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Screening of genetically modified organisms (GMOs) in cotton and textiles

Criblage pour la détection des organismes génétiquement modifiés (OGM) dans le coton et les 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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Introduction

0.1 General

This purpose of this document is to provide guidance to laboratories worldwide to assess, in a standardized way, whether cotton, cotton fibre and/or cotton-derived materials are produced from, or contain materials from, genetically modified (GM) cotton plants. This document is intended for non-GM cotton and textiles production lines, but it can be applied to any production line that wants to check the presence of GM cotton.

0.2 Protocol

The GM screening protocol described in this document is based on Polymerase Chain Reaction (PCR)-based methods, as these methods are the minimal set of DNA-based methods to cover all known GM-cotton events. The protocol is written for and tested to work on all four of the major commercial cotton species: *Gossypium hirsutum*, *G. barbadense*, *G. arboreum G. herbaceum*.

Cotton (*Gossypium* spp.) has been cultivated for lint for over 8 000 years. There are over 50 species in the *Gossypium* genus (Wendel et al., 2009). The *Gossypium* genome is complex, containing 2,25 to 2,43 gigabase (Arumuganathan and Earle, 1991). While GM-cotton cultivation covers a large part of global cotton production today, there are countries where the cultivation of GM cotton is not allowed by law as well as voluntary private and/or public standards that do not allow the intentional use of genetically modified organisms (GMOs) in the cotton and textile production process. This creates a need for an adequate and harmonized protocol on the screening of cotton and cotton-derived materials for the potential presence of GM-cotton related sequences.

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This document describes a procedure to screen seed, leaf and (processed) fibre samples in the cotton production chain for the potential presence of GM-related DNA elements. The protocol describes three major steps:

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a) an effective way to isolate DNA from cotton materials;

b) a method to confirm that the isolated DNA consists of amplifiable cotton DNA, i.e. suitable for PCR, preferably a low copy nuclear target;

c) A screening method consisting of a minimum set of detection methods covering all the currently known GM cotton events, to be performed on the cotton DNA isolate.

If the results of the screening methods described in this protocol are 'not detected', the likelihood that the cotton sample is (at least partly) derived from GM cotton is minimal, based on the ability of the screening methods to detect elements and constructs of the GM cotton events. GM cotton levels below the detection limit of the method or unknown GM cotton events that do not contain any of the elements or the construct tested cannot be determined by this detection method. When one or more screening methods indicate that GM elements are present, the sample should be considered as derived from GM cotton.

Further investigation for the identification of GM-cotton events present in the sample is not part of this document as such, but some guidance is provided in Annex A as to how further identification of the related cotton events can be achieved.

0.3 Structure

The structure of this document is illustrated in Figure 1. Clause 4 describes the principle of the screenings protocol. Clause 5 describes sample preparation for different types of material. Clause 6 describes the DNA isolation method that allows for successful DNA isolation from the respective cotton-related products. Clause 7 describes the DNA quality control for the different cotton species. Clause 8 describes the screening of GM-related DNA sequences in a cotton sample. Clause 9 describes recommendations on the test report (outcome). Annex A gives an overview of known GMO cotton events. Annex B gives an overview of detection methods applied by RIKILT[1]. Annex C provides

1) https://www.wur.nl/en/Research-Results/Research-Institutes/rikilt.htm

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more information on the inhouse validation as carried out by RIKILT. Annex D provides a list of the contributos to the International Workshop.

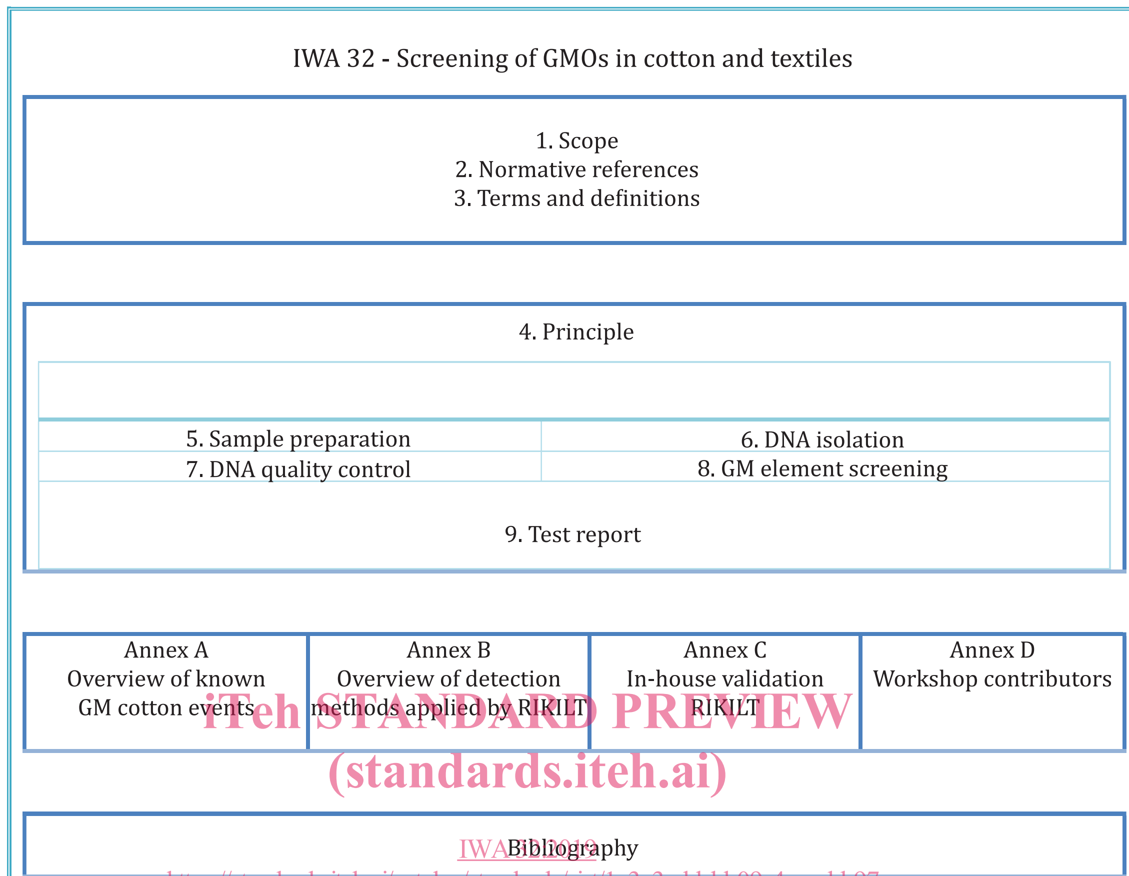

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Figure 1 — Structure of this document

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Screening of genetically modified organisms (GMOs) in cotton and textiles

WARNING — The method described in this document implies the use of reagents that pose a hazard to health. This document does not claim to address all associated safety problems. It is the responsibility of the user of this document to take appropriate measures for health and safety protection.

1 Scope

This document provides requirements and recommendations to laboratories that perform genetically modified organism (GMO) analyses in cottonseed, leaf, cotton fibre and cotton fibre-derived materials.

The following are within the scope of this document:

a) identifying the materials to be assessed, based on the probability of obtaining good quality, fit for purpose DNA from the materials in subsequent steps in the cotton cloth production process;

b) specifying a method for efficient DNA isolation from cotton and cotton-derived materials described under point a);

c) specifying the cotton-specific method(s) to be used as control for amplifiable DNA;

d) specifying the screening procedure that provides optimal chances to detect GMOs as a result of the performance of the lowest number of genetically modified (GM) element screening assays.

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NOTE 1 The protocol allows for the screening of all currently known GM cotton events and is set up in a way that optimizes the probability of also detecting unknown GM cotton events that possibly contain similar DNA sequences. Further information is given in CEN/TS 16707.

Sampling is outside of the scope of this document.

NOTE 2 A recommended sampling method is given in ISO 6497. General guidance for the sampling of bulk materials or for cotton-based products is available in standards such as ASTM D1441-12 and CEN/TS 15568.

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 21570:2005, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

ISO 24276:2006, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

3 Terms and definitions

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

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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 http://www.electropedia.org/

3.1
cottonseed
seed from cotton plants

3.2
cotton leaf
leaves from the cotton plant

3.3
seed cotton
raw cotton that contains both the seed and the fibre before it has been ginned

3.4
cotton lint
raw fibre that has gone through the ginning process

3.5
greige yarn
unprocessed long continuous length of interlocked cotton lint that results from the cleaning and subsequent spinning of the cotton lint

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3.6
greige fabric
unprocessed textiles formed by weaving, knitting or crocheting the yarn and non-wovens

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3.7
processed yarn
yarn that has undergone processing, to develop its full textile potential

3.8
processed fabric
fabric that has undergone processing, to develop its full textile potential

4 Principle

This document describes a method for the screening of GMO in cotton and textiles. The screening is based on realtime PCR methods which depends on obtaining good quality amplifiable DNA. Good quality DNA samples (those fit for purpose) are defined as those where the amplification of an endogenous cotton gene (positive control) is observed. The amplification and detection of endogenous cotton is achieved through isolation methods that result in good quality DNA, applied to cotton and textiles, while the targeted amplification of six genetic elements can allow for the detection of GM-cotton in these samples.

NOTE Experimental results have shown that good quality DNA can be isolated from the production stages of cottonseed up to greige yarn and greige fabric, while it showed not to be possible to isolate amplifiable DNA in processed yarn and processed fabric. Processed yarn and processed fabric are therefore excluded from this protocol. See Clause C.3 for the assessment of isolation of good quality DNA at different cotton production stages by RIKILT.

5 Sample preparation

Homogenize the sample using suitable methods and avoiding excessive heating.

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Sample preparation is dependent on sample type. Prepare samples by using either one of the following techniques: 'teasing', 'cutting', 'crushing' or 'shredding'.

Prepare at least two replicates per sample. Include appropriate controls, as specified in ISO 21571 on DNA extraction.

The recommended sample preparation for different types of material is as follows.

— Cottonseed: Crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA isolation procedure.

— Cotton leaf: Crush the leaves thoroughly with a suitable method. Use 100 mg in the DNA isolation procedure.

— Seed cotton: Seperate the seeds from the fibres, crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA isolation procedure.

— Cotton lint: The fibre material can be teased thoroughly applying suitable method. Use 100 mg in the DNA isolation procedure.

— Yarn: Cut the yarn with a suitable method into small parts of a maximum of approximately 0,5 cm length. Use 100 mg in the DNA isolation procedure.

— Fabric: Cut the fabric with a suitable method in small parts of a maximum of approximately 0,5 × 0,5 cm in size. Use 100 mg in the DNA isolation procedure.

6 DNA isolation

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6.1 General

In order to obtain amplifiable DNA from cottonseed, cotton and textiles as per the protocol's scope, a DNA isolation method has been selected that allows for successful DNA isolation from the respective cotton-related products. This method allows for rapid purification of genomic DNA suitable for PCR with a limited number of protocol steps. The protocol works well for cotton-derived materials that can contain relatively high levels of PCR inhibitors.

NOTE 1 The DNA isolation procedure described in this document is the QIAamp® Fast DNA Stool Mini Kit. The rest of this protocol refers to the QIAamp® Fast DNA Stool Mini Kit[2].

NOTE 2 As an alternative strategy to the DNA isolation method described below, the cotton-adjusted CTAB-protocol (e.g. CRLVL-14/05XP: JRC 2006) or any other suitable DNA isolation method can be applied, provided that this method has been proven by means of in-house validation against the QIAamp® Fast DNA Stool Mini Kit to perform equally well or better compared to the QIAamp® Fast DNA Stool Mini Kit. For seed, certified reference materials are used for validation.

6.2 Principle

The DNA isolation procedure is based on an inhibition buffer, a lysis buffer and a DNA-binding spin column. DNA binds specifically to the silica-gel membrane in the spin column, while contaminants pass through. No phenol-chloroform extraction is required. PCR inhibitors are separated from DNA by the inhibition buffer.

2) QIAamp® Fast DNA Stool Mini Kit 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.

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6.3 Chemicals, reagents and equipment

Use only reagents of recognized analytical grade. Appropriate facilities should be used in order to avoid contamination during the steps of preparation and measurement (e.g. uses of laminar flow benches or comparable clean facilities)[3].

Unless otherwise stated, only reagents that conform to the specifications of ISO 24276 were used.

6.3.1 Reagents

6.3.1.1 Inhibition buffer: contains lithium chloride (>=1 – 10 % w/w) and sodium dodecyl sulfate (>=1 - <10 % w/w) (e.g. Inhibitex Buffer Qiagen Cat No./ID: 51604), as provided by the manufacturer.

6.3.1.2 Lysis buffer: lysis buffer contains guanidine hydrochloride (>=30 - <50 % w/w) and maleic acid (>=0.1 - <1 % w/w), as provided by the manufacturer.

6.3.1.3 Wash Buffer 1; ethanol solution to denature proteins contains guanidine hydrochloride (>=50 - <70 % w/w)), as provided by the manufacturer.

6.3.1.4 Wash Buffer 2: Tris-based ethanol solution to remove salts, contains sodium azide), as provided by the manufacturer.

6.3.1.5 Ethanol 96 % to 100 %.

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6.3.1.6 Elution Buffer: contains 10 mM Tris-HCl pH8.3, 0.1 mM EDTA, 0.04 % NaN_3 (sodium azide).

6.3.1.7 Proteinase K (>=1 - <10 % w/w).

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6.3.1.8 Molecular biology grade water or water of equivalent purity.

6.3.1.9 DNA degrading solution (e.g. 1 % bleach) **household bleach** (hypochloric acid).

6.3.2 Apparatus and equipment

6.3.2.1 Silica-based mini spin columns, as provided by the manufacturer.

6.3.2.2 Disposable spatulas.

6.3.2.3 Sterile filter pipette tips protecting against aerosols.

6.3.2.4 Microcentrifuge tubes of 1,5 ml and 2,0 ml.

6.3.2.5 Disposable gloves (powder-free).

6.3.2.6 Analytical scale and top weigher.

6.3.2.7 Waterbath and/or thermoshaker (e.g. 24 ml × 2,0 ml).

6.3.2.8 Centrifuge for microcentrifuge tubes (at least 20 000 x g).

3) Reference to a given product is given for for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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6.3.2.9 Suitable prepared homogenization equipment.

6.3.2.10 Autoclave, 121 °C, 20 minutes.

6.3.2.11 Pipettes (1-10 µl, 2-20 µl, 20-200 µl, 200-1 000 µl).

6.3.2.12 Vortex.

6.3.2.13 Refrigerator.

6.3.2.14 Freezer.

6.3.2.15 Clean lab coat.

6.4 Procedure

6.4.1 General

The DNA extraction procedure comprises the following steps:

— lysis of, and separation of, impurities from samples in guanidine hydrochloride-containing buffer;

— purification of DNA on mini spin columns.

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6.4.2 Protocol

All centrifugation steps should be carried out at room temperature (15 °C to 25 °C).

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Perform the DNA isolation according to the protocol of the chosen isolation method or see the manufacturer’s instructions.

— (1) Weigh 100 mg (+/− 10 mg) homogenized sample, as prepared in Clause 5, in a 2 ml microcentrifuge tube.

— (2) Add 1 ml inhibition buffer to each sample. Vortex continuously for 1 min or until the sample is thoroughly mixed.

— (3) Centrifuge sample at 20 000 x g for 1 min to pellet particles.

— (4) Pipette 25 µl proteinase K into a new 2 ml microcentrifuge tube.

— (5) Pipette 600 µl supernatant from step (3) into the 2 ml microcentrifuge tube containing proteinase K.

— (6) Add 600 µl lysis buffer and vortex for 15 s.

— (7) Incubate at 70 °C for 10 min.

— (8) Add 600 µl of ethanol (96 %) to the lysate, and mix by vortexing.

— (9) Carefully apply 600 µl lysate from step (8) to the silica-based spin column. Close the cap and centrifuge at 20 000 x g for 1 min. Place the silica-based spin column in a new 2 ml collection tube and discard the microcentrifuge tube containing the eluate.

— (10) Repeat step (9) until all of the lysate has been loaded on the column.

— (11) Carefully open the silica-based spin column and add 500 µl wash buffer 1. Centrifuge at 20 000 x g for 1 min. Place the silica-based spin column in a new 2 ml collection tube and discard the collection tube containing the eluate.

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