INTERNATIONAL STANDARD

ISO 18074

First edition
2015-12-01

Textiles — Identification of some animal fibres by DNA analysis method — Cashmere, wool, yak and their blends

Textiles — Identification de certaines fibres animales par la méthode d'analyse de l'ADN — Cachemire, laine, yack et leurs mélanges

Reference number
ISO 18074:2015(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

The committee responsible for this document is ISO/TC 38, *Textiles*.

Introduction

The composition of fibres in textile products is one of the most important properties. Labelling of composition of textile products is required globally by legislation or by voluntary regulation for fair trade.

The testing method to determine the composition of some animal fibres in the textile products has been developed as ISO 17751[3]. This is only one method to determine the animal fibre composition currently available. In this method, animal fibres are observed by microscope and identified from the shape of scales by experienced examiners. Many samples can be tested with a high degree of efficiency using this method. However, even experienced examiners have difficulties in identifying fibres, because textile products have a broad variety of colours and finishings, and there are many blends in animal fibres.

Given this situation, several testing methods to obtain the more accurate results have been investigated and developed. Among those methods, the DNA (deoxyribonucleic acid) analysis method has been found to be a practical and feasible method to identify the inherent type of animal fibres.

As it is well known, DNA is specific for animals. The DNA-PCR (polymerase chain reaction) method has recently been developed with high accuracy. A very trivial quantity of the DNA extracted from animal fibres is amplified by PCR to yield a huge quantity of copy DNA. Mitochondrial DNA is used for this analysis because it provides greater numbers than nuclear DNA.

Textiles — Identification of some animal fibres by DNA analysis method — Cashmere, wool, yak and their blends

WARNING — The use of this International Standard can involve hazardous materials, operations, and equipment. This International Standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations and supplier's requirement for safety prior to use.

1 Scope

This International Standard specifies a testing method for DNA analysis of some animal fibres to identify cashmere, wool, yak, and their blends by using extraction, amplification by the polymerase chain reaction (PCR) method and DNA detection processes.

This International Standard is applicable to cashmere, yak, and wool and their blends as a qualitative method.

2 Caution

Test results for fibre identification by the DNA analysis method can be obtained with a high accuracy for the above-mentioned textile products which were processed at lower dye concentration levels or dyed in light colours.

However, when such textile products were processed under severe conditions or high temperatures, the mitochondrial DNA could have been damaged. In such cases, identification can be difficult because amplification of DNA by PCR cannot take place. If textile products were contaminated by using products from another species, such as cashmere grease on wool fibres, this situation may be solved by checking using microscopy techniques.

3 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

4 Terms and definitions

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

4.1
DNA
deoxyribonucleic acid, that exists in nuclei and in mitochondria of animal fibre cells and is composed of a linear array of 4 bases (Adenine: A, Thymine: T, Guanine: G and Cytosine: C)

Note 1 to entry: The DNA sequence is identical and intrinsic for each animal fibre.

4.2
animal fibres
cashmere, wool, or yak fibres

4.3
buffer solution
solution used to maintain pH of reaction solution at required value

4.4
reducing agent
agent that degrades animal fibres through reductive cleavage of S-S bonds in the fibres

4.5
DNA amplification
amplification of the specific fragment of DNA by the PCR method

4.6
PCR method
method of the polymerase chain reaction

Note 1 to entry: The amplification process of the DNA fragment with a constant length is explained in Annex A.

4.7
DNA polymerase for PCR method
heat-stable DNA polymerase that is used for PCR and has no proof reading activity

4.8
primer
short length fragment of a single strand DNA which is a reaction initiator and designed as the identical sequence of 18-30 bases to DNA of the animal fibre

4.9
primer set
set of primer with the reaction direction of forward and reverse

4.10
primer for cashmere
primer with an identical base sequence of mitochondrial DNA of cashmere

Note 1 to entry: The sequence of base for primers will be submitted to the public database.

4.11
primer for wool
primer with an identical base sequence to mitochondrial DNA of wool

4.12
primer for yak
primer DNA with an identical base sequence to mitochondrial DNA of yak

Note 1 to entry: Information concerning the primers may be obtained from ISO/TC 38 secretariat.

4.13
gel electrophoresis migration
method to detect the amplified constant length DNA fragments

5 Principle

Mitochondrial DNA is extracted from animal fibre samples by using a chemical and enzyme reaction. The extracted DNA is purified by using a precipitation method and centrifuge. The purified DNA is applied for the amplification reaction of PCR method. In the PCR method, primers for cashmere, yak and wool are respectively tested. If the sample is cashmere, only cashmere primer can amplify the constant length of DNA fragments. Then, the constant length of DNA fragments is detected by the electrophoretic migration method.

The sample fibres are identified by knowing whether amplification was observed or not for the tests using all primers respectively.

6 Apparatus and equipment

6.1 Pipettes, capable of measuring and taking (0 to 20) µl (±0,20 µl), (20 to 200) µl (±1,60 µl), (200 to 1 000) µl (±8 µl) within systematic errors defined in ISO 8655-2.

6.2 Micro tube, capable of withstanding the centrifugation of 14 000*g* and autoclave.

The capacity is 2 ml for purification and 0,2 ml for PCR method. A tube of 0,2 ml for PCR method should follow the manufacturer's recommendation of the PCR instrument.

6.3 Cap lock, use for micro tube.

6.4 Heat block, with mounting holes for micro tubes and capable of heating up to about 80 °C (±1,0 °C).

6.5 Shaking agitator, capable of heating up to 50 °C and maintaining at the temperature of 50 °C and shaking micro tube at around 500 r/min or higher.

6.6 Shaking machine, capable of mounting micro tubes and shaking at around 500 times/min or higher.

This machine can be replaced by an equivalent instrument such as microtube rotator which is possible to rotate at 30 r/min or higher.

6.7 Centrifuge, capable of centrifuging of 14 000*g* or higher, setting up temperature from 0 °C to room temperature and mounting micro tubes or units of centrifugal ultrafiltration.

6.8 Unit of centrifugal ultrafiltration[1], capable of capturing molecules with the molecular weight of 100 kDa (Dalton) or more.

6.9 PCR instrument[2], capable of programing for temperature and time.

6.10 UV illuminator, UV irradiator.

6.11 Photo booth.

6.12 Generic plastic box with a resealable lid.

6.13 Comb, used for making wells in the agarose gel of the gel electrophoresis migration test.

6.14 Mixer mill, used for mixing and homogenizing animal fibres.

6.15 Erlenmeyer flask, with capacity 200 ml.

---

1) Amicon Ultra is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) Life Technologies Corporation is a provider of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

7 Reagents

7.1 Pure water.

Use pure water as defined in ISO 3696 with the purity of Grade 1. This water should not have DNase (DNA digesting) activity. It should not contain a significant amount of DNA which can be amplified by primers and should not show inhibitory effects for this testing method.

7.2 Chloroform/isoamyl alcohol reagent.

100 % concentration of the highest grade.

— Chloroform	9,6 ml
— Isoamyl alcohol	400 µl

7.3 Sodium perchlorate solution.

— Sodium perchlorate	6,1 g

Make the solution up to 10 ml by adding pure water.

7.4 1 mol/l Tris – HCl [tris(hydroxymethyl)aminomethane].

Dissolve 12,1 g of tris(hydroxymethyl)aminomethane in 800 ml of pure water, then adjust the pH to 8,0 by adding HCl and using a pH-meter. Then, make it up to 1 000 ml by adding pure water.

7.5 500 mmol/l EDTA (ethylenediaminetetraacetic acid).

Dissolve 186,1 g of EDTA·Na_2·$2H_2O$ (disodium salt dihydrate of EDTA) in 800 ml pure water, then adjust pH to 8,0 by adding NaOH and using a pH-meter. Then, make it up to 1 000 ml by adding pure water.

7.6 Buffer solution A.

— 1 mol/l Tris-HCL (7.4)	5 ml
— 500 mmol/l EDTA (7.5)	2 ml
— Sodium lauryl sulfate (SLS) ($C_{12}H_{25}SO_4Na$)	0,2 g
— Sucrose	1,2 g
— Sodium chloride	170 mg
— Dithiothreitol (DTT)	920 mg

Make it up to 10 ml by adding pure water. Prepare fresh before use.

Due to its volatile nature, DTT should be added after autoclaving, so that its effectiveness is not reduced.

7.7 Buffer solution B.

— 500 mmol/l EDTA (7.5)	0,2 ml

Make it up to 100 ml by adding pure water.

7.8 Protein resolving enzyme solution, the papain solution, with a papain of 10 units dissolved in pure water (e.g. 10 units/20 µl).

7.9 Heat-stable DNA polymerase, without 3‘ to 5‘ exonuclease activity.

7.10 Animal fibre A primer 1, forward primers for cashmere, yak, and wool.

7.11 Animal fibre A primer 2, reverse primers for cashmere, yak, and wool.

7.12 DNA composition component, with a grade for the PCR method.

7.13 Buffer solution for polymerase, suitable for the polymerase of the PCR reaction.

This buffer solution may be designated by manufacturers of polymerase.

7.14 Salt, potassium chloride (KCl), used to stabilize the PCR reaction.

7.15 Magnesium chloride ($MgCl_2$), used to stabilize the PCR reaction.

7.16 Gel electrophoretic migration marker.

7.17 Agarose, a kind of agar with a grade for DNA electrophoretic migration.

7.18 Buffer solution for loading on the electrophoretic migration.

—	Glycerol	36 g
—	500 mmol/l EDTA (7.5)	6 ml
—	Bromophenol Blue	0,25 g
—	Xylene cyanol	0,25 g

Make it up to 100 ml by adding pure water. After adding the solution of more than 1/6 to the sample reaction solution, load it on the gel.

7.19 Buffer solution for electrophoretic migration.

—	tris(hydroxymethyl)aminomethane base (Trizma base)	242 g
—	Acetic acid (glacial acetic acid)	57,1 ml
—	EDTA·2Na	7,43 g

Dissolve using pure water and make the solution up to 1 l by adding pure water. Then, dilute it 50 times with pure water.

7.20 DNA dyeing colorant, ethidium bromide.

Ethidium bromide dissolves in the buffer solution for electrophoretic migration so as to be approximately 5 µg/ml.

NOTE Other DNA intercalating agents can be used as substitute for ethidium bromide.

7.21 Common DNA fragment, primer.

Common DNA fragments are common base alignments existing in all the DNAs of cashmere, yak, and wool. Common primer 1 is a forward primer; common primer 2 is a reverse primer.

NOTE Information on primers can be obtained from ISO/TC 38 secretariat.

8 Sampling

Animal fibre samples shall represent the textile products for test. If the textile product is composed by several parts, separate them into identical parts and describe the details of the parts in the test report. Avoid contamination among the parts.

Two test specimens are selected from the sample. When the two results are not consistent, do not adopt the results and perform another test for two specimens again.

NOTE ISO 17751:2007, Annex B can be used as a reference for this procedure.

9 Test methods

9.1 General

The test should be performed in parallel for the primers for cashmere, yak, and wool.

9.2 Chipping sample

Chip the animal fibre sample of 100 mg with the length of less than 2 mm by scissors, mixer mill, or other instruments. Then, put the chipped sample of 50 mg into a micro tube[3] with a 2,0 ml capacity.

9.3 DNA extraction

Follow the procedure described in 9.3.1 to 9.3.6.

9.3.1 Add 600 µl of buffer solution A (7.6) to the micro tube with the test sample (9.2).

9.3.2 Cap the micro tube and then tumble it by hand to immerse the sample in the buffer solution perfectly.

9.3.3 Lock the cap of the micro tube using a cap lock. Then, heat the micro tube with the test specimen at 60 °C for 20 min using the heat block. Take out the micro tube at 3 min, 6 min, 9 min, and 15 min from the heat block and tumble it for 10 s by hand. Then, put it back on the heat block again and continue heating.

9.3.4 After 20 min, take out the micro tube from the heat block and cool it down to room temperature.

9.3.5 Add 10 units of papain solution (7.8) to the micro tube with the test specimen. Then, heat up the micro tube to 50 °C. Maintain the temperature at 50 °C and shake it at 500 r/min for 1 h using the shaking agitator (6.5).

9.3.6 Add 10 units of papain solution (7.8) to the micro tube of 9.3.5. Maintain the temperature at 50 °C. Shake it at 500 r/min for over 12 h. Cool it down to room temperature.

---

3) Eppendorf tubes are an example of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

