BRITISH STANDARD

BS EN ISO
8871-4:2006

# Elastomeric parts for parenterals and for devices for pharmaceutical use —

## Part 4: Biological requirements and test methods

The European Standard EN ISO 8871-4:2006 has the status of a British Standard

ICS 11.040.20



BSI
British Standards

# National foreword

This British Standard was published by BSI. It is the UK implementation of EN ISO 8871-4:2006.

The UK participation in its preparation was entrusted to Technical Committee CH/212, IVDs.

A list of organizations represented on CH/212 can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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June 2006

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English Version

# Elastomeric parts for parenterals and for devices for pharmaceutical use - Part 4: Biological requirements and test methods (ISO 8871-4:2006)

Éléments en élastomère pour administration parentérale et dispositifs à usage pharmaceutique - Partie 4: Exigences biologiques et méthodes d'essais (ISO 8871-4:2006)

Elastomere Teile für Parenteralia und für Geräte zur pharmazeutischen Verwendung - Teil 4: Biologische Anforderungen und Prüfverfahren (ISO 8871-4:2006)

This European Standard was approved by CEN on 5 June 2006.

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Ref. No. EN ISO 8871-4:2006: E

## Foreword

This document (EN ISO 8871-4:2006) has been prepared by Technical Committee ISO/TC 76 "Transfusion, infusion and injection equipment for medical and pharmaceutical use" in collaboration with CMC.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2006, and conflicting national standards shall be withdrawn at the latest by December 2006.

This document supersedes EN ISO 8871:1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

### Endorsement notice

The text of ISO 8871-4:2006 has been approved by CEN as EN ISO 8871-4:2006 without any modifications.

EN ISO 8871-4:2006

INTERNATIONAL STANDARD

**ISO 8871-4**

First edition
2006-06-15

# Elastomeric parts for parenterals and for devices for pharmaceutical use —

## Part 4: Biological requirements and test methods

*Éléments en élastomère pour administration parentérale et dispositifs à usage pharmaceutique —*

*Partie 4: Exigences biologiques et méthodes d'essai*

Reference number
ISO 8871-4:2006(E)

# Foreword

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ISO 8871-4 was prepared by Technical Committee ISO/TC 76, *Transfusion, infusion and injection equipment for medical and pharmaceutical use*.

This first edition, together with parts 1, 2, 3 and 5, cancels and replaces ISO 8871:1990 and ISO 8871:1990/Amd.1:1995, which has been technically revised.

ISO 8871 consists of the following parts, under the general title *Elastomeric parts for parenterals and for devices for pharmaceutical use*:

— *Part 1: Extractables in aqueous autoclavates*

— *Part 2: Identification and characterization*

— *Part 3: Determination of released-particle count*

— *Part 4: Biological requirements and test methods*

— *Part 5: Functional requirements and testing*

## Introduction

The pharmaceutical industry requires, to an increasing extent, concrete details from the rubber manufacturer about the biological status of rubber closures as far as elastomeric closures are used as primary packaging materials in direct contact with the medicinal products. This request has been taken into account by preparing Annexes A to D of this part of ISO 8871.

Tests presented in this part of ISO 8871 can be taken into account as a guideline if the question of biological safety arises in context with primary packaging materials for pharmaceutical products. The use of certain tests of Annex A to Annex D in case of special applications of the packaging material should be agreed upon between users and manufacturers.

# Elastomeric parts for parenterals and for devices for pharmaceutical use —

# Part 4: Biological requirements and test methods

## 1 Scope

This part of ISO 8871 specifies biological requirements for elastomeric parts for parenterals and for devices for pharmaceutical use. It also specifies the test methods, i.e. it offers the extraction procedures for elastomeric parts, and it makes reference to relevant biological test instructions in Pharmacopoeias and standards.

## 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 10993-5, *Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity*

*USP, The United States Pharmacopeia, United States Pharmacopeial Convention, Inc., Rockville, MD, USA*

## 3 Terms and definitions

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

**3.1**
**bacterial endotoxins**
lipo-polysaccharides from gram-negative bacteria

**3.2**
**bioburden**
population of viable microorganisms on or in product and/or a package

[ISO 11737-1:—, definition 3.1]

**3.3**
**cytotoxicity**
biological response of mammalian cell cultures in vitro using appropriate biological parameters to extracts of elastomeric parts

**3.4**
**intracutaneous toxicity**
local response to extracts of elastomeric parts after intracutaneous injections into rabbits

**3.5**
**systemic toxicity**
systemic response to extracts of elastomeric parts after injection into mice

# 4 Biological requirements

## 4.1 General

The elastomeric parts shall not release any substances that may adversely affect the therapeutic effectiveness and safety of the pharmaceutical solution, including those substances which can exhibit toxic or pyrogenic reactions.

Selection of tests and their interpretation to be used in biological evaluations should take into account the chemical composition of the materials, including the conditions of exposure as well as the nature, degree, frequency and duration of exposure of the material.

Cytotoxicity, systemic toxicity and intracutaneous toxicity shall be considered for type testing as material characteristics. Endotoxins and bioburden shall be considered for monitoring purposes.

## 4.2 Extractable bacterial endotoxins

The limit, specified as endotoxin units per square centimetre (EU/cm$^2$) of elastomeric part or endotoxin units per millilitre (EU/ml) extractable endotoxins, shall be agreed upon between supplier and user.

The test method shall be validated.

NOTE Annex A includes an example of a test method for the determination of extractable bacterial endotoxins that can be used.

## 4.3 Bioburden

The limit, specified as colony-forming units per square centimetre (cfu/cm$^2$) or cfu per elastomeric part, shall be agreed upon between supplier and user.

The bioburden method shall be validated.

NOTE ISO 11737-1 can be used as a guideline to establish and validate the method.

## 4.4 Toxicity

### 4.4.1 General

Materials shall be tested *in vitro* for cytotoxicity in accordance with 4.4.2. Materials that meet the requirements of this test are not required to undergo further testing. Materials that do not meet the requirements of this test shall be tested *in vivo* for systemic and intracutaneous toxicity in accordance with 4.4.3 and 4.4.4 respectively.

### 4.4.2 Cytotoxicity

The test shall be performed according to Annex B.

For assessment refer to the requirements specified in USP, chapter <87>, “Biological Reactivity Tests, In Vitro”.

#### 4.4.3 Intracutaneous toxicity

The test shall be performed according to Annex C.

For assessment, refer to the requirements specified in USP, chapter <88>, “Biological Reactivity Tests, In Vivo, Intracutaneous Test”.

#### 4.4.4 Systemic toxicity

The test shall be performed according to Annex D.

For assessment, refer to the requirements specified in USP, chapter <88>.

# Annex A
(informative)

# Test for extractable bacterial endotoxins

## A.1 General

This Annex defines a conventional method for the extraction of bacterial endotoxins from the surface of elastomeric parts. The extractable bacterial endotoxins are determined according to the methods specified in USP, chapter <85>, “Bacterial Endotoxins Test” or Ph.Eur.[2], chapter 2.6.14 “Bacterial Endotoxins”.

## A.2 Principle

The extraction is performed by shaking elastomeric parts in endotoxin-free water. The determination of extractable bacterial endotoxins is performed according to the methods specified in USP or Ph.Eur. using limulus amebocyte lysate (LAL) which has been obtained from horseshoe crab, *Limulus polyphemus*, and which has been prepared and characterized for use as an LAL reagent.

## A.3 Reagents and materials

**A.3.1** **Depyrogenated glassware/heat stable instruments** (e.g. forceps).

**A.3.2** **Endotoxin-free water**.

**A.3.3** **Endotoxin reagents** as specified in USP or Ph.Eur.

## A.4 Preparation of depyrogenated glassware and heat stable instruments

**A.4.1** Clean the glassware in a laboratory dish washer. Ensure that residual soap is completely removed.

**A.4.2** Wrap the glassware in aluminium foil as follows:

— completely wrap test tubes;

— wrap only the neck part of sample containers;

— completely wrap forceps.

**A.4.3** Depyrogenate the wrapped parts at appropriate conditions, e.g. at least 30 min at 250 °C.

**A.4.4** After depyrogenation, keep the parts in the aluminium foil.

## A.5 Extraction of the elastomeric parts

Aseptically transfer a number of elastomeric parts corresponding to a surface of $(100 \pm 10)\ cm^2$ into a depyrogenated sample container. Carefully add 100 ml endotoxin-free water, protect with cover film and shake (orbital shaking) for 5 min at room temperature.

If the surface area of $(100 \pm 10)\ cm^2$ cannot be achieved, add $(1 \pm 0{,}1)$ ml for every additional square centimetre.

In case of elastomeric parts with barely accessible cavities, e.g. 13 mm freeze drying closures, flashballs, needle shields etc., it may be necessary to cut the parts before extraction in order to obtain adequate recoveries. If parts are cut, take into account the original surface area of the parts.

## A.6 Expression of results

Express the results as EU/$cm^2$ elastomeric part or EU/ml extractable endotoxins.

# Annex B
(normative)

# Test for cytotoxicity

## B.1 Principle

The following test is designed to determine the biological reactivity of mammalian cell cultures following contact with extracts of elastomeric parts.

For details of performing the biological test itself, see USP, chapter <87> or ISO 10993-5.

## B.2 Preparation of extract

Prepare as specified for preparation of extracts in USP, chapter <87>, or in ISO 10993-5 using serum supplemented mammalian cell culture medium. Extract with a sample/medium ratio of 25 $cm^2$ per 20 ml for 24 h at (37 ± 1) °C.

## B.3 Procedure

Follow the instructions specified in USP, chapter <87> or in ISO 10993-5.

## B.4 Expression of results

**B.4.1** Express the results as follows:

— cytotoxic;

— non-cytotoxic.

**B.4.2** The sample is considered to be non-cytotoxic if the following criteria have been met:

— ⩽ 50 % of the cells are round and devoid of intracytoplasmic granules;

— no extensive cell lysis and empty areas between cells.

# Annex C
(normative)

# Test for intracutaneous toxicity

## C.1 Principle

The following test is designed to determine local responses and skin irritations to extracts of elastomeric parts by intracutaneous injections into rabbits.

For details of performing the biological test itself, see USP, chapter <88>.

## C.2 Preparation of extract

Prepare as specified for preparation of extracts in USP, chapter <88> using 9 g/l NaCl solution.

## C.3 Procedure

Follow the instructions specified in USP, chapter <88>.

## C.4 Expression of results

**C.4.1** Express the results as follows:

— toxic

— non-toxic

**C.4.2** For assessment, see USP, chapter <88>.

# Annex D
(normative)

# Test for systemic toxicity

## D.1 Principle

The following test is designed to determine the systemic responses to the extracts of elastomeric parts by intravenous injections into mice.

For details of performing the biological test itself, see USP, chapter <88>.

## D.2 Preparation of extract

Prepare as specified for preparation of extracts in USP, chapter <88>.

## D.3 Procedure

Follow the instructions specified in USP, chapter <88>.

## D.4 Expression of results

**D.4.1** Express the results as follows:

— toxic

— non-toxic

**D.4.2** For assessment, see USP, chapter <88>.

# Bibliography

[1] ISO 11737-1:2006, *Sterilization of medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

[2] *Ph.Eur., European Pharmacopeia, European Directorate for the Quality of Medicines*, Council of Europe, Strasbourg, France

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