Bacterial endotoxin test report (LAL)

Customer name:
Azar Teb Asoudeh Company
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Test place:
KiaNonoBioVista laboratory
Address: Unit 10, Floor 4, No. 58, Rose building, Taherian Street, Sadeghiyeh Square, Tehran, Iran
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Reference Standard:
- ISO 10993-11:2017 " Annex G: Tests for material-mediated pyrogens in medical devices "
- European Pharmacopoeia 8.0. 2.6.14: "Bacterial endotoxins– Gel-clot Method: limit test"

Time Schedule:

Sample receiving date:	04/01/2022
Start test date:	04/01/2022
End test date:	11/01/2022

Sample Identification:

Name:	Conical tube with cap sterile
Manufacturer:	Azar Teb Asoudeh Company - Iran
Sample code in lab.:	S001012/677/06
Manufacturing Date:	09/2019
Expiry date:	09/2022
LOT Number:	ASC10TKP1
REF Number:	-
Batch Number:	-
Sterilization method:	R

Test Summary Report:

Test type	Test method	Test results	Issue date
In vitro pyrogen test: Test on sample extract	ISO 10993-11:2017 Limulus Amebocyte Lysate (LAL) test, Gel Clot Method	Non Pyrogenic 1:100	12/01/2022

Sajjad Mohammadi
KiaNanoBioVista Lab Manager

KiaNanoBioVista
کیا نانو زیست ویستا
سهامی خاص ثبت ۵۰۳۲۱۵

Reference Documents:

- ISO 10993-12:2012 "Biological evaluation of medical devices - Part 12: Sample preparation and reference materials"
- ISO 10993-11:2017 " Limulus Amebocyte Lysate (LAL) test - Part 11- Annex G: Tests for material-mediated pyrogens in medical devices "
- European Pharmacopoeia 8.0. 2.6.14: "Bacterial endotoxins– Gel-clot Method: limit test"

Test Materials:

Sodium chloride Injection (Sigma), Single dose Limulus Amobocyte Lysate (LAL) kit

Test Method:

Sample Preparation

The extract was obtained by overfilling the test sample in sodium chloride injection (0.9%) under laminar flow hood in aseptic condition. The sample extract has reached a surface/volume ratio of 3cm²/ml and then was incubated at 37±1°C for 72±2 hours in dynamic condition. Fresh extract was used for the test (within 24 hours from the preparation). No abnormalities were observed in extract appearance: no signs of particles, clouding, discoloring or chemical precipitation were recorded, the extract were not filtered before use.

Negative control Preparation

Sodium chloride injection (0.9%) was prepared as a negative control in the incubator at 37°C±1°C for 72±2 hours at the same time and same process of the test sample.

Positive Control Preparation

After the standard endotoxin stock solution was vigorously mixed, appropriate serial dilutions of this solution were prepared using sodium chloride injection (0.9%).

Experimental Design

Experimental design consisted of 2 groups (extract and controls) each consisting of 2 repetition. The solution A, B and C were prepared as shown in table 1 and test was performed on these solutions.

Table 1, samples concentration

Solution	Endotoxin concentration to which endotoxin is added	
A	Test solution at dilutions less than the MVD/ Sodium chloride Injection	Test solution
B	None/ Sodium chloride Injection	Negative control
C	2l, 1l, 0.5l and 0.25l/ sodium chloride Injection	Positive control

The amount of l (the labeled lysate sensitization) in this test is 0.125 EU/ml. 4 concentrations equivalent to 2l, 1l, 0.5l and 0.25l were prepared as positive control. The Maximum Valid Dilution (MVD)[1] of the sample was determined using the following formula:

$$MVD = \frac{endotoxin\ limit \times concentration\ of\ test\ solution}{l}$$

Volumes of the test solution (1:100 aliquots) were prepared. Then all the A, B, and C solutions were added directly to the separate vials or ampules. The reaction mixtures were incubated at 37±1°C for 60±2 minutes. The integrity of the gel in each vial were evaluated by invert it through approximately 180° in one smooth motion.

Acceptance criteria:

- The test conditions will be satisfied if the negative control (sodium chloride Injection) does not show any cloth.
- The test is considered valid if the intact gel is not formed in test solutions.
- IF a firm gel in test tubes formed and remains in place upon inversion, the result is record as positive.

Results:

There are no the intact gel formation in sample solutions.

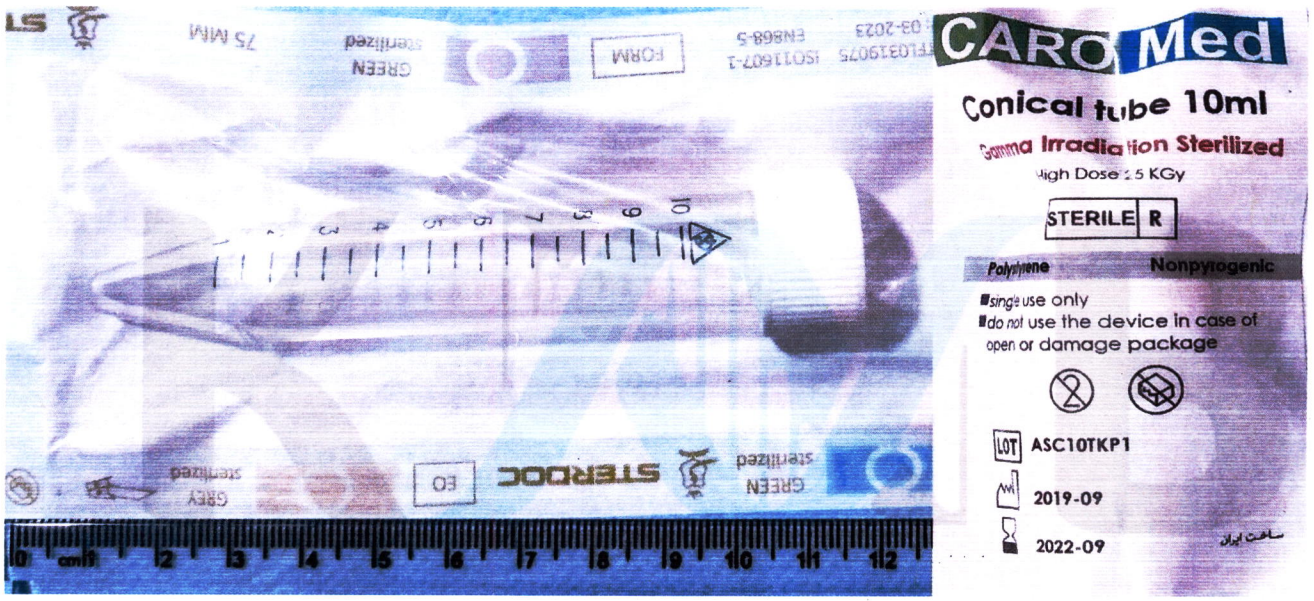
[1] maximum allowable dilution of the sample at which the endotoxin limit can be determined

Reporting statements of conformity:
On the basis of the results, interpreted according to ISO 10993-11:2017 the test sample `` Conical tube with cap sterile - Azar Teb Asoudeh Company `` is considered **NON PYROGENIC** and **satisfied** the requirements of the test.

Interoperation of results:
Not applicable

Record filing:
The study program and all raw data will be retained in Kiananobiovista Lab archives for a period of 10 years from the issue of the final report. An original copy of the report is available at Kiananobiovista Lab

Sample test image:



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