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Medical gloves made from natural rubber latex — Determination of water-extractable protein using the modified Lowry method

Gants médicaux à base de latex de caoutchouc naturel — Détermination des protéines extractibles par l'eau par la méthode modifiée de Lowry

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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 12243 was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 3, *Raw materials (including latex) for use in the rubber industry*.

Introduction

There have been problems of allergic reactions experienced by some users of medical gloves manufactured from natural rubber latex. ISO 12243 specifies a method for the determination of the water-extractable protein in such gloves.

Medical gloves made from natural rubber latex — Determination of water-extractable protein using the modified Lowry method

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard specifies a method for the determination of the amount of water-extractable protein in natural rubber (NR) gloves for medical use. The method is potentially suitable for the determination of extractable protein in other articles made from NR latex; however the extraction procedures and times have not been validated and will vary with the type of article to be tested. Other methods for the determination of specific proteins in medical gloves exist (see Annex C) but they are not of general applicability.

This International Standard is concerned solely with the method of assay. It is not concerned with sampling nor does it purport to address the safety implications of the values obtained or requirements for labelling.

2 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.

ISO 10282:2002, *Single-use sterile rubber surgical gloves — Specification*

ISO 11193-1:2002, *Single-use medical examination gloves — Part 1: Specification for gloves made from rubber latex or rubber solution*

3 Principle

Water-soluble proteins are extracted into a buffer solution and then precipitated to concentrate them and separate them from other water-soluble substances which may interfere with the determination (see Annexes A and D). The precipitated protein is redissolved and quantified colorimetrically by the modified Lowry method using a protein standard (for a general review of the method, see reference [1] in the Bibliography).

4 Terms and definitions

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

4.1
concentration factor
F
extent to which a protein extract is concentrated by precipitation followed by redissolution in a smaller volume of sodium hydroxide solution

NOTE Thus if the protein in 4 cm^3 of solution is precipitated and redissolved in 0,8 cm^3, then the concentration factor F would be 4/0,8 (= 5).

4.2
protein
proteins and protein-like substances (e.g. polypeptides) occurring in articles made from NR latex and which are extractable with water

4.3
modified Lowry method
modification of the original Lowry assay method, which involves the precipitation and isolation of the proteins to reduce the level of other water-extractable substances that may interfere in the determination

5 Apparatus

Unless otherwise stated, all laboratory equipment (i.e. flasks, tubes, etc.) shall be made of polypropylene or polyethylene.

NOTE Polypropylene or polyethylene equipment is specified rather than glass to minimize surface adsorption. A method for the determination of protein-binding capacity is described in Annex B.

5.1 Protein-free gloves, made from synthetic rubber latex or plastic and that are free of powder and other materials capable of being transferred to the test samples or extractant solutions.

5.2 Centrifuge, capable of reaching not less than 60 000 m/s^2 (6 000 × g).

NOTE A refrigerated centrifuge is preferred as it is possible for the temperature to rise considerably when centrifugation is carried out for prolonged periods.

5.3 Centrifuge tubes, capacity 200 cm^3, 50 cm^3, 10 cm^3, 2 cm^3 and 1,5 cm^3, made of polypropylene or polyethylene (if available) with a low protein-binding capacity.

5.4 Conical flasks, capacity 250 cm^3.

5.5 Micropipettes.

5.6 Test tube shaker, operating at between 3 Hz and 6 Hz.

5.7 Vortex mixer or **ultrasonic bath**.

5.8 Disposable filter, with a low protein-binding capacity and a pore size of 0,45 µm or less.

5.9 Clamps, for sealing gloves watertight during extraction. Pairs of aluminium bars lined with foam rubber which can be screwed together, or 170-mm-long plastic clips as used for haemodialysis, are suggested.

5.10 Spectrophotometric equipment.

5.10.1 Spectrophotometer, with disposable polystyrene cuvettes (quartz cuvettes may be used but require careful cleaning).

Or

5.10.2 Microplate reader, with flat-bottom polystyrene microtitre plates having 96 wells of 0,25 cm^3 to 0,5 cm^3 capacity.

NOTE Wells with a capacity of 0,5 cm^3 are preferred. Wells with a smaller capacity may be used but will reduce the sensitivity of the assay.

5.11 Balance, accurate to 0,000 1 g.

6 Reagents

During the assay, use only reagents of recognized analytical grade and distilled or deionized water.

6.1 Dye solution: Bromophenol blue, sodium salt, prepared by dissolving 0,1 g of bromophenol blue in 1 l of water. Discard the solution after four weeks.

6.2 Extractant solution: A buffer solution capable of maintaining pH 7,4 ± 0,4 throughout the extraction.

NOTE 1 Suitable buffers include phosphate buffer saline (PBS) solution (0,01 mol/l) and *N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulfonic acid hemisodium salt (TES) solution (0,1 mol/l). The PBS buffer is prepared by dissolving a PBS tablet in distilled water in accordance with the manufacturer's instructions. In the event that, at the conclusion of the extraction, pH 7,4 ± 0,4 is not achieved, it would be necessary to use a more concentrated buffer solution. The TES solution is prepared by dissolving 24 g of TES in 500 cm^3 of water and making the volume up to 1 l.

NOTE 2 PBS tablets and TES are widely available.

6.3 Modified Lowry protein assay reagents

6.3.1 Reagent A: Alkaline copper citrate, prepared fresh daily by mixing 10 parts of reagent C with 0,2 parts of reagent D.

Alkaline copper tartrate is also considered to be suitable. It shall also be prepared fresh daily. The material available in kits can contain undeclared preservatives which may affect the determination.

6.3.2 Reagent B: Dilute Folin reagent prepared by diluting 72 cm^3 of 2 N Folin reagent with 28 cm^3 of water.

NOTE 2 N Folin reagent is available commercially. It can, for example, be obtained from Sigma Chemical Co. (Catalogue No. F 9252), Box 14508, St Louis, MO 63178, USA. The concentration of some commercial Folin reagents may not be 2 N.

6.3.3 Reagent C: A solution of 6 g of sodium carbonate in 100 cm^3 of water.

6.3.4 Reagent D: A solution containing 1,5 g of copper sulfate and 3 g of sodium citrate in 100 cm^3 of water.

6.3.5 Sodium hydroxide solution, c(NaOH) = 0,2 mol/l.

6.3.6 Sodium deoxycholate (DOC) solution, prepared by dissolving 0,15 g of sodium deoxycholate in water and diluting with water to 100 cm^3. Store the solution in a refrigerator, discarding it after 4 weeks.

6.3.7 Trichloroacetic acid (TCA) solution, prepared by diluting 72 g of trichloroacetic acid to 100 cm^3 with water and mixing thoroughly. Store the solution in a refrigerator. The solution is stable over a long period.

6.3.8 Phosphotungstic acid (PTA) solution, prepared by diluting 72 g of phosphotungstic acid to 100 cm^3 with water and mixing thoroughly. Store the solution in a refrigerator, discarding it after 4 weeks.

It may be convenient to premix the TCA and PTA solutions in equal volumes and to add them simultaneously in 7.4.2. Such a mixture shall be prepared daily in the absence of data on its storage life.

6.4 Ovalbumin protein stock solution.

Use ovalbumin prepared by ammonium sulfate fractionation and repeated crystallization at pH 4,5 such as Sigma A 5503 from Sigma Chemical Co., Box 14508, St Louis, MO 63178, USA.

Prepare a solution of 100 mg of ovalbumin in 100 cm^3 of the preferred extractant (6.2) to give a concentration of 1 mg/cm^3. Filter the solution through a low-protein-binding filter of 0,45 µm or smaller pore size and determine the absorbance at 280 nm using a UV spectrophotometer with a 1 cm path length cuvette and employing extractant solution (6.2) as a blank. Divide the absorbance by 0,64 [1)] to obtain the precise concentration of the ovalbumin stock solution. The solution is stable for 2 days when stored at a temperature of not more than 7 °C or for 2 months frozen at −10 °C. Thawing requires heating to 45 °C for 15 min.

NOTE The length of time under refrigeration is cumulative. In order to avoid repeated thawing and freezing, it is recommended that the stock solution be stored as aliquot portions each sufficient for the preparation of a single calibration curve or for use in the verification procedure (see Annex A).

7 Procedure

7.1 Principle

The procedure involves the extraction of a whole glove followed by purification and concentration of the extract. The concentration of protein in the extract is determined by reference to a standard calibration curve prepared using dilutions of the protein stock solution (6.4 and 7.3) which has been concentrated in the same manner. The analytical technique of the analyst must previously have been verified as described in Annex A.

The extraction is run in triplicate using three gloves or pairs of gloves from a given lot; the purification and concentration of each extract and the subsequent determination are run singly.

7.2 Extraction procedure

7.2.1 General

The entire surface of the glove shall be exposed to the extractant at 25 °C ± 5 °C for a period of 120 min ± 5 min. Two extraction procedures are permitted, the so-called “cut-glove” procedure and also the “glove-in-glove” procedure. The procedure used shall be noted in the test report and all samples in a given series shall be extracted by the same procedure. The extraction shall be carried out in triplicate and single determinations run on each extract.

Use protein-free gloves (5.1) to handle the glove samples used for the extraction.

NOTE The frequency of sampling and left- or right-handedness of gloves are outside the scope of this document.

7.2.2 Procedure A — Cut-glove procedure

7.2.2.1 Record the mass of the glove (m) to an accuracy of not less than 0,001 g.

1) The precise value of the extinction coefficient of ovalbumin is subject to confirmation.

7.2.2.2 Cut the glove along the periphery. To facilitate the extraction, it is permissible to cut the glove into smaller pieces (but see 7.2.2.3).

7.2.2.3 If the result is to be reported in micrograms per unit area of the glove (e.g. μg/dm^2), determine the surface area of the glove as follows:

Cut a rectangular piece from the back of the glove of about 0,5 dm by 0,5 dm and measure its dimensions accurately. Calculate the area A_1.

Determine the mass (m_p) of the rectangular piece to the nearest 0,001 g.

The total surface area A of both sides of the glove is given by $A = 2A_1 \times m/m_p$.

7.2.2.4 Transfer all the pieces of the glove to a suitable conical flask (5.4).

7.2.2.5 Add accurately a volume V of extractant (6.2). The total volume V of extractant used shall be between 10 cm^3 and 15 cm^3 per gram of glove and sufficient to cover the pieces.

7.2.2.6 Extract the test sample at 25 °C ± 5 °C for 120 min ± 5 min, shaking for 15 s initially and thereafter at intervals not greater than 30 min. If practical, continuous slow shaking is desirable.

7.2.2.7 Decant off the extract and remove any particulate matter by centrifuging at not less than 20 000 m/s^2 (2 000 × g) for 15 min. The extract is preferably used immediately but may be stored for up to 48 h at a temperature of not more than 7 °C or frozen for up to 15 days at below −10 °C.

7.2.3 Procedure B — Glove-in-glove procedure

7.2.3.1 Take two gloves and determine the mass of each one to an accuracy of not less than 0,001 g (m_1 and m_2). Mark each glove at a point on the cuff 20 cm from the tip of the middle finger. Take one glove and insert it inside the other so that they fit together (this can be done conveniently using rods to insert the thumb into the thumb, etc.; however, the method of doing this is not critical as long as the gloves are exposed to minimum handling). Repeat the process with two further pairs of gloves of the same size.

7.2.3.2 Pour sufficient dye solution (6.1) into the inner glove to fill all of the fingers. Introduce 25 cm^3 of extractant (6.2) between the inner and outer glove. Manipulate gently to remove any air bubbles and seal the gloves with a clamp (5.9) at the 20 cm mark.

7.2.3.3 Fix the gloves to a shaker and shake for 120 min ± 5 min at 25 °C ± 5 °C. If small droplets of liquid are noted on the outer surface, suggesting the presence of pinholes in the outer glove, discard the samples and repeat the extraction with a fresh pair of gloves.

7.2.3.4 Remove the clamp and separate the gloves carefully, taking care not to contaminate the extract with the dye solution in the inner glove.

7.2.3.5 Decant the extract from the outer glove into a centrifuge tube (5.3). If it is coloured blue, it is indicative of a pin-hole or cross-contamination. In such cases, discard the solution and repeat the extraction with a fresh pair of gloves. Clarify the extract by centrifugation at not less than 20 000 m/s^2 (2 000 × g) for 15 min. Store the extract at a temperature of not more than 7 °C and carry out the determination within 48 h. Alternatively, frozen aliquots of the extract may be stored at −10 °C or lower for up to 15 days.

7.2.3.6 Cut both gloves at the 20 cm mark to remove the cuffs. Remove surplus liquid from the cuffs by blotting and allow to dry at room temperature. Determine the mass of the cuffs (m_c) to an accuracy of not less than 0,001 g. Calculate the average mass (m_s) of the extracted part of the gloves: $m_s = (m_1 + m_2 - m_c)/2$ where m_1 and m_2 are the masses of the original gloves and m_c is the combined mass of the un-extracted cuffs.