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Milk and milk products — Guidelines for the standardized description of immunoassays or receptor assays for the detection of antimicrobial residues

Laits et produits laitiers — Lignes directrices pour la description normalisée des essais immunologiques et des essais récepteur pour la détection des résidus antimicrobiens

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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 18330|IDF 188 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 AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team *Antimicrobials and other veterinary medical residues*, of the Standing Committee *Analytical methods for additives and contaminants*, under the aegis of its project leader, Mr E. Märtlbauer (DE)

Introduction

Because of the method of detection, the tests described in this International Standard may have limitations so that they cannot be used for quantification. For example, receptor assays have group-specific detection and not chemical-specific detection. Consequently, positive results cannot be subject to quantitation without knowledge of the identity of the specific contaminant. Moreover, assays based on a visual evaluation of colour development may not measure the degree of colour and thus may not provide a quantitative value.

Within an integrated system for antimicrobial residue detection, immunoassays and receptor assays may be used as primary-screening methods (e.g. for screening of compounds which can not be detected at regulatory levels by microbiological inhibition assays). These methods may also be used as post-screening methods for preliminary identification and quantification of compounds in samples with a positive result in a microbiological inhibition assay.

Depending on whether a certain test complies with the specifications given, immunoassays and receptor assays may be used for routine quality control, especially if the absence/presence of a certain compound in concentrations exceeding a certain level [e.g. maximum residue limit (MRL)] has to be determined. Substances which are not approved or for which no MRLs have been fixed, may require specific consideration. For legal purposes in many countries, positive results obtained by immunoassays or receptor assays require confirmation by an accepted physico-chemical method.

Milk and milk products — Guidelines for the standardized description of immunoassays or receptor assays for the detection of antimicrobial residues

1 Scope

This International Standard gives guidelines for the standardized description of immunoassays or receptor assays for the detection of antimicrobial residues in milk and milk products.

It is intended to provide a framework and basis for the evaluation/validation of tests based on the binding of an antimicrobial compound to its specific antibody or to other types of detecting molecules.

In addition to immunoassays [e.g. enzyme-immunoassay (EIA) and radio-immunoassay (RIA)], there are several quantitative, semi-quantitative and qualitative test formats based on the binding of antimicrobial compounds to microbial receptors or to receptor proteins. Enzymatic assays and particle-based assays based on receptor proteins are referred to as receptor assays in this International Standard.

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 13969, *Milk and milk products — Guidance for a standardized description of microbial inhibitor tests*

3 Terms and definitions

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

3.1
false positives
percentage of positive results when testing negative samples

3.2
false negatives
percentage of negative results at the claimed detection level(s)

3.3
specificity
extent to which the presence of substances with chemical structures similar to that of the target analyte will result in a positive result (cross-reaction)

3.4
limit of detection for qualitative tests
concentration level at which a defined percentage of samples is detected, e.g. 95 % together with the respective confidence level.

3.5
limit of detection for quantitative tests
concentration level which gives a final result that is statistically different from that of negative milk

4 Information needed from the developer/manufacturer

4.1 Methodology

The developer or manufacturer of the test should provide information regarding methodology by mentioning the following:

a) description of the method (e.g. sample preparation and test performance);

b) principle of the method (e.g. competitive direct enzyme-immunoassay);

c) evaluation of test results (e.g. visual or instrumental reading, criteria for positive or negative result);

d) capacity (e.g. sample throughput);

e) special requirements for sampling, preservation and testing;

f) procedure for the purpose of quality assurance, including the use of positive/negative control samples;

g) field of application concerning

- the intended test use [e.g. screening for milk quality payment or for regulatory purposes (detection of banned substances)],

- the substrate or matrix (e.g. raw tanker bulk milk or heat-treated milk), and

- the limitations with respect to sample composition (e.g. cell count and bacteriological quality).

4.2 Test kit reagents

The following information should be given regarding test kit reagents.

a) In the case of immunoassays:

   1) type of antibodies (e.g. monoclonal or polyclonal, sheep, rabbit or egg);

   2) type of antigen used in the test (e.g. antigen-enzyme conjugate, solid-phase antigen);

   3) type of marker for signal production (e.g. enzyme: peroxidase, alkaline phosphatase; radiochemical: H^3, C^{14}, I^{125}; inert bead: colour latex bead, gold);

   4) type of enzyme substrate and type of substance used for transformation of enzyme activity to measured signal (e.g. hydrogen peroxide/tetramethylbenzidine), if applicable.

b) In the case of microbial receptor assays:

   1) type of label (e.g. radioactive isotope),

   2) amount of radioactivity and safety requirements, and

   3) type of receptor (e.g. non-viable microbial cells or antibody).

c) In the case of receptor protein assays:

   1) type of receptor protein used (e.g. enzyme-receptor conjugate),

   2) type of reagent competing for receptor protein binding sites (e.g. analyte-enzyme conjugate, solid-phase analyte),

   3) type of enzyme substrate and type of substance used for transformation of enzyme activity to measured signal, and

   4) enzymatic reaction(s) used to produce signal.

4.3 Additional chemicals not necessarily included in the test kit

The following should be mentioned regarding additional chemicals not necessarily included in the test kit:

a) purity and quality of chemicals required;

b) composition and preparation of solutions;

c) storage conditions and stability of solutions;

d) water quality required.

4.4 Operating requirements

The following information should be given regarding operating requirements:

a) requirements for user experience and training;

b) requirements for laboratory equipment:

   1) microtitre plate reader,

   2) fluorometer, scintillation counter, computer hard- and software,

   3) incubators and precision pipettes;

c) requirements for safety (e.g. for handling and disposal of radioactive waste);

d) requirements for quality control by developer/manufacturer and/or user.

4.5 Test specifications

The following information should be given regarding test definitions:

a) false positives: see 3.1;

b) false negatives: see 3.2;

c) specificity: see 3.3;

d) limit of detection: see 3.4 and 3.5 respectively;

e) precision: figures for repeatability and reproducibility obtained from the results of collaborative studies, if carried out and available.

4.6 Documentation

The following information should be given regarding documentation:

a) user manual, including a trouble-shooting guide;

b) suppliers of instruments, reagents, standards, technical services and customer support;

c) status of official recognition in specified countries (if available);

d) availability of reference material;

e) availability of internationally recognized and/or validated references from ISO, IDF and AOAC International or others;

f) availability of, for example, literature and practical experiences.

5 Evaluation of the attributes of the enzyme-immuno or receptor assay

5.1 Prerequisites (see ISO 13969)

5.1.1 Milk free from antimicrobials ("negative milk")

The cows from which milk is collected in order to serve as "negative milk" shall meet the following requirements. If, however, a test is applied for milk of an animal species other than cows, the requirements with respect to the status of that animal should be adjusted accordingly.

a) The clinical and sub-clinical health status shall be good, with special emphasis on udder health (less than 150 000 somatic cells per millilitre).

b) The treatment or feeding with antimicrobial substances shall be prohibited for at least 8 weeks before milk collection. In the case of dry cow treatment, the milk shall not be collected earlier than 60 days after calving provided the dry cow period was at least 4 weeks.

c) The cows shall be mid-lactation: more than 60 days and less than 200 days after calving, producing more than 5 kg milk per day.

d) The milking of at least five to seven cows shall be combined to overcome individual variations in milk composition.

e) The total viable count shall be less than 10^4 colony-forming units (CFU) per millilitre before the preservation process (deep-freezing, lyophilisation). The possible presence of β-lactamase-producing microorganisms shall be kept in mind in the case of β-lactam antibiotic testing.

5.1.2 Test substances

The test substances which are used in the testing procedure should be obtained from a recognized developer/manufacturer, preferably with an analytical certificate with a guaranteed specification. The concentration required should be calculated based on the free acid or base forms of the drug corrected for purity. Special considerations should be given to substances with stability/potency problems.

Unless otherwise stated, it is preferable that the evaluation of detection limits (5.2.2) should be undertaken using those antimicrobials and/or concentrations that the developer/manufacturer claims the test will detect.

5.1.3 Solvents

If special solvents or other chemicals are required to dissolve the substances, it should have been ensured that these solvents or chemicals in the test samples have no influence on the test result.

5.1.4 Preparation of test samples

5.1.4.1 General

For large-scale evaluations (e.g. to obtain the data basis for a generalized description), all dilutions required should have been prepared in one batch to avoid day-to-day variations in weighing, diluting and differences in the status of the “negative milk”.

The preparation of test samples may cause problems and is a very laborious task for the test evaluation laboratories. Therefore, it might be appropriate to employ a centralized test sample preparation laboratory which agrees to supply interested laboratories with the test samples in stable form (e.g. lyophilized).

5.1.4.2 Selection of concentrations

The selection of concentrations for the determination of the detection limits is described in 5.2.2. For estimated purposes, if not otherwise stated, the concentration found to represent the detection limit should be tested together with one concentration level higher and two or three concentration steps lower than the claimed detection limit and the corresponding negative milk. These data may be used to estimate the 25 %, 50 %, 75 % and 95 % positive sample concentration. As an approximate guideline, it is recommended to divide the concentration range resulting in 50 % to 100 % positive samples into three or four equally distant levels (linear and logarithmic scales respectively) as demonstrated in Figure 1.

5.1.4.3 Dilution

The following precautions should be met when preparing dilution series of test substances.

a) The preparation of the dilution series should be carried out in such a way that only the final dilution step is prepared with milk in order to avoid protein binding.

b) The proportion of the added aqueous standard solution in the final milk dilution step should be the same for all test samples and less than 1 %.

5.1.4.4 Preservation

Preservation of test samples should preferably be done by lyophilization, if this is not deprecated by the developer/manufacturer of the test under study or the test principle. The following procedure for preservation has proved to be feasible.

a) Immediately after preparation of the various milk samples, all dilutions should be dispensed into test tubes with the desired volume and be frozen at –18 °C ± 2 °C in a sloping position.

b) Lyophilization should be carried out as soon as possible, and not later than one week after deep freezing. During the lyophilization process, the temperature should not exceed 25 °C.

c) Test tubes should be hermetically sealed immediately after lyophilization and stored in the dark at $\leqslant$ 6 °C.

d) Test samples should be reconstituted with distilled water. The added volume of water should be 10 % less than the volume of sample that was lyophilized in order to compensate for the dry matter of milk.

e) Reconstituted test samples may be used on the day of reconstitution only. They should be kept in a refrigerator between uses and discarded at the end of the day.