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Medical Gloves Made from Natural Rubber Latex - Determination of Water-extractable Protein Using the Modified Lowry Method

天然乳胶医用手套水抽提蛋白质的测定 改进 Lowry 法

(ISO 12243:2003, IDT)

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WARNING---users of this Standard shall be familiar with general laboratory operations. This Standard does not address any safety issues, even if they are related to it. Users shall establish corresponding safety and health specifications and ensure that they comply with national regulations.

1 Scope

This Standard specifies the determination of water-extractable protein content in medical gloves made from natural rubber latex. It is also applicable to the determination of water-extractable protein content in other natural rubber latex products. However, the extraction process and frequency have not been validated and may vary with the type of test sample. Appendix C introduces alternative methods for the determination of several specific proteins in medical gloves, but these methods are not universally applicable.

This Standard only addresses analytical methods and is not related to sampling. It also does not address safety or marking requirements for the determination results.

2 Normative References

The clauses in the following documents become clauses of this Standard through reference. For dated referenced documents, all subsequent amendments (excluding errata) or revisions are not applicable to this Standard. However, parties to agreements based on this Standard are encouraged to investigate whether the latest versions of these documents can be used. For undated referenced documents, the latest versions apply to this Standard.

GB 7543-2006 Single-use Sterile Rubber Surgical Gloves (ISO 10282:2002, IDT)

GB 10213-2006 Single-use Medical Rubber Examination Gloves (ISO 11193-1:2002, IDT)

3 Principle

Use a buffer solution to extract water-soluble proteins, and then, through precipitation and concentration, separate them from other water-soluble substances that may interfere with the determination (see Appendix A and Appendix D); re-dissolve the precipitated proteins, and through colorimetric analysis with a standard protein as the reference, and using the modified Lowry method, quantitatively determine the protein content (for an overview of this method,

see Bibliography [1]).

4 Terms and Definitions

The following terms and definitions apply to this Standard.

4.1 concentration factor *F*

The ratio of the volume of the extracting solution used to precipitate the protein to the volume of the sodium hydroxide solution used to re-dissolve the protein precipitate.

NOTE: if 4 mL of protein extracting solution is used for precipitation, and then, the precipitate is re-dissolved with 0.8 mL of sodium hydroxide solution, then, the concentration factor *F* is: 4/0.8 = 5.

4.2 protein

Water-extractable protein and protein-like substances (for example, peptides) present in natural rubber latex products.

4.3 modified Lowry method

This method is a modification of the original Lowry method. By precipitating and separating the proteins, it reduces the potential interference from other extractable substances in the determination.

5 Equipment

Unless otherwise specified, all laboratory utensils (such as flasks, test tubes, etc.) are made from polypropylene or polyethylene.

NOTE: polypropylene or polyethylene utensils have lower protein adsorption than glassware. The method for determining protein adsorption capacity is shown in Appendix B.

5.1 Protein-free Gloves

Gloves that are made from synthetic latex or plastic, powder-free and free of other substances that may transfer to the specimen or extracting solution.

5.2 Centrifuge

It shall reach at least 60,000 m/s^2 (6,000 *g*).

NOTE: temperatures may rise with prolonged centrifugation time; a refrigerated centrifuge is preferred.

5.3 Centrifuge Tubes

Polypropylene or polyethylene (if applicable) centrifuge tubes with low protein adsorption, with a capacity of 200 mL, 50 mL, 10 mL, 2 mL or 1.5 mL.

5.4 Conical Flasks

With a capacity of 250 mL.

5.5 Micropipette.

5.6 Test tube vibrator

With a vibration frequency of 3 Hz ~ 6 Hz.

5.7 Vortex mixer or sonicator.

5.8 Disposable filter membranes

Filter membranes with low protein adsorption and a pore size of 0.45 μm or smaller.

5.9 Clamp holder

A device used to hold the gloves and maintain a watertight seal during the extraction process. The clamp holder can be a pair of aluminum strips connected to foam rubber and can be fastened together by screws, or a 170 mm long plastic clamp for hemodialysis.

5.10 Spectrophotometric apparatus

5.10.1 Spectrophotometer

Equipped with disposable transparent polystyrene cuvettes (quartz is acceptable but must be very clean).

5.10.2 Microplate reader

A 96-well polystyrene flat-bottom microplate reader with a capacity of 0.25 mL ~ 0.5 mL.

> **NOTE:** a 0.5 mL well plate is preferred; smaller well plates may be used, but this will reduce analytical sensitivity.

5.11 Balance

Accurate to 0.0001 g.

6 Reagents

During the test, all reagents used are analytically pure and distilled or deionized water.

6.1 Coloring agent: bromophenol blue (sodium salt). Dissolve 0.1 g of bromophenol blue in 1 L of water. It shall remain valid for 4 weeks.

6.2 Extracting solution: a buffer solution capable of maintaining a pH within the range of 7.4 ± 0.4 throughout the extraction process.

NOTE 1: suitable buffer solutions include 0.01 mol/L phosphate buffer solution (PBS) and 0.1 mol/L *N*-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid sodium salt (TES) buffer solution. Phosphate buffer solution is prepared by dissolving phosphate in distilled water in accordance with the product instructions. If the pH value of the buffer solution does not reach 7.4 ± 0.4, a phosphate solution with a higher concentration may be necessary. TES buffer solution is prepared by dissolving 24 g of TES in 500 mL of water, and then, use water to dilute it to 1 L.

NOTE 2: PBS and TES are commercially available.

6.3 Modified Lowry protein analytical reagent

6.3.1 Reagent A: alkaline copper citrate solution. Mix 10 portions of Reagent C and 0.2 portions of Reagent D. Prepare on the day of testing.

Alkaline copper tartrate may also be used and shall also be prepared on the day of testing. The complete reagent kit may contain unspecified preservatives that may affect the testing.

6.3.2 Reagent B: a dilute solution obtained by adding 72 mL of 2 mol/L Folin reagent to 28 mL of water.

NOTE: 2 mol/L Folin reagent is commercially available, for example, from Sigma Chemical Company (Box 14508, St. Louis, MO 63178, USA) (Catalog No. F 9252). Some high-concentration Folin reagents used in industry may not reach 2 mol/L.

6.3.3 Reagent C: 6 g of sodium carbonate dissolved in 100 mL of water.

6.3.4 Reagent D: 1.5 g of copper sulfate and 3 g of sodium citrate dissolved in 100 mL of water.

6.3.5 Sodium hydroxide solution: c(NaOH) = 0.2 mol/L.

6.3.6 Sodium deoxycholate solution (DOC): use water to dissolve 0.15 g of sodium deoxycholate and dilute to 100 mL. Perform refrigerated storage, and it shall remain valid for 4 weeks.

6.3.7 Trichloroacetic acid (TCA) solution: use water to dissolve 72 g of trichloroacetic acid, dilute to 100 mL, and thoroughly mix it. Refrigerated storage allows for stable preservation for a long period of time.

6.3.8 Phosphotungstic acid (PTA) solution: use water to dissolve 72 g of phosphotungstic acid and dilute to 100 mL. Thoroughly mix it and store it in a refrigerating chamber. It shall remain valid for 4 weeks. For convenience, the TCA and PTA solutions can be pre-mixed simultaneously in equal volumes in accordance with the step of 7.4.2. However, there is no shelf life data for this mixed solution, so it can only be mixed on the day of testing.

6.4 Ovalbumin stock solution: ovalbumin is obtained by fractionation with ammonium sulfate and repeated crystallization at pH = 4.5. For example, it can be obtained from Sigma Chemical Company (Box 14508, St. Louis, MO 63178, USA) (Catalog No. A 5503).

Dissolve 100 mg of ovalbumin in 100 mL of the extracting solution (6.2) to prepare a stock solution with a concentration of 1 mg/mL. Filter the solution through a 0.45 μm (or smaller pore size) low-protein-adsorption filter membrane (5.8). Use a UV spectrophotometer and a cuvette (with a 1 cm optical path), and at a wavelength of 280 nm, determine the protein absorbance. Use the extracting solution (see 6.2) as a blank. Then, divide the absorbance by 0.64[1)] to obtain the precise concentration of the ovalbumin stock solution. At or below 7 °C, the solution can be stably stored for 48 hours, or frozen at –10 °C for 2 months. Thawing requires heating to 45 °C and holding for 15 minutes.

NOTE: refrigeration time is cumulative. To avoid repeated freezing and thawing, it is recommended to store the protein stock solution in several portions, each sufficient for preparing a calibration curve or for use in the validation test procedure (see Appendix A).

7 Extraction Procedures

7.1 Principle

The test procedures include extraction of the entire glove, purification and concentration of the extracting solution. Use diluent of the protein stock solution (see 6.4 and 7.3) concentrated using the same method to draw the standard curve. Determine the protein concentration in the extracting solution against the standard curve. The analytical skills of the analyst must be validated in accordance with Appendix A.

Take 3 gloves or 3 pairs of gloves from a given batch to perform parallel determination by 3 portions. Each extracting solution is independently purified, concentrated, and finally determined.

7.2 Extraction

7.2.1 Overview

At (25 ± 5) °C, fully unfold the glove surface and extract in the extracting solution for (120 ± 5) min. Extraction can be performed using either the “cut-glove” or “glove-in-glove” extraction method. The extraction method used must be stated in the test report. All samples of the same type shall be extracted using the same method. During extraction, 3 parallel tests shall be performed, and each extracting solution shall be separately determined.

During the extraction process, wear protein-free gloves (see 5.1) to handle the test glove samples.

NOTE: sampling and the distinction between left and right gloves are beyond the scope of this

[1)] The exact value of the extinction coefficient of ovalbumin has been confirmed.

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