Milk and milk products - Guidelines for a standardized description of competitive enzyme immunoassays - Determination of aflatoxin M1 content

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EESTI STANDARDI EESSÕNA **NATIONAL FOREWORD**

Käesolev Eesti standard EVS-EN ISO 14675:2003 sisaldab Euroopa standardi EN ISO 14675:2003 ingliskeelset teksti. Käesolev dokument on jõustatud 16.05.2003 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes. Standard on kättesaadav Eesti standardiorganisatsioonist.	This Estonian standard EVS-EN ISO 14675:2003 consists of the English text of the European standard EN ISO 14675:2003. This document is endorsed on 16.05.2003 with the notification being published in the official publication of the Estonian national standardisation organisation. The standard is available from Estonian standardisation organisation.

Käsitlusala: This International Standard gve guidelines on the use of screening methods used for the determination of aflatoxin M1 content in milk and milk progucts, based upon competitive enzyme immunoassays	**Scope:** This International Standard gve guidelines on the use of screening methods used for the determination of aflatoxin M1 content in milk and milk progucts, based upon competitive enzyme immunoassays

ICS 67.100.10

Võtmesõnad: bioassay, chemical analysis and testin, enzyme assay, enzymes, food inspection, food products, food technology, food testing, guide books, immunology, milk, milk products, mycotoxin, qualitative analysis, standardization, test antigens, testing, toxicology

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 14675

January 2003

ICS 67.100.10

English version

Milk and milk products - Guidelines for a standardized description of competitive enzyme immunoassays - Determination of aflatoxin M1 content (ISO 14675:2003)

Lait et produits laitiers - Lignes directrices pour une description normalisée des tests immuno-enzymatiques - Détermination de la teneur en aflatoxine M1 (ISO 14675:2003)

Milch und Milchprodukte - Leitfaden für eine vereinheitlichte Beschreibung kompetitiver Enzym-Immunoassays - Bestimmung des Gehalts an Aflatoxin M1 (ISO 14675:2003)

This European Standard was approved by CEN on 6 December 2002.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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CORRECTED 2003-04-30

Foreword

This document (EN ISO 14675:2003) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis", the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2003, and conflicting national standards shall be withdrawn at the latest by July 2003.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 14675:2003 has been approved by CEN as EN ISO 14675:2003 without any modifications.

INTERNATIONAL STANDARD

ISO 14675

IDF 186

First edition
2003-01-15

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

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Reference numbers
ISO 14675:2003(E)
IDF 186:2003(E)

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Printed in Switzerland

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

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.

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 National Committees casting a vote.

International Standard 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.

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.