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Textiles — Determination of reduction activity of specific proteins derived from pollen, mite and other sources on textile products

Textiles — Détermination de l'activité de réduction des protéines spécifiques provenant du pollen, des acariens et d'autres sources sur les produits textiles

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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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 38, *Textiles*.

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.

Introduction

Specialty textile products which can have the positive effect on human comfortable and hygienic life, such as antibacterial, antifungal, antiviral treated textiles, have been introduced in the market and are expanding year by year in various applications.

Now, contamination on textile products with specific proteins which show antigen-antibody reaction also can have the negative effect on human comfortable and hygienic life. There are high performance textile products which can reduce the amount of those specific proteins on textile products.

Because those products are relatively new and include the technical aspects of textile and biological technology, the testing methods have been developed by the individual procedures to evaluate the product performance. That has resulted in inexistence of a unified test method, hindering for both consumers and producers a true explanation or understanding of those functional products.

The demand to establish an international standard has been growing in the consumers, retailers, producers, etc. as stakeholders in the market.

This document provides a quantitative test method by using enzyme-linked immunosorbent assay to assess the reduction activity of the specific proteins on textile products by taking proteins derived from pollen and mite-faeces or carcass as an example.

Textiles — Determination of reduction activity of specific proteins derived from pollen, mite and other sources on textile products

WARNING — This document calls for use of the antigen-antibody reactive derived-protein or substances/procedures that can be injurious to the health/environment if appropriate conditions are not observed. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety/environment at any stage.

1 Scope

This document specifies a test method for the determination of reduction activity of textile products against specific proteins which shows antigen-antibody reaction. This document only specifies the reduction activity against those proteins on the surface of textile products. It does not specify a testing method to evaluate the allergenic reaction against human beings.

Specific proteins which show antigen-antibody reaction are proteins derived from pollen, mite and other sources. Other specific proteins can be used after appropriate validation described in this document.

Enzyme-linked immunosorbent assay is used to quantify the amount of those proteins in this document.

This document is applicable to textile products include woven, knitted and nonwoven fabrics, fibres, yarns, braids, etc.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3696, *Water for analytical laboratory use — Specification and test methods*

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
antigen
substance that is recognized as foreign by the immune system and elicits an immune response through stimulating antibody production

[SOURCE: ISO 16577:2016, 3.12]

3.2
antibody
protein (immunoglobulin) produced and secreted by B lymphocytes in response to a molecule recognised as foreign (antigen) and which is capable of binding to that specific antigen

Note 1 to entry: Immunoglobulin is the common synonym for antibody.

[SOURCE: ISO 16577:2016, 3.10]

3.3
specific protein
proteins which act as antigen and are derived from pollen, mite and other sources

3.4
reduction activity of specific protein
reduction rate of *specific protein* (3.3) concentration

3.5
specific protein reduction agents
inorganic or organic chemicals able to reduce the specific protein concentration

3.6
untreated fabric
fabric of the testing sample without treatment by antigen reduction agent

3.7
negative control
blank test to confirm the effect of an empty plastic bag

3.8
control test
test to confirm that the extract chemicals from test sample do not affect to the sensitivity of ELISA measurement

3.9
enzyme-linked immunosorbent assay
ELISA
method that used *antibodies* (3.2) or *antigens* (3.1) covalently bound to enzyme

4 Principle

The suspension of specific protein from pollen, mite and others are deposited onto a test specimen. After specified contact time, the remaining antigen-antibody reactive specific proteins from pollen, mite and others is measured by using the ELISA method, and the reduction activity is calculated by the comparison between the concentration of the test specimen and the negative control.

5 Specific proteins

Examples of specific proteins derived from pollen and mite are shown in Annex A. Other specific proteins from other species can be used after appropriate validations. If the other species are used, the name of the species and the specific reason for their use shall be described in the test report.

6 Apparatus

6.1 Measuring flask, with capacity of 1 l.

6.2 Balance, with the available range of 0,001 g to 100 g with accuracy of 1,0 %.

6.3 Micropipette, having the most suitable volume for each use, with a tip made of glass or plastic, and with an accuracy of 0,5 % or less.

6.4 Freezer, capable of operating at a temperature of (-20 ± 2) °C.

6.5 Refrigerator, capable of operating at a temperature between 2 °C and 8 °C.

6.6 pH meter, with a glass electrode, with a resolution of at least ±0,01 pH unit

NOTE The pH meter are described in ISO 3071.

6.7 Biological safety cabinet, class II.

6.8 Incubator, capable of maintaining at a temperature of (25 ± 1) °C and (37 ± 1) °C.

6.9 Microplate reader, capable of measuring at a 450 nm to 620 nm in wavelength.

6.10 Polyethylene bag with zipper, with (60 ± 2) mm × (85 ± 2) mm.

6.11 Culture container, made of glass bottle

6.12 Specific protein antibody coated plate, having 96 wells coated with specific protein antibody on bottom of wells.

6.13 Gel filtration columns.

7 Reagents and media

All reagents shall have the quality suitable for biological needs. Some of the media are available in the market.

7.1 Water, which shall be analytical-grade water for microbiological media preparation, which is ion-exchanged and/or freshly distilled and/or ultra–filtered and/or filtered with reverse osmosis (RO) or ISO 3696 grade 3.

7.2 Phosphate buffered saline PBS (-)

7.2.1 Prepare a measuring flask of 1 l, and put the following chemicals into a flask (6.1):

— sodium chloride (NaCl), 8 g;

— potassium chloride (KCl), 0,2 g;

— disodium hydrogen phosphate $12H_2O$ (Na_2HPO_4, $12H_2O$), 2,9 g;

— potassium dihydrogen phosphate (KH_2PO_4), 0,2 g.

7.2.2 Add water (7.1) and make up a whole amount to 1 000 ml. Dissolve well.

7.2.3 Transfer the solution (7.2.2) to a culture container (6.11).

7.3 Suspension solution of specific protein

7.3.1 Put the polysorbate 20 (7.7), 0,5 g, in the phosphate buffered saline PBS (-) prepared at 7.2, approximately 700 ml to 800 ml and dissolve well.

7.3.2 Add the solution (7.2) by making up whole amount to 1 000 ml and mixed well.

7.3.3 Then, transfer the solution (7.3.2) to a culture container (6.11).

7.4 ELISA assay reagents, reagents and buffer solutions

ELISA assay reagents, reagents and buffer solutions can be obtained from commercial suppliers which shall be prepared for use in accordance with the manufacturer's instructions.

Examples of ELISA assay reagents, reagents and buffer solutions are shown in Annex B.

7.5 Sodium hydroxide solution.

7.6 Hydrochloric acid solution.

7.7 Polysorbate 20.

8 Preparation

8.1 Preparation of test specific protein suspension

Adjust the concentration of specific protein extract to 10 ng/ml to 20 ng/ml by using suspension solution of specific protein (7.3).

The default concentration of specific protein extract shall be to 10 ng/ml to 20 ng/ml, however, the concentration of specific protein extract can be allowed to increase up to 100 ng/ml according to the experience of the laboratories.

Specific protein extracts are available in the market. The storage of the specific protein extracts shall be in the freezer (6.4) with the temperature below -20 °C and all operation to handle specific protein extracts shall be done in the biological safety cabinet (6.7).

8.2 Preparation of test specimens

8.2.1 Prepare the test specimens of specific protein reduction test sample as specified in Table 1.

Table 1 — Dimension or mass of specimen

Kind of sample	Mass[a]	Specimen
Fabrics (woven, knitted, nonwoven)	0,40 g or more	(50 ± 2) mm × (50 ± 2) mm
	less than 0,40 g	0,40 g ± 0,05 g
Yarns, braid, fibres, wadding and feather		0,40 g ± 0,05 g
[a] Mass of the fabric specimen with a dimension of (50 ± 2) mm × (50 ± 2) mm.		

8.2.2 Prepare six (6) test specimens of the specific protein reduction test sample.

Three (3) specific protein reduction test specimens are used for the control test.

The remaining 3 specific protein reduction test specimens are used for the main test of this document.

If untreated fabrics are used for the main test instead of negative control, prepare 6 test specimens of the untreated fabric. Three untreated fabric test specimens are used for the control test and the remaining 3 untreated fabric test specimens used for the main test.

8.3 Control test

8.3.1 General

The purpose of the control test is to confirm that any chemicals from the test specimen does not reduce the sensitivity of ELISA. In case of using a commercial kit, refer to the technical sheet.

8.3.2 Procedure of verification of the sensitivity of ELISA

8.3.2.1 Put 3 specific protein reduction test specimens in each bag with zipper (6.10) and add 1 ml of suspension solution of specific protein (7.3).

8.3.2.2 Fold the test specimen in four plies or more in the bag and immediately squeeze it as it is in the bag.

8.3.2.3 Collect the solution in the bag and take 0,1 ml of the solution by micropipette (6.3). Put the solution into the 2 wells of specific protein antibody coating plate (6.12).

Additionally, take 0,1 ml of suspension solution for specific protein (7.3) by micropipette (6.3) as negative control and put it into the 2 wells of the plate (6.12).

8.3.2.4 Keep them at 25 °C in the incubator (6.8) for (30 ± 5) min. Then, remove the solution from the well and wash the well three times with 300 µl of suspension solution of specific protein (7.3).

8.3.2.5 Determine the concentration of the test specific protein suspension by ELISA test method using the plate (see 8.3.2.4) described in Annex C.

8.3.3 Requirement for verification of the sensitivity of ELISA

Calculate the specific protein concentrations for the negative control and the test specimen. Obtain the values by using Formula (1). The requirements for verification of the sensitivity of ELISA shall satisfy Formula (1).

$$| (S_n - S_t) / S_n \times 100 | < 20\ \% \quad (1)$$

where

S_n is the average of the specific protein concentration (ng/ml) from 3 negative controls;

S_t is the average of the specific protein concentration (ng/ml) recovered from 3 specific protein reduction test specimens.

If untreated fabrics are used for the main test instead of negative control, the requirements for verification of the sensitivity of ELISA shall also satisfy Formula (2).

$$| (S_n - S_u) / S_n \times 100 | < 20\ \% \quad (2)$$

where S_u is the average of the specific protein concentration (ng/ml) recovered from 3 untreated fabric test specimens.