

Session 7:

Product Safety and Biocompatibility Issues



World Health
Organization



Session Objectives

- Understand safety and biocompatibility requirements for prequalification per the WHO Specification
- Identify required documentary evidence
- Discuss ISO 10993 compliance
- Identify other safety issues

Safety and Biocompatibility Requirements for Prequalification

- WHO Specification requires manufacturer to provide documentary evidence of:
 - ISO 10993 compliance
 - Protein allergies
 - Dusting powders
 - Smell

Introduction

- Verification of conformity to these requirements will be part of the documentation assessment
- Verification may also be done at random checks or in case of product complaints

ISO 10993: Biological Evaluation of Medical Devices

Relevant parts for condom manufacturers:

- Part 1: Selection and evaluation of tests
- Part 5: Cytotoxicity
- Part 10: Irritation and sensitization

ISO 10993, Part 1: Evaluation and Testing

Issues to be considered in the biological evaluation of male latex condoms:

- Materials of manufacture
- Compound ingredients, process contaminants, and residues
- Leachable substances
- Lubricants and other dressing materials
- The properties and characteristics of the final product

ISO 10993, Part 1: Evaluation and Testing (continued)

Biological reevaluation shall be done when any changes occur in:

- The source or in the specification of the materials used in the manufacture of the product
- The formulation, processing, and primary packaging
- The final product during storage

or

- When there is any evidence that the product may produce adverse effects when used in humans

ISO 10993, Part 1: Evaluation and Testing (continued)

Categorization of male latex condoms according to:

- The nature of body contact:
 - Surface contacting device: skin and intravaginal mucosal membranes
- The duration of contact:
 - Limited exposure (A): devices with single use or contact up to 24 hours

ISO 10993, Part 1: Evaluation and Testing (continued)

Initial evaluation test for male latex condoms:

- Cytotoxicity (ISO 10993: Part 5)
- Sensitization (ISO 10993: Part 10)
- Irritation (ISO 10993: Part 10)

ISO 10993, Part 5: Test for *In Vitro* Cytotoxicity

- Test on extracts: qualitative and quantitative evaluation of cytotoxicity
- Test by direct contact: qualitative and quantitative evaluation of cytotoxicity
- Test by indirect contact: qualitative evaluation of cytotoxicity

ISO 10993, Part 5: Test for *In Vitro* Cytotoxicity (continued)

- Qualitative evaluation: examine the cell microscopically, using cytochemical stain if desired
- Cytotoxicity scale:
 - 0: noncytotoxic
 - 1: mildly cytotoxic
 - 2: moderately cytotoxic
 - 3: severely cytotoxic

ISO 10993, Part 5: Test for *In Vitro* Cytotoxicity (continued)

- Quantitative evaluation—measure one or more of the following parameters:
 - Cell death
 - Inhibition of cell growth
 - Cell proliferation or colony formation

ISO 10993, Part 10: Tests for Irritation and Delayed- Type Hypersensitivity

- Specifies procedure for assessing medical devices and constituent materials
- Focus on potential to produce irritation and delayed-type hypersensitivity
- Includes pretest considerations, test procedures, and key factors for interpretation of results

ISO 10993, Part 10: Tests for Irritation and Delayed- Type Hypersensitivity (continued)

In vitro irritation tests:

- Transcutaneous Electrical Resistance (TER) test
- EPISKIN test
- Both internationally validated as alternative tests to assess the skin corrosivity of chemicals
- Can be performed with the finished product and/or extracts thereof
- No validated methods to assess skin irritancy yet exist

ISO 10993, Part 10: Tests for Irritation and Delayed- Type Hypersensitivity (continued)

Important factors affecting the results of irritation studies:

- Nature of the device used in a patch test
- Dose of the test material
- Method of application of test material
- Degree of occlusion
- Application site
- Duration and number of exposures
- Techniques used in evaluating test

Small Group Exercise

- Groups are given a list of hypothetical changes to formulation or processes at a factory
- Groups should identify which changes would require retesting for any of the biocompatibility requirements and which tests would need to be done

Other Safety Issues

- Nitrosamines
- Protein allergies
- Dusting powders
- Smell

Group Discussion

- **What are you now doing to monitor these?**
 - Nitrosamines
 - Protein allergies
 - Dusting powders
 - Smell

Nitrosamines

- Problem: some are carcinogenic
- Some processing chemicals used for rubber compounding produce nitrosamines
- No limits for condoms; are limits for baby teats

How to Keep Nitrosamine Levels Low

- Nitrosamines are only a problem if they come out of the condom, so only extractable nitrosamines matter
- Extract as much as possible by effective leaching
- Use accelerators that minimize N-nitrosamines

Allergies

- Problem: Proteins in rubber can cause severe allergic reactions (allergic reactions to condoms are rare [except in people already sensitized])
- Caused by residues of accelerators or proteins
- Two approaches to testing: Lowry and ELISA
- ASTM standard

Minimizing Leachable Proteins

- Only proteins that leach out of the rubber can cause harm
- Increasing the leach time and temperature will remove more of them during production
- Use de-proteinized latex?

Dusting Powders

- Problem:
 - **Cornstarch** may promote protein transfer to users
 - **Silica** may cause silicosis if inhaled (in factory)
 - WHO does not allow **lycopodium** and **talc**
- Maximum recommended amount of powder is 50 mg per condom
- ASTM and ISO standards to measure powder content of gloves

Smell

- Problem:
 - Odorific chemicals can build up in sealed condom pack
 - Can make the condom smell strongly when it is opened
 - A bad smell is a major barrier to condom use
- Assessment of smell is mainly subjective

Smell

(continued)

- Unpleasant smells are often due to accelerators
- Choose chemicals that do not impart a strong smell
- Do not use excess quantities
- Test the smell on a panel of people who DO NOT work in the factory

Questions?

Product Safety/Biocompatibility Group Exercise

Instructions: Discuss the following possible changes to a production process. Consider whether any of them would require repetition of any of the biocompatibility tests.

1. A change in the maturation conditions for the latex, from 24 hours at 50°C to 48 hours at 45°C.
2. A change of antioxidant level from 1.3% to 1.0%.
3. A change of antioxidant level from 0.7% to 1.3%.
4. A change of accelerator from PX to Zinc dibutyl dithiocarbamate.
5. A reduction in sulphur level from 1.2% to 0.8%.
6. The addition of a stabilizer to the compounded latex.
7. A change of powder used in after-processing.
8. A change in the leaching solution.
9. A change from hot leach to a cool leach.
10. A change from wet ET to dry ET.

Would any of these changes also require a repetition of the shelf life studies?

WARNING: This material is only a summary of the standard; do not use this material as a substitute for the complete official standard document.

Overview of ISO 10993: Biological Evaluation of Medical Devices

- **Part 1: Evaluation and testing**
- Part 2: Animal welfare requirements
- Part 3: Tests for genotoxicity, carcinogenicity, and reproductive toxicity
- Part 4: Selection of tests for interactions with blood
- **Part 5: Tests for *in vitro* cytotoxicity**
- Part 6: Tests for local effects after implantation
- Part 7: Ethylene oxide sterilization residuals
- Part 8: Selection and qualification of reference materials for biological tests
- Part 9: Framework for identification and quantification of potential degradation products
- **Part 10: Tests for irritation and delayed-type hypersensitivity**
- Part 11: Tests for systemic toxicity
- Part 12: Sample preparation and reference materials
- Part 13: Identification and quantification of degradation products from polymeric medical devices
- Part 14: Identification and quantification of degradation products from ceramics
- Part 15: Identification and quantification of degradation products from metals and alloys
- Part 16: Toxicokinetic study design for degradation products and leachables
- Part 17: Establishment of allowable limits for leachable substances
- Part 18: Chemical characterization of materials

Part 1: Evaluation and testing

Background:

- A description of the principles governing biological evaluation and testing of medical devices.
- A description of the categorization of devices based on the nature and duration of their contact with the body.
- Selection of appropriate tests.

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Important general principles:

- The selection and evaluation of a device for use in humans requires a structured programme of assessment, which includes a biological evaluation.
- The biological evaluation shall be planned, carried out, and documented by knowledgeable and experienced people capable of making decisions based on the nature of the various materials and test procedures available.

Issues to be considered in the biological evaluation of male latex condoms:

- Materials of manufacture.
- Compound ingredients, process contaminants, and residues
- Leachable substances.
- Lubricants and other dressing materials.
- The properties and characteristics of the final product.

Biological reevaluation shall be done when any changes occur in:

- The source or specification of the materials used in the manufacture of the product.
 - The formulation, processing, and primary packaging.
 - The final product during storage.
- OR
- Any evidence that the product may produce adverse effects when used in humans.

Categorization of male latex condoms according to:

- The nature of body contact:
 - Surface contacting device: skin and intravaginal mucosal membranes.
- The duration of contact:
 - Limited exposure (A): devices with single use or contact up to 24 hours.

Initial evaluation tests for male latex condoms:

- Cytotoxicity (described in ISO 10993:Part 5)
- Sensitization (described in ISO 10993:Part 10)
- Irritation (described in ISO 10993:Part 10)

Part 5: Tests for in vitro cytotoxicity

Background:

- Describes test methods to assess the in vitro cytotoxicity for biological evaluation of medical devices.
- These methods specify the incubation of cultured cells either directly or through diffusion.
- A minimum of three replicates shall be used for test samples and controls. If the results are inconsistent among the replicates, the tests shall be repeated.

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- **Test on extracts:** qualitative and quantitative evaluation of cytotoxicity.
- **Test by direct contact:** qualitative and quantitative evaluation of cytotoxicity.
- **Test by indirect contact:** qualitative evaluation of cytotoxicity.

Determination of cytotoxicity

Qualitative evaluation: examine the cell microscopically, using cytochemical stain if desired.

Cytotoxicity scale:

- 0: Noncytotoxic
- 1: Mildly cytotoxic
- 2: Moderately cytotoxic
- 3: Severely cytotoxic

Quantitative evaluation: measure one or more of the following parameters:

- Cell death
- Inhibition of cell growth
- Cell proliferation
- Colony formation

Part 10: Tests for irritation and delayed-type hypersensitivity

Background

- Specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and delayed-type hypersensitivity, including pretest considerations, details of the test procedures, and key factors for the interpretation of the results.

***In vitro* irritation tests**

- Two *in vitro* methods, the rat skin Transcutaneous Electrical Resistance (TER) test and the EPISKIN test, have been internationally validated as alternative tests to assess the skin corrosivity of chemicals. However, no validated methods to assess skin irritancy yet exist.
- The tests can be performed with the finished product and/or extracts thereof.

Important factors affecting the results of irritation studies

- Nature of the device used in a patch test.
- Dose of the test material.
- Method of application of the test material.
- Degree of occlusion.
- Application site.
- Duration and number of exposures.
- Techniques used in evaluating the test.

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Key factors in interpretation of test results

- Industry and consumer complaint data.
- Experience with devices containing similar components.
- Diagnostic test results in dermatologic clinics.
- Retrospective epidemiologic data.

Additional Safety Issues: Nitrosamines, Protein Allergies and Dusting Powders

Nitrosamines

- Nitrosamines are organic chemical compounds of the structure $R_1N(-R_2)-N=O$.
- Nitrosamines are a group of compounds which form from a reaction between secondary amines (often in proteins) and nitrosating agents, typically nitrites or oxides of nitrogen.
- Nitrosable compounds are those that can convert into nitrosamines (e.g., secondary amines).
- Nitrosamine formation can occur only under certain conditions.
- Some processing chemicals used for rubber compounding produce nitrosamines.
 - Tetramethylthiuram
 - Many dithiocarbamates
 - N-oxodiethylene benzothiazolylsulfenamide
- All rubber products containing dialkyl amine derivatives exhibit considerable levels of the corresponding nitrosamines
- **Nitrosamines can be carcinogenic**, and they may adversely affect the health of the liver, kidney, lungs, skin, and eyes.

N-Nitrosamines

- N-nitrosamines are secondary or tertiary nitrosamines with straight molecular links (*no rings*).
- The N is a location prefix indicating alkyl groups.
- About 300 N-nitrosamine compounds have now been tested, and about 90% are carcinogenic.
- The secondary N- nitrosamines are the types of nitrosamine of primary concern.
- Regulations limiting the level of nitrosamines in some rubber products have been established (e.g. baby teats).

The migration of substances from the product must not exceed the following limits:

European Union

0.01 mg/kg rubber for N-nitrosamines compounds

AND

0.1 mg/mg for N-nitrosatable compounds

USA

An action level of 10 ppb for individual nitrosamines

- There are no limits established for condoms.
- Responsible manufacturers will keep nitrosamine levels as low as possible.
- Target levels are about 20 micrograms per gram.
- Less is better.

How to keep nitrosamine levels low

- Nitrosamines are only a problem if they come out of the condom—so only extractable nitrosamines matter.
- Extract as much as possible by effective leaching.
- Use accelerators that minimize N-nitrosamines.

Reference: Proksch E. Toxicological evaluation of nitrosamines in condoms. *International Journal of Hygiene and Environmental Health*. 2001; 204(2-3):103-110.

Abstract: Volatile N-nitrosamines have been found in rubber products including gloves, balloons, toys, baby bottle teats, soothers, and condoms. N-Nitrosamines are potent carcinogens, and therefore, European legislation has limited the release of N-nitrosamines and N-nitrosatable compounds in teats and soothers to 0.01-0.1 mg/kg rubber, respectively. Previously, endogenous nitrosamine formation in the vagina has been suggested as a cause of cervical cancer. It was speculated that exogenous N-nitrosamines and N-nitrosatable compounds from condoms may also lead to genital cancer. Therefore, we reviewed the literature and calculated the risk for the induction of tumors by nitrosamines from condoms. In vitro BiauDET et al. (1997) found up to 88 ng nitrosatable compounds migrating from condoms to cervical mucous within 24 hrs. During sexual intercourse about 0.6 ng may migrate in the female genital mucous membranes because of the short contact to the condom, e.g. 10 min. Comparable amounts of nitrosamines may also migrate in the penile skin. Estimating 1500 contacts to condoms during lifetime (50 condoms/year for 30 years) this may result in the adsorption of up to 0.9 microgram nitrosamines in total. Animal studies in Syrian hamsters showed the induction of local and/or systemic tumors, in particular liver tumors, after topical application of nitrosamines to the skin or mucous membrane at a total dose of about 1 g. This dose exceeds the dose to be expected from contact with condoms by more than 1 million. Also, epidemiological studies do not support a role for condoms in the induction of cancer. The incidence of cervical cancer and liver tumors is high in developing countries, where condoms are seldom used. In addition, humans are regularly exposed to nitrosamines from food and tobacco smoke at a dose which is 1,000 to 10,000 fold higher than expected from condom use. In summary, the risk for the induction of tumors from nitrosamines in condoms is very low.

Protein allergies

- Rubber allergies are mainly associated with the use of medical gloves.
- Allergic reactions to condoms are rare (except in people who are already sensitized).
- Sensitization usually occurs only after continued exposure.
- There are two types of allergy:
 - Type IV: reaction to residual chemicals; usually relatively mild
 - Type I: reaction to proteins; can be very serious, but rarely fatal

Type IV allergies

- Reaction to residual chemicals; usually relatively mild.
- Usually limited to local skin reactions.
- Generally caused by accelerators.
 - Good to minimize residual accelerators (but remember antioxidant properties).

Type I allergies

- Reaction to proteins; can be very serious, but rarely fatal.
- May result in “immediate” reaction (as soon as five minutes after contact).
- Only about 10% of the proteins present are allergenic, but there are no specific tests for them.
- Protein transfer may be enhanced by use of cornstarch.
 - Ensure use of appropriate powder.

Types of Adverse Reactions

Reaction type	Symptoms	Cause
Type IV: Chemical hypersensitivity (cell-mediated allergy)	Eczema; appears at 48 to 96 hours post exposure by skin contact	Residues of chemicals used for processing, particularly thiurams and carbamates
Type I: Latex protein hypersensitivity (IgE-mediated allergy)	Immediate local itching; burning or discomfort; urticaria (hives) 5 to 60 minutes after contact; rhinitis; asthma; and in serious cases, anaphylaxis (rare)	Residual extractable proteins found in natural rubber latex products

Dusting powders

- Used to prevent the condoms from sticking together during manufacturing and to allow them to unroll easily.
- Common materials:
 - Cornstarch or cornflour
 - Magnesium carbonate - $MgCO_3$
 - Calcium carbonate – $CaCO_3$
 - Silica – SiO_2
- Maximum recommended is 50 mg per condom.
- Talc and lycopodium spores shall not be used.
 - Lycopodium linked to soft tissue granulomas.
 - Talc possibly linked to pulmonary issues and skin and ovarian cancer.
 - Silica may cause silicosis if inhaled (in factory).
 - Cornstarch may promote protein transfer to users.

Testing Protein Concentrations: The Lowry Method, the LEAP Method, and the ELISA-Inhibition Latex Antigen Test

The Lowry Method (ASTM D5712 [1999])

The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocaltey phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids [Lowry, 1951]. The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10–10.5.

The Lowry method is sensitive to low concentrations of protein. Dunn [1992] suggests concentrations ranging from 0.10–2 mg of protein per mL, while Price [1996] suggests concentrations of 0.005–0.10 mg of protein per mL. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. However, one can use very small volumes of sample that will have little or no effect on pH of the reaction mixture.

A variety of compounds will interfere with the Lowry procedure. These include some amino acid derivatives, certain buffers, drugs, lipids, sugars, salts, nucleic acids, and sulphhydryl reagents [Dunn, 1992]. Price [1996] notes that ammonium ions, zwitterionic buffers, nonionic buffers, and thiol compounds may also interfere with the Lowry reaction. These substances should be removed or diluted before running Lowry assays.

The Lowry test involves the reaction of latex proteins with an alkaline copper tartrate compound and the subsequent reaction of the protein-copper tartrate complex with Folin reagent, which results in a blue color detectable in a spectrophotometer. In the ASTM modified Lowry test as it applies to the detection of latex proteins, these proteins are first precipitated in order to remove interfering, water-soluble substances, and the Lowry test is performed only after the protein precipitation and reconstitution step. The Lowry test method detects latex proteins in the microgram range. The limit of quantitation of the test was determined by ASTM to be above 70 µg/g. This test is subject to interference by chemical accelerators, such as carbamates, thiurams, benzothiazoles, and guanidines, used in the latex glove manufacturing process and phenolic chemicals naturally found in the latex itself. Generally these substances increase color development, resulting in an inflated or false positive protein signal. The Lowry test has been standardized as an ASTM test method D5712 for the analysis of protein in NRL and is recognized by the USA FDA for determination of protein levels in medical gloves. Manufacturers can make protein level labeling claims of 50 µg/dm² glove surface or greater based on this test method.

The LEAP Method

LEAP, or Latex ELISA for Antigenic Proteins, measures latex proteins by using latex-specific antibodies of the rabbit to recognize them. The proteins of glove extracts are diluted to various concentrations and are adsorbed to plastic wells in microtiter plates. Then a known quantity of anti-latex antiserum is added and allowed to bind to the protein antigens. Once this antigen-antibody complex is formed, it is reacted with a second antibody that recognizes the antigen-antibody complex. This second antibody carries a color-tag on it that turns color when activated. The color reaction product can then be detected and measured in a spectrophotometer—the more color, the more latex protein originally on the plate. This method can detect nanogram quantities of latex protein.

However, one of the drawbacks to LEAP is interference by surfactant chemicals in latex glove products, which tends to decrease the amount of glove protein binding and thus result in underdetection of protein antigens. Another drawback is the lessened tendency of peptides or small pieces of protein to bind to the microtiter plate wells, so they are less likely to be available for detection. The detergent interference can usually be overcome by sufficient dilution of the sample protein to levels that still contain detectable protein, but do not contain enough detergent to interfere.

ELISA-Inhibition Latex Antigen Test (Enzyme-Linked ImmunoSorbent Assay) (ASTM D6499 [2003])

The ELISA-Inhibition test for antigenic proteins, measures latex antigens by using latex-specific antibodies of the rabbit to recognize them Yunginger [1994], Turjanmaa [1996]. ELISA-Inhibition is not the same as the LEAP test. In the ELISA-Inhibition assay, a known amount of standardized latex protein is bound or adsorbed to a solid support, e.g. plastic wells of a microtiter plate. This approach reduces the variability and surfactant interferences inherent in the LEAP assay because it relies on the binding of a standard preparation of NRL proteins to the plate and not an unknown glove extract. Then a known dilution of rabbit antiserum (latex-specific IgG antibody) is added to the plate, and mixed with one of a series of dilutions of the test glove extract. Higher levels of protein antigens in the test glove extract will inhibit the ability of the known quantity of IgG to bind with the known amount of standard latex protein on the plate. Hence the use of the term ELISA-Inhibition. The plate is then washed to remove the unbound glove extract and IgG. The amount of IgG that did bind to the microtiter plate is measured with a second anti-IgG antibody that recognizes it and produces a color. The color reaction product can then be detected and measured in a spectrophotometer: the lower the color (greater inhibition of binding), the higher the level of antigenic protein in the test glove extract that was available to prevent IgG's binding to the standard latex protein-coated plate. This method is sensitive and reproducible: nanogram quantities of latex antigens ($> 1 \mu\text{g}/\text{dm}^2$) can be detected, in contrast to the microgram quantities detectable by the Lowry method. The antigenic protein levels detected by the ELISA-Inhibition test depend on the standardization of latex protein concentrations bound to the microtiter plate, the reactivity of the anti-latex IgG rabbit antisera, and the quality of the assay conditions. Since the immune response in rabbits can produce a fairly consistent reactivity to latex proteins, the rabbit antiserum used in the ELISA-Inhibition test is

expected to be reliable in its ability to be blocked by the protein antigen in the test glove extract. The availability of standardized, uniformly reactive, pooled sera from a number of latex-immunized rabbits should allow latex antigenic protein test results to be more easily compared to one another. Currently, the ELISA-Inhibition test for latex antigenic proteins is standardized as an approved ASTM Standard Test Method. The ELISA-Inhibition test for latex antigenic protein levels in gloves should allow manufacturers to make lower protein labeling claims or claims specific to protein antigens in the future because of this tests increased specificity and sensitivity. The FDA currently does not allow manufacturers to claim protein levels below 50 micrograms per dm² of glove, due to the limit of detection of the Lowry method, but it may be possible in the future to label less than 50 micrograms of antigenic latex protein.

Lowry, O. H., N. J. Rosebrough, A.L. Farr and R. J. Randall, 1951. Protein measurement with the Folin-Phenol reagents. *J. Biol. Chem.* 193: 265-275.

Dunn, M. J., 1992. Protein determination of total protein concentration. Harris, E. L. V., Angal, S., [Eds], *Protein Purification Methods*, Oxford: IRL Press.

Price, N. C., 1996. *Proteins*, Labfax, Oxford: Academic Press.

ASTM Designation D5712-99: Standard Test Method for Analysis of Protein in Natural Rubber and its Products, September 1999

ASTM D6499-03 Standard Test Method for the Immunological Measurement of Antigenic Protein in Natural Rubber and its Products, ASTM, 2003.

Yunginger J., Jones R., Fransway A., Kelso J., Warner M., Hunt L. and Reed C. (1994) Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J. Allergy Clin. Immunol.* **93**, 836.

Turjanmaa K., Mäkinen-Kiljunen S., Alenius H., Reunala T. and Palosuo T. (1996) *In-vivo* and *in-vitro* evaluations of allergenicity of natural rubber latex (NRL) gloves used in healthcare: A nation-wide study. (Abstract) *J. Allergy Clin. Immunol.* **97**, 325.