 1: Vocabulary*
- *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

The following parts are under preparation:

- *Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory*
- *Part 4: Protocol for single-laboratory (in-house) method validation*
- *Part 5: Protocol for factorial interlaboratory validation of non-proprietary methods*
- *Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing*

Introduction

The use of validated methods is an important requirement for obtaining reliable results with a specific method. It also facilitates the comparability of results obtained with the same method in different laboratories. Validation procedures covered by ISO 16140 (all parts) involve various aspects of validation, such as validation of alternative (proprietary) methods, single laboratory validation, validation of (alternative) methods using a limited number of laboratories, and verification of methods (demonstration of a laboratory to correctly apply a validated method). In addition, there is a close link to ISO 17468 describing the procedure for the validation of the standard methods themselves.

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Microbiology of the food chain — Method validation —

Part 1: Vocabulary

1 Scope

This part of ISO 16140 defines general terms and definitions relating to method validation of microbiology in the food chain.

This part of ISO 16140 is applicable to the validation of methods for the analysis (detection or quantification) of microorganisms in

- products intended for human consumption,
- products intended for animal feeding,
- environmental samples in the area of food and feed production, handling, and
- samples from the primary production stage.

2 Terms and definitions

2.1

acceptability limit

AL

maximum positive or negative acceptable difference between the *reference value* (2.60) (or if not known, the accepted reference value) of a *sample* (2.69) and an individual result obtained when applying the operating procedure of an analytical method

Note 1 to entry: Because *accuracy* (2.2) is defined as 'the closeness of agreement between a measured quantity value and an assigned quantity value of a measurand', acceptability limits can be interpreted as the maximum measure of the lack of accuracy for *quantitative methods* (2.57).

2.2

accuracy

measurement accuracy

closeness of agreement between a measured quantity value and an assigned quantity value of a measurand

Note 1 to entry: The concept 'measurement accuracy' is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Note 2 to entry: The term 'measurement accuracy' should not be used for measurement *trueness* (2.77) and the term measurement *precision* (2.51) should not be used for 'measurement accuracy', which, however, is related to both these concepts.

Note 3 to entry: 'Measurement accuracy' is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

[SOURCE: JCGM, 2012, modified]

2.3

accuracy profile

graphical representation of the capacity of measurement of the *quantitative method* (2.57), obtained by combining acceptability intervals and *β -expectation tolerance intervals* (2.8), both reported to different levels of the *reference value* (2.60)

Note 1 to entry: For a given measurement method, different accuracy profiles can be drawn, depending on the experimental design where data were collected: under *repeatability conditions* (2.64) or *reproducibility conditions* (2.67), for different matrices, etc.

Note 2 to entry: Calculations of accuracy profile elements depend on experimental design.

2.4

alternative method

method submitted for validation

method of analysis that detects or quantifies, for a given category of products, the same *analyte* (2.6) as is detected or quantified using the corresponding *reference method* (2.59)

Note 1 to entry: The method can be proprietary. The term 'alternative' is used to refer to the entire 'test procedure and reaction system'. This term includes all ingredients, whether material or otherwise, required for implementing the method.

2.5

alternative method result

final result of the qualitative or quantitative analysis for the *alternative method* (2.4)

2.6

analyte

component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2003, 3.2]

Note 1 to entry: For food microbiology, this means a microorganism, group of microorganisms, or its products (e.g. toxins) quantified or detected by the method of analysis.

Note 2 to entry: Possible targets of the techniques that are used for detection or enumeration of the analyte can be DNA/RNA, proteins, lipopolysaccharides, or others.

2.7

assigned value

value that serves as an agreed-upon reference for comparison

Note 1 to entry: It is normally derived from or based on experimental work.

2.8

β -expectation tolerance interval

β -ETI

range of values within which a stated proportion of the population is expected to lie

Note 1 to entry: The stated proportion represents the probability that a value falls between an upper and lower bound of a distribution.

Note 2 to entry: Tolerance intervals tend towards a fixed value as the *sample* (2.69) size increases.

2.9

bias

measurement bias

estimate of a systematic measurement error, or the systematic difference between the quantitative *assigned value* (2.7) and the average of measurement *replicate* (2.65) results

2.10 blind replicates

set of *samples* (2.69) submitted to evaluate performance in which the presence and/or concentration of the *analyte* (2.6) is unknown to the analyst

Note 1 to entry: Within *validation* (2.81) studies, *blind replicates* (2.10) are used within the *interlaboratory study* (2.33). The *organizing laboratory* (2.45) prepares *samples* (2.69) and sends them to the *collaborators* (2.13). These samples are labelled (marked) in such a way that the *collaborator* (2.13) does not know if they contain the *analyte* (2.6), or not.

2.11 category

group of *sample* (2.69) *types* (2.78) of the same origin

EXAMPLE Heat-processed milk and dairy products.

2.12 certified reference material CRM

reference material (2.58) characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: Adapted from ISO Guide 30 and ISO Guide 35.

2.13 collaborator

individual laboratory technician, who works completely independently from other collaborators, using different sets of *blind samples* (2.69) or *test portions* (2.75)

2.14 combined standard deviation

combined standard uncertainty

standard measurement uncertainty that is obtained using the individual standard uncertainties associated with the input quantities in a measurement model

[SOURCE: JCGM, 2012, modified]

2.15 confidence interval

value $(1 - \alpha)$ of the probability associated with a confidence interval or a statistical coverage interval

EXAMPLE Confidence intervals can be obtained for arithmetic means, standard deviations, regression coefficients, etc.

Note 1 to entry: $(1 - \alpha)$ is often expressed as a percentage.

2.16 confidence level

specific probability of obtaining some result from a *sample* (2.69) if it did not exist in the population as a whole

Note 1 to entry: The usual levels of probability are 95 % or 99 %, but any level can be used.

2.17 confirmation procedure or test

procedure or test which is carried out to verify a presumptive result

Note 1 to entry: Not all methods have a confirmation procedure.

2.18

count

observed number of objects

EXAMPLE Colonies or plaques.

2.19

coverage factor

number larger than one by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty

[SOURCE: JCGM, 2012, modified]

2.20

detection level

<qualitative methods> minimum concentration of organisms that produce evidence of growth in a liquid medium with a probability of $P = 0,95$ when inoculated into a defined culture medium and incubated under defined conditions

Note 1 to entry: The theoretical level that conforms to this definition is three viable cells in an inoculum volume.

Note 2 to entry: The term 'sensitivity' ([2.71](#)) is discouraged for detection level.

2.21

environmental sample

sample ([2.69](#)) from a surface of equipment or from the production environment, or from water used in the manufacturing process

2.22

exclusivity study

study involving pure *non-target strains* ([2.44](#)), which can be potentially cross-reactive, but are not expected to be detected or enumerated by the *alternative method* ([2.4](#))

2.23

false-negative test result

negative result by the tested method that is actually confirmed as a positive result

2.24

false-positive test result

positive result by the tested method that is actually confirmed as a negative result

2.25

feed

feedstuff

any material or product intended to be, or reasonably expected to be, used for animal consumption

2.26

fitness for purpose

degree whether data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose

2.27

food

foodstuff

any material or product intended to be, or reasonably expected to be, used for human consumption

2.28

fractional recovery

validation ([2.81](#)) criterion that is satisfied when *replicate* ([2.65](#)) *samples* ([2.69](#)) of either the *alternative method* ([2.4](#)) or *reference method* ([2.59](#)) yield 50 % (range 25 % – 75 %) positive responses

2.29**homogeneity**

condition of being of uniform structure or composition with respect to one or more specified properties

Note 1 to entry: A *reference material* (2.58) is said to be homogeneous with respect to a specified property if the property value, as determined by tests on *samples* (2.69) of specified size, is found to lie within the specified uncertainty limits, the samples being taken either from different supply units (bottles, packages, etc.) or from a single supply unit.

[SOURCE: ISO Guide 30:1992, 2.6]

2.30**identification procedure or test**

procedure or test yielding the identity of the *analyte* (2.6)

2.31**inclusivity study**

study involving pure *target strains* (2.74) to be detected or enumerated by the *alternative method* (2.4)

2.32**in-house reference material****IRM**

non-certified material or substance, produced by one laboratory, one or more of whose property values are sufficiently homogeneous and well established to be used for *validation* (2.81)

2.33**interlaboratory study**

study performed by multiple laboratories testing identical *samples* (2.69) at the same time, the results of which are used to estimate alternative-method performance parameters

Note 1 to entry: The aim of an interlaboratory study is to determine the variability of the results obtained in different laboratories using identical samples.

2.34**item**

single specified *food* (2.27), *feed* (2.25), environmental, or primary production *matrix* (2.38)

EXAMPLE Food *category* (2.11): heat-processed milk and dairy products; food *type* (2.78): pasteurized dairy product; food *item*: milk-based desserts.

2.35**level of detection****LOD_x**

<qualitative methods> measured *analyte* (2.6) concentration, obtained by a given measurement procedure, for which the *probability of detection* (2.53) is *x*

EXAMPLE LOD₅₀ is the level of detection for which 50 % of tests give a positive result.

Note 1 to entry: The term 'level of detection' is used for qualitative methods in microbiology based on *replicate* (2.65) analyses with three different inoculation levels of the target *analyte* (2.6) in a tested *matrix* (2.38). The replicates are analysed, and the number of positive results is recorded (e.g. 20 %, 70 %, and 100 %) respectively at each inoculation level. These data are then used to determine the number of cells that would give 50 % positive using a generalized linear model (see ISO 16140-2). This differs from the procedure used for chemical and physical methods for which a 'limit of detection' is defined as the lowest quantity of an analyte that can be distinguished from the absence of that analyte with a stated *confidence level* (2.16).

2.36**limit of quantification****LOQ**

limit of determination

<quantitative methods> lowest *analyte* (2.6) concentration that can be quantified with an acceptable level of *precision* (2.51) and *trueness* (2.77) under the conditions of the test

2.37

line of identity

two-dimensional Cartesian coordinate system, where the identity line is the $y = x$ line

2.38

matrix (product)

all the components of the *sample* (2.69)

2.39

method comparison study

study, performed by the *organizing laboratory* (2.45) to compare the *alternative method* (2.4) with the *reference method* (2.59)

2.40

negative agreement

NA
agreement when the qualitative *alternative method* (2.4) and *reference method* (2.59) both present a *negative test result* (2.43)

2.41

negative control

sample (2.69) in which the target *analyte* (2.6) is either absent or below the *detection level* (2.20) of the method used

2.42

negative deviation

ND
negative result of the *alternative method* (2.4) when the corresponding *reference method* (2.59) result is positive

2.43

negative test result

test result (2.76) indicating the *analyte* (2.6) was not detected in a given *test portion* (2.75) as defined by the procedure of the *qualitative method* (2.56)

2.44

non-target strain

strain, defined according to the scope of the *reference method* (2.59) that would not reasonably be expected to be detected or enumerated by the *alternative method* (2.4)

2.45

organizing laboratory

expert laboratory
independent laboratory
laboratory with responsibility for managing all of the technical and statistical activities involved in the *validation* (2.81) study, i.e. *method comparison study* (2.39) and the *interlaboratory study* (2.33)

Note 1 to entry: The organizing laboratory is not involved in development and/or marketing of a *proprietary method* (2.55) that they will be validating.

2.46

outlier

member of a set of values which is inconsistent with other members of that set

[SOURCE: ISO 5725-1:1994, 3.21, modified]

Note 1 to entry: This extreme value normally appears randomly in less than 1 % of repetitive tests, but more frequently if abnormal situations occur. Statistical test procedures can be used to quantify this probability.

2.47**paired study**

paired/matched data

study when the qualitative *reference method* (2.59) and *alternative method* (2.4) have a common first enrichment step

Note 1 to entry: In this case, only one *test portion* (2.75) of a *sample* (2.69) is used to obtain a result with the *reference method* (2.59) and the *alternative method* (2.4). The incubated broth is then used in the second procedure step of both the reference method and the alternative method. The results from both methods are strongly dependent upon each other.

2.48**positive agreement**

PA

qualitative *alternative method* (2.4) and *reference method* (2.59) both present a confirmed *positive test result* (2.50) (confirmed positive results)

2.49**positive deviation**

PD

(confirmed) positive result of the *alternative method* (2.4) when the corresponding *reference method* (2.59) result is negative

2.50**positive test result**

test result (2.76) indicating the presence of the *analyte* (2.6) in a given *test portion* (2.75) as defined by the procedure of the method

Note 1 to entry: When the *reference method* (2.59) or *alternative method* (2.4) provides a preliminary positive test result requiring further testing to confirm this result, this test result can be considered as a presumptive positive test result. If the further testing specified by the method's procedure confirms that the test result can indeed be considered as being positive, the test result can be considered as a confirmed positive test result.

2.51**precision**

measurement precision

closeness of agreement between indications or measured quantity values obtained by *replicate* (2.65) measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The 'specified conditions' can be, for example, *repeatability conditions* (2.64) of measurement, intermediate precision conditions of measurement, or *reproducibility conditions* (2.67) of measurement (see ISO 5725-3).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement *reproducibility* (2.66).

Note 4 to entry: Sometimes, 'measurement precision' is erroneously used to mean measurement accuracy.

2.52**primary production sample**

sample (2.69) of animal faeces, or from the environment of animals or non-faecal samples from breeding flocks

2.53**probability of detection**

POD

proportion of positive analytical outcomes for a *qualitative method* (2.56) for a given *matrix* (2.38) at a given *analyte* (2.6) level or concentration

2.54

processing

any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion, or a combination of those processes

Note 1 to entry: Processed products may contain ingredients that are necessary for their manufacture or to give them specific characteristics.

2.55

proprietary method

method with a trademark/brand name, which is owned and generally marketed by a commercial company

EXAMPLE Enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) methods.

Note 1 to entry: Generally, some of the components of the method are undisclosed.

2.56

qualitative method

method of analysis whose response is that the *analyte* (2.6) is either detected or not detected, either directly or indirectly in a specified *test portion* (2.75)

2.57

quantitative method

method of analysis whose response is the amount [*count* (2.18) or mass] of the *analyte* (2.6) measured either directly (e.g. enumeration in a mass or a volume), or indirectly (e.g. colour absorbance, impedance, etc.) in a specified *test portion* (2.75)

2.58

reference material

RM

material or substance whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials

[SOURCE: ISO Guide 30:1992, 2.1, modified]

2.59

reference method

internationally recognized and widely accepted method

Note 1 to entry: For the purpose of this part of ISO 16140, these are ISO standards and standards jointly published by ISO and CEN or other regional/national standards of equivalent standing.

2.60

reference value

quantity value used as a basis for comparison with values of quantities of the same kind

[SOURCE: JCGM, 2012, modified]

2.61

relative level of detection

RLOD

level of detection (2.35) at $P = 0,50$ (LOD_{50}) of the (proprietary) *alternative method* (2.4) divided by the level of detection at $P = 0,50$ (LOD_{50}) of the *reference method* (2.59)

Note 1 to entry: For the purposes of alternative-method acceptance, the derived RLOD is checked with the *acceptability limit* (2.1) for conformity.

2.62**relative trueness****RT**

degree of correspondence between the response obtained by the *reference method* (2.59) and the response obtained by the *alternative method* (2.4) on identical *samples* (2.69)

2.63**repeatability**

measurement repeatability

r

measurement precision under a set of *repeatability conditions* (2.64) of measurement

[SOURCE: JCGM, 2012, modified]

2.64**repeatability conditions**

repeatability condition of measurement

condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

[SOURCE: JCGM, 2012, modified]

Note 1 to entry: A condition of measurement is a repeatability condition only with respect to a specified set of repeatability conditions.

Note 2 to entry: In chemistry, the term 'intra-serial precision condition of measurement' is sometimes used to designate this concept.

2.65**replicate**

repeating the analysis from different portions of the same *sample* (2.69) to obtain an independent measurement

2.66**reproducibility**

measurement reproducibility

R

measurement precision under *reproducibility conditions* (2.67) of measurement

[SOURCE: JCGM, 2012, modified]

Note 1 to entry: Relevant statistical terms are given in ISO 5725-1 and ISO 5725-2.

2.67**reproducibility conditions**

reproducibility condition of measurement

condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and *replicate* (2.65) measurements on the same or similar objects

Note 1 to entry: The different measuring systems may use different measurement procedures.

Note 2 to entry: A specification should give the conditions changed and unchanged (to a practical extent).

[SOURCE: JCGM, 2012, modified]

2.68**reproducibility standard deviation**

standard deviation of *test results* (2.76) obtained under *reproducibility conditions* (2.67)

[SOURCE: ISO 5725-1:1994, 3.19]

2.69

sample

food (2.27), feed (2.25), environmental, or primary production specified item (2.34) to be included in the validation (2.81) as per the intended use of the method

EXAMPLE Food category (2.11): heat processed milk and dairy products; food type (2.78): pasteurized dairy product; food item (2.34): milk-based desserts; sample: vanilla ice cream.

2.70

scope of validation

analytes (2.6), matrices, and concentrations for which a validated method of analysis can be used satisfactorily

2.71

sensitivity

SE

ability of the reference method (2.59) or alternative method (2.4) to detect the analyte (2.6)

2.72

specificity

SP

ability of the reference method (2.59) or alternative method (2.4) not to detect the analyte (2.6)

2.73

systematic error

systematic measurement error

component of measurement error that, in replicate (2.65) measurements, remains constant or varies in a predictable manner

Note 1 to entry: An assigned quantity value for a systematic measurement error is a quantity value, or a measured quantity value of a measurement standard of negligible measurement uncertainty, or a conventional quantity value.

2.74

target strain

strain, defined according to the scope of the reference method (2.59) that is expected to be detected or enumerated by the alternative method (2.4)

2.75

test portion

specified quantity of the sample (2.69) that is taken for analysis, e.g. 10 g, 25 g, 375 g of samples, or sponges for environmental samples (2.21), or boot socks for primary production samples (2.52)

2.76

test result

outcome of an analytical procedure or method

2.77

trueness

measurement trueness

closeness of agreement between the average of an infinite number of replicate (2.65) measured quantity values and a reference quantity value

Note 1 to entry: Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725 (all parts).

Note 2 to entry: Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error.

Note 3 to entry: Measurement accuracy should not be used for 'measurement trueness' and vice versa.

[SOURCE: JCGM, 2012, modified]