DRAFT INTERNATIONAL STANDARD

ISO/DIS 18074

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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, yak et leurs mélanges

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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.

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 18074 was prepared by Technical Committee ISO/TC 38, *Textiles*, WG22, *Composition and chemical testing*.

Introduction

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

The testing method to detemine the composition of some animal fibres in the textile products has been developed as ISO 17751:2007 "*Textiles -- Quantitative analysis of animal fibres by microscopy -- Cashmere, wool, speciality fibres and their blends*". This is only one method to determine the animal fibre composition currently. In this method animal fibres is observed by microscope and identified from the shape of scales by experienced examiners. The many samples can be tested with a high efficiency in this method. However, even though the exprienced examiners, they have been experiencing some difficulties to identify fibres, because textile products have broad varieties of the colours and finishings, and many blends in animal fibres.

Under the situation, the several testing methods to obtain the more accurate results has been investigated and developed. Among those methods, DNA (deoxyribonucleic acid) analysis method has been found out the practical and feasible method to identify the kind of animal fibres inherently.

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

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

WARNING — The use of this standard may involve hazardous materials, operations and equipments. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this 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 polymerase chain reaction (PCR) method and detection process of DNA.

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

2 Caution

Testing results for fibre identification by 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 by using severe conditions or high temperature, mitochondorial DNA may possibly have been damaged. In the cases, the identification may be difficult, because amplification of DNA by PCR can not take place.

3 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.

ISO139, *Textiles -- Standard atmospheres for conditioning and testing*

ISO 6938, *Textiles -- Natural fibres -- Generic names and definitions*

ISO 3696, Water for analytical laboratory use – specification and test methods

ISO 8655, Piston-operated volumetric apparatus

4 Terms and definitions

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

4.1
DNA
Deoxyribonucleic acid, that exsits in nuclei and in mitochondria of animal fibre cells and is composed of a linear array of 4 bases, as Adenine : A, Thymine: T, Guanine: G and Cytosine: C. 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 keep pH at desirable value of the reaction solution.

4.4
reducing agent
agent resolves the animal fibres by the reductive decomposition of S-S bonds of the protein.

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 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 should lack proof reading activity is specified for the PCR method.

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 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 The information of the primers may be obtained from ISO/TC38 secretariat.

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

5 Principle

Mitocondorial 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 the amplification was observed or not for the tests with using all primers respectively.

6 Apparatus and equipments

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

6.2 Micro tube, capable of withstanding the centrifugation of 14 000 x *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 recomendation 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 rpm or higher.

6.6 Shaking machine, capable of mounting micro tubes and shaking at around 500 times/minute or higher. This machine can be replaced by an equivalent instrument such as microtube rotator which is possible to rotate at 30 rpm or higher.

6.7 Centrifuge, capable of centrifuging of 14 000 x *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, capable of capturing molecules with the molecular weight of 100 kDa (Dalton) or more.

NOTE The unit is commercially available as Amicon Ultra, etc.

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

NOTE An instrument is commercially available from Life Technologies Corporation. Equivalent instruments of other suppliers are also available.

6.10 UV illuminator, UV irradiator.

6.11 Photo booth

6.12 Tupperware

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

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

6.15 Erlenmeyer flask, capacity of 200 ml

7 Reagents

7.1 Pure water

Pure water is defined in ISO 3696 as with the purity of Grade 1, which should not have activity of DNase (DNA digesting) and should not contain significant amount of DNA which can be amplified by primers or 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 it up to 10 ml by pure water

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

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

7.5 500 mmol/l EDTA (ethylenediaminetetraacetic acid)

$EDTANa_2 \cdot 2H_2O$ (disodium salt-dihydrate of EDTA) of 186,1 g is dissolved in pure water of 800 ml, then adjust pH to 8,0 by adding NaOH and using pH meter. Then, make it up to 1 000 ml by pure water.

7.6 Buffer solution A

— 1 mol/l Tris-HCL (7.4) 5 ml

— 500 m mol/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 pure water.

NOTE Prepare fresh before use

7.7 Buffer solution B

— 1 mol/l Tris-HCl (7.4) 1 ml,