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Milk and milk products — Guidelines for a standardized description of competitive enzyme immunoassays — Determination of aflatoxin M_1 content

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Foreword

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ISO 14675|IDF 186 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.

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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 National Committees casting a vote.

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

Introduction

Proprietary methods such as ELISA methods cannot be described in separate International Standards. Therefore, this International Standard is intended to provide guidelines on basic parameters required for evaluation/validation of competitive enzyme immunoassays for the quantitative determination of aflatoxin M_1 in milk and milk products.

Currently several quantitative immunochemical test formats are commercially available, which all share the basic principles of the competitive enzyme immunoassay. However, since the test format of the 96-well microtitre plate assay is most commonly used for quantitative measurement purposes, the parameters given in this International Standard are specifically adopted to this test format, and may not necessarily apply in full to a different test format.

Milk and milk products — Guidelines for a standardized description of competitive enzyme immunoassays — Determination of aflatoxin M_1 content

1 Scope

This International Standard give guidelines on the use of screening methods used for the determination of aflatoxin M_1 content in milk and milk products, based upon competitive enzyme immunoassays.

For legal purposes, positive enzyme immunoassay results require confirmation by an accepted reference method. However, depending on whether the test complies with the specifications given hereafter, enzyme immunoassays can be used for routine quality control, especially when the absence of aflatoxin M_1 above the regulatory limit needs to be documented.

2 Principle

Immunochemical methods are based on the ability of antibodies to bind to specific substances. The reversible association between antibodies and their corresponding antigens is called the immunological reaction. The binding forces involved are weak molecular interactions, such as Coulomb and van der Waals forces, as well as hydrogen bonds and hydrophobic binding.

The antigen-antibody reaction is based on the law of mass action and the amount of antigen or antibody present in the reaction mixture can be inferred from the extent of the reaction.

The “quality” of any immunoassay is a function of the immunochemical principle of the method, the properties of the reagents, the assay design and the experimental errors. These basic principles determine the sensitivity, specificity, precision and accuracy of the assay.

Concerning the principle of the method, a distinction exists between competitive methods and non-competitive methods.

For practical reasons, these methods need either labelled antigen or labelled antibody to permit observation of the antigen-antibody reaction.

Competitive methods are based on the competition of free (A_g) and labelled (A_g*) antigen for a limited number of antibody-combining sites (AB).

Schematically, this immunochemical principle may be presented according to the following formula:

$$A_g + A_g{}^* + AB = A_gAB + A_g{}^* AB + A_g + A_g{}^*$$

In most cases, the assay response represents the bound-labelled antigen, but any measure of the distribution of the labelled antigen is, in principle, possible.

For the detection of low molecular weight compounds like mycotoxins, which possess only one antibody binding site (epitope), the competitive assay format is mandatory. To provide a distinction between unreacted and complexed reactants, most assays use either antibody (direct competitive assay) or antigen (indirect competitive assay) bound to a solid-phase as immunosorbent. So all the reagents that are not bound by the antibody can be easily removed by “washing” the solid phase.