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Guidelines for the validation of qualitative screening methods for the detection of residues of veterinary drugs in milk and milk products

Lignes directrices pour la validation des méthodes qualitatives de dépistage des résidus de médicaments vétérinaires dans le lait et les produits laitiers

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Forewords

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 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 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products — Methods of sampling and analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF.

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.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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This document was prepared by the IDF *Standing Committee on Analytical Methods for Additives and Contaminants* and ISO Technical Committee ISO/TC 34, *Food products,* Subcommittee SC 5, *Milk and milk products*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products — Methods of sampling and analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF.

This IDF Reviewed method is equal to an ISO Publicly Available Specification (ISO/PAS) or an ISO Technical Specification (ISO/TS) and is therefore published jointly under ISO conditions.

The work was carried out by the IDF-ISO Action Team on A10 of the *Standing Committee on Analytical Methods for Additives and Contaminants* under the aegis of its project leader Dr W. Reybroeck (BE).

Guidelines for the validation of qualitative screening methods for the detection of residues of veterinary drugs in milk and milk products

1 Scope

This document describes general workflows and protocols for the validation and the verification of qualitative screening tests for the detection of residues of veterinary drugs in liquid milk (raw, pasteurized, UHT and reconstituted milk powders and whey protein extracts) including biological methods. This guideline does not cover the validation of residue analysis by HPLC, UHPLC or LC-MS/MS.

This document is intended to be useful for manufacturers of screening test kits, laboratories validating screening methods or tests, competent authorities and dairies or end users of reagents or tests for the detection of veterinary drug residues in milk products. This document facilitates and improves the validation and verification of screening methods. The goals of this document are a harmonization in validation of methods or test kits in order for all stakeholders to have full trust in the result of residue screening and to limit the overlap and multiplication of validation work in different laboratories by sharing the validation results generated by an independent laboratory. Furthermore, a harmonized validation and verification procedure allows for comparison of the performance of different screening methods.

This document does not imply that all end users are bound to perform all verification work proposed.

The verification of the correct use of reagents/kits for the detection of antimicrobials is not part of the scope of this document.

2 Normative references

There are no normative references in this document.

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 https://www.electropedia.org/

3.1
biological method
method that is used to detect cellular responses to analytes

EXAMPLE Inhibition of bacterial growth, immunological test, and receptor test.

3.2
qualitative method
method that gives a yes/no response, with no indication of the concentration of the putative analyte

EXAMPLE 1 Bacterial growth inhibition tests which give a result of either “no zone” or “zone of inhibition”.

EXAMPLE 2 Inhibition tests which give a colour change of growth medium.

EXAMPLE 3 Immunochemical/ligand binding tests, where a response is considered as “above” or “below” a cut-off level; or where analytes with different cross-reactivities are included within the method scope.

EXAMPLE 4 Biosensors.

3.3
matrix
non-analyte portion of the sample

Note 1 to entry: Matrices are included in the scope.

3.4
detection capability
CCβ
smallest content of the analyte that can be detected, identified and/or quantified in a sample with an error probability of β

Note 1 to entry: The β error is the probability that the tested sample is truly non-conformant even though a conformant measurement has been obtained.

3.5
cut-off level
response or signal from a screening test which indicates that a sample contains an analyte at or above the screening target concentration

3.6
blank matrix sample
negative control sample
sample from animals with known history of treatment which have not been exposed to the substance in question

Note 1 to entry: If samples from such animals are not available, samples which have been previously confirmed as conformant and not containing residues of the substance of interest by suitably sensitive physicochemical tests can also be acceptable.

Note 2 to entry: See Table 1.

3.7
positive control sample
control sample that is spiked with the test analyte at the screening target concentration

Note 1 to entry: This can, however also be an incurred-positive sample (i.e. sample taken from animals which have been treated with the substance in question) or Certified Reference Material.

3.8
screening target concentration
concentration at which a screening test categorizes the sample as “screen positive” (potentially non-conformant)

Note 1 to entry: This should always be lower than the regulatory limit.

3.9
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application, such as a test or measurement method, have been fulfilled

EXAMPLE Procedure applied in the originator laboratory (manufacturer’s laboratory) or in an independent laboratory.

Note 1 to entry: Validation often determines the fitness for purpose of a method.

3.10
verification
procedure applied to a method which has been previously validated in the case of a transfer validation

Note 1 to entry: The verification procedure is applied by a receptor laboratory for the same matrix as initially validated, to demonstrate that the method will work reliably in that laboratory with locally sourced milk and is fit for purpose.

3.11
originator laboratory
laboratory that has performed the complete validation of the method

Note 1 to entry: This is by preference an ISO/IEC 17025 accredited independent laboratory and preferably not the laboratory that developed the method. The laboratory should have experience in residue testing and in validation of screening tests for the detection of residues of veterinary drugs in milk.

3.12
receptor laboratory
laboratory that will perform the verification of the method

Note 1 to entry: This could be any laboratory interested in using the method.

3.13
spectrum
range of substances that a test can detect

Note 1 to entry: Some tests detect several classes of antibiotics and a large number of substances, whereas others are more specific.

3.14
regulatory limit
level defined by food legislation for residues in food

Note 1 to entry: Regulatory limits can be MRL (see 3.15), MRPL (see 3.16), RPA (see 3.17).

3.15
maximum residue limit for veterinary drugs
MRL
maximum concentration of residue resulting from the use of veterinary drugs that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in food

Note 1 to entry: Antibiotics are used to treat and prevent diseases in animal husbandry and as a result, low residues of antibiotics can be present in food. MRLs are set for pharmacologically active substances used or intended to be used in veterinary medicinal products placed on the market. In the EU the MRLs are set by EMA (European Medicines Agency).

3.16
minimum required performance limit
MRPL
minimum content of an analyte in a sample, which at least has to be detected and confirmed

Note 1 to entry: MRPL is intended to harmonize the analytical performance of methods for substances for which no permitted limit has been established.

3.17
reference point for action
RPA
level of a residue of a pharmacologically active substance established for control reasons in the case of certain substances for which a maximum residue limit has not been laid down following certain EU regulations

Note 1 to entry: EU Regulation 470/2009 is applicable for maximum residue limits.

Note 2 to entry: RPAs are currently based on analytical considerations (i.e. the lowest concentration that can be quantified using a validated analytical method). The aim is "to define an analytical concentration for a non-allowed pharmacologically active substance that can be determined by official control laboratories and that is low enough to adequately protect the consumers of food commodities which contain that substance"[19].

3.18
positive / negative result
result of the test after interpretation of the reading of the test taking into account the (pre-set) cut-off level

Note 1 to entry: Positive result: presence of antimicrobial residues (microbial inhibitor test) or presence of residues of veterinary drugs.

Note 2 to entry: Negative result: absence of antimicrobial residues (microbial inhibitor test) or absence of residues of veterinary drugs. Since only screening tests are involved, no judgement about 'conformant' or 'non-conformant' can be made.

3.19
repeatability limit
value less than or equal to which the absolute difference between two measurement results obtained under repeatability conditions is expected with a probability of 95 %

3.20
probability of detection
POD
proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration

Note 1 to entry: POD is concentration dependent (AOAC, 2014[7]).

4 Principle

Samples of matrix spiked with known levels of analyte are run on the test under validation or verification to determine the detection capability, sensitivity and robustness of the test. Evaluation of the test results determines the tests' suitability for routine use in screening milk for the presence of veterinary residues.

NOTE Annex B provides information on FDA tolerances and/or safe levels of animal drug residues in milk.

The key requirement for a screening method is its ability to reliably detect the analyte in question at the chosen screening target concentration. The screening target concentration should be chosen to avoid false-negative results, i.e. low enough to ensure that if the analyte in question is present in the sample at the Regulatory Limit, the sample will be classified as 'Screened Positive'.

Both validation and verification should provide the objective evidence that this key requirement is met. Validation should cover the entire matrix/species/analyte combinations claimed within the scope of the method standard operating procedure (SOP). Validation should be as broad as possible to cover the scope of all end users.

Verification should cover the matrix/species/analyte combinations included in the scope of the implementing (receptor) laboratory. The extent of validation required is variable, depending on whether it is a validation or a verification of a transferred method.

The verification does not need to cover the entire spectrum if the implementing laboratory is to be applicable to only a limited scope (e.g. some species and not others, some residues more relevant than others, raw but not UHT [Ultra-High temperature] milk, etc.).

If a receptor laboratory wants to use the method for screening in a different matrix (IDF 2014) not tested by the originator laboratory, the receptor laboratory should test all necessary validation parameters to prove that the method functions for that specific matrix.

5 General requirements for the test/kit

The developer or the manufacturer should provide information regarding methodology, test reagents, additional chemicals not necessarily included in the kit, operating requirements (information about the reading system, cut-off level), test specifications and documentation (extracted from ISO 18330 and ISO 13969). Additionally, the target country(ies) and its/their specific regulatory limits should be known, in order for the test to be evaluated against the appropriate regulatory limits.

Elements of information to be provided by the manufacturer/distributor/lab manager (in case of an in-house developed method) before starting the validation are as follows:

— Test principle, principle of reading and interpretation of the test (including cut-off level or calculation of cut-off level).

— Test formats, if relevant (e.g. ampoules/plates).

— Scope of the test:

  — Matrices suitable to be tested: matrices in the scope of the document (see Clause 1).

  — Animal species producing the milk.

  — Matrices with potential impact (interference) on the result.

— Potential impact of the use of sample preservatives.

— Spectrum of the test: list of veterinary drugs and expected detection capabilities (so far known).

— List with the current regulatory limits (RL) for the detectable veterinary drugs in the matrix(ces) of concern in the country(ies) of concern.

— Detailed protocol in a language understood by laboratory staff: if minor modifications need to be made to the method according to the matrix/species, they should be announced in the test protocol (kit manual).

6 Reagents

6.1 Standard blank matrix

— The raw milk used is commingled milk coming from at least 4 animals not treated with veterinary drugs within the last 2 months, in mid lactation, and delivering milk with a low to moderate number of somatic cells (e.g. < 150 000 ml^{-1} for bovine milk). The raw milk is collected in sterile containers and kept below 4 °C. The maximum period for the cold storage of the fresh raw milk should be in line with the definition of fresh raw milk as fixed locally.

— The milk used should be in line with the normal milk produced in the country or area of concern. This means that the composition and quality of the milk should approach the average composition of the milk of the country/region.

— Table 1 gives examples of parameters to consider for ‘normal’ milk. Actual figures are likely to vary depending on country and region.

— Milk of at least 4 animals is commingled and is considered as a sample of standard blank matrix. At least four such samples should be used for the determination of the detection capability when testing 20 replicates. If 40 or 60 replicates need to be tested to determine the detection capability, eight or twelve different blank milk samples should be used, respectively. At least four different commingled milks should be sourced and used in the verification work (20 replicates).

— The use of thawed or reconstituted lyophilized milk could also be authorized, but strictly on condition. The pre-requisite condition to work with these alternative solutions, is to demonstrate

previously the equivalence of results between raw milk and thawed or reconstituted lyophilized milk, after the analysis of negative and positive milk samples.

Table 1 — Examples of reference data for the composition and quality of normal milk of different animal species

Species		SCC[a] cells per ml	TBC[b] cfu[c] per ml	FC[d] g/l	PC[e] g/l	pH	Antibiotics	Lactating period
Cow	Target value	< 150 000	< 30 000	40	33	6,7 to 6,8	Absence	Between 60 and 200 days after calving
	Acceptable range	< 400 000	< 100 000	35 to 45	30 to 36	6,6 to 6,9		
Goat	Target value	< 2 000 000	< 60 000	38	34	6,7 to 6,8	Absence	Between 20 and 150 days after kidding
	Acceptable range			30 to 50	28 to 40	6,6 to 6,9		
Ewe	Target value	< 2 000 000	< 60 000	70	55	6,7 to 6,8	Absence	Between 20 and 150 days after lambing
	Acceptable range			50 to 90	40 to 70	6,6 to 6,9		

[a] Somatic cell count.
[b] Total bacterial count.
[c] Colony forming units.
[d] Fat content.
[e] Protein content.

6.2 Antibiotics

Only use analytical grade or certified reference material for validation or verification purposes.

6.3 Standard stock solution

— Standard stock solutions of the antibiotic at 100 mg/l are made in water or a suitable solvent and kept below 4 °C (refer to 8.1)[14]. The shelf life depends on the stability of the molecule.

— In the preparation of the stock solution, correction for impurity and water content is performed.

— For each substance a single stock solution is prepared, but by preference for certain problematic compounds (for example solubility problem, stability), at least two stock solutions should be prepared to determine the detection capability. A list of problematic compounds is given in Annex C.

— If only one stock solution is used it should be either prepared from certified material or verified with an independent physicochemical method.

— Some compounds like tetracyclines are light sensitive and need to be kept protected from light. Other compounds can require specific requirements for the glassware used.

6.4 Working stock solutions

Dilutions of 10 mg/l to 0,1 mg/l are freshly prepared on a daily basis.

6.5 Spiked sample

For the preparation of end concentration, the final spiking is performed in the standard blank matrix.

The blank milk will be spiked with each analyte.

The added volume of working stock solution should be below 5 % of the final volume of the milk sample to be tested.

7 Apparatus

Any apparatus specified in the test kits procedure that is not provided by the test kit manufacturer.

7.1 Test kit, in which reagents of at least two and by preference three different production lots are used.

Production lots should be determined randomly and be representative of the production level of the product.

7.2 Incubator or water-bath, capable of maintaining the appropriate incubation temperature for the test.

If the manufacturer has an incubator that is designed for the test, this incubator should be supplied by the manufacturer for use in the validation or verification.

7.3 Automated readers. If the manufacturer provides an apparatus for evaluating the results from the test, this apparatus should be supplied by the manufacturer for use and evaluation in the validation or verification.

Calibration procedures for the automated reader shall be made available and all readings shall be taken from only calibrated equipment.

7.4 Micropipettes, capable of delivering the appropriate amount of sample required for use in the test.

If the test kit supplies micropipettes or other liquid transfer equipment these should be by preference evaluated as part of the validation or verification.

8 Sample Preparation

8.1 Stock solution preparation

A 100 mg/l solution of each antibiotic is prepared by first calculating the amount of reference material needed to give 10 mg ± 0,1 mg of active compound. This calculation is done using Formula (1) and the information (purity, water content) from the certificate of analysis of the antibiotic.

$$m_{\mathrm{m}} = m_{\mathrm{a}} \times \left(\frac{100}{P} \right) \times \left(\frac{100}{100 - W} \right) \tag{1}$$

where

m_{m} is the mass of the material required, in mg;

m_{a} is the mass of the analyte required, in mg;

P is the purity, in %;

W is the water content, in %.

Weigh this amount directly in a weight boat and transfer into a 100 ml volumetric flask and make up to 100 ml using the appropriate solvent.