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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 35511-2017

**Biological requirements and test methods for closures of butyl
rubber**

丁基橡胶瓶塞生物性能要求与试验方法

(English Translation)

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1 Scope

This Standard specifies the biological requirements and test methods for closures of butyl rubber.

This Standard applies to pharmaceutical closures made primarily of butyl rubber (hereinafter referred to as 'closures').

2 Normative references

The following 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.

GB/T 14233.2-2005, Test methods for infusion, transfusion, injection equipment for medical use - Part 2.Biological test methods

GB/T 16886.5-2003, Biological evaluation of medical devices - Part 5.Test for in vitro cytotoxicity (ISO 10993-5.1999, IDT)

GB/T 16886.10-2005, Biological evaluation of medical devices - Part 10.Tests for irritation and delayed-type hypersensitivity (ISO 10993-10.2002, IDT)

GB/T 16886.11-2011, Biological evaluation of medical devices - Part 11.Tests for systemic toxicity (ISO 10993-11.2006, IDT)

3 Terms and definitions

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

3.1

bacterial endotoxins

Lipo-polysaccharides on gram-negative bacteria.

3.2

bioburden

Population of viable microorganisms on or in product and/or packaging.

[GB/T 19973.1-2015, definition 3.1]

3.3

cytotoxicity

Biological response of mammalian cells cultured in vitro to extracts of closures.

3.4

intracutaneous toxicity

Local response of rabbits to intracutaneous injections of extracts of closures.

3.5

systemic toxicity

Systemic response of mice to injection of extracts of closures.

4.1 General

The closures shall not release any substances that may adversely affect the therapeutic effectiveness and safety of the pharmaceutical solution, or substances that can exhibit toxic or febrile reactions.

When selecting biological tests and analyzing the results, comprehensive considerations shall be taken into account, including the chemical composition of the materials, the environment in which they are exposed, the properties of the materials, and the degree, frequency, and duration of exposure.

Type inspection of closures shall include cytotoxicity, intracutaneous toxicity, and systemic toxicity. Monitoring shall include bacterial endotoxins and bioburden.

4.2 Bacterial endotoxins

The bacterial endotoxins shall be tested according to Annex A, where the endotoxin content in the extracts of closures shall meet the requirements agreed upon between supplier and user, expressed in endotoxin units per milliliter (EU/mL) extractable endotoxins or endotoxin units per square centimeter (EU/cm²) of closure.

4.3 Bioburden

The bioburden shall meet the requirements agreed upon between supplier and user, expressed in colony forming units per square centimeter (CFU/cm²) or colony forming units per closure.

The test method shall be validated.

Note: GB/T 19973.1 can be used as a guideline to establish and validate the test method.

4.4.1 General

The in vitro cytotoxicity of the extracts of closures shall be determined according to

4.4.2. Closures that meet the requirements of 4.4.2 do not require further testing;

otherwise, the intracutaneous toxicity and systemic toxicity of the extracts of closures in vivo shall be determined according to 4.4.3 and 4.4.4, respectively.

4.4.2 Cytotoxicity

Tested according to Annex B, there shall be no cytotoxicity.

4.4.3 Intracutaneous toxicity

Tested according to Annex C, there shall be no intracutaneous toxicity.

4.4.4 Systemic toxicity

Tested according to Annex D, there shall be no systemic toxicity.

Annex A (Normative)

Test for bacterial endotoxins

A.1 General

This Annex specifies a conventional method for determining bacterial endotoxins in the extracts from the surface of closures.

A.2 Principle

Prepare the extracts by placing the closures in endotoxin-free water and shaking.

Determine bacterial endotoxins in the extract using LAL reagent according to the test method specified in Chapter 4 of GB/T 14233.2-2005. LAL reagent (also known as Tachypleus Amebocyte Lysate) is obtained from horseshoe crab (*Limulus polyphemus*).

A.3 Reagents and materials

A.3.1 Pyrogen-free glassware and heat-resistant apparatus (e.g., tweezers).

A.3.2 Water for bacterial endotoxin testing.

A.3.3 Bacterial endotoxin working standards, as specified in Chapter 4 of GB/T 14233.2-2005.

A.4.1 Clean the apparatus using a laboratory dishwasher, ensuring that any residual

foam on the surface of the apparatus is completely removed. After cleaning, use water for bacterial endotoxin testing to rinse thoroughly.

A.4.2 After cleaning, air-dry or oven-dry the apparatus before wrapping them in aluminum foil, as follows.

- Wrap test tubes completely;
- Wrap sample containers only on the neck;
- Wrap tweezers completely.

A.4.3 Wrapped apparatus shall be depyrogenated, for example, dried at 250 °C for at least 30 minutes.

A.4.4 After the depyrogenation, the apparatus shall still be placed in the aluminum foil.

A.5 Extracts of closures

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