Translated from Russian

MEDICAL DEVICES TESTING CENTER OF THE FEDERAL RESEARCH AND CLINICAL CENTER OF PHYSICAL-CHEMICAL MEDICINE, FEDERAL MEDICAL BIOLOGICAL AGENCY OF RUSSIA
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"APPROVED" (round seal)
The head of Medical Devices Testing Center
of FRCC PCM, Candidate of medical science
_____ Martynov A.K.
10 November 2015

REPORT №89\2.015P
of the toxicological studies of medical device

Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015: Material hydrous biopolymer with silver ions, sterile ARGIFORM (as a substitute of synovial fluid at treatment of patients with degenerative joint diseases), under the trademark NOLTREX™

Performed at Medical Devices Testing Center of FRCC PCM, Federal Medical Biological Agency of Russia
License: accreditation certificate №RA.RU.21МИ25 dated 10 October 2015

1. Dates of testing: 26th of October 2015 – 09th on November 2015

Product design: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor
Manufacturer: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor
Address of manufacture: 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor, Russia

Testing is performed in accordance with:
GOST ISO 10993-1-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 1. Evaluation and testing." GOST R ISO 10993-2-2009 "Medical devices. Evaluation of biological effect of medical devices. Part 2. Requirements for treating laboratory animals."GOST ISO 10993-4-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 4. Testing of the medical devices, contacting with human blood." GOST ISO 10993-5-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 5.Cytotoxicity testing: in vitro methods." GOST ISO 10993-6-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 6. Tests for local effects after implantation. GOST ISO 10993-10-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 10. The study of irritating effect and sensitizing effect."GOST ISO 10993-11-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 11. The study of general toxic effect." GOST ISO 10993-12-2011 Medical devices. Evaluation of biological effect of medical devices. Part 12. Preparation of testing sample and control sample. GOST ISO 10993-18-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 18. Chemical characterization of materials; Guidance package for toxico-hygenic testing of polymer materials and medical devices from them, Methodology guideline 1.1.037-95 "Biotesting of products from polymer and other materials", GOST 31214-2003 Medical devices. The requirements to samples and documentation submitted for toxicological, sanitary-chemical testing, sterility testing and pyrogenicity testing; GN 2.3.3.972-00 "MPC of chemicals released from materials contacting with food." GOST R 52770-2007 "Medical devices. Safety requirements. Methods of sanitary – chemical testing and toxicological testing."; GOST EN 556-1-2011 Sterilization of medical devices. The requirements to medical device of sterile category. Part 1. Requirements to medical devices subject to finish sterilization.

2. Samples presented for testing:

- request for testing
- reference documentation for medical device
- technical and operational instructions with the list of standards and requirements to which the medical device complies

- application for registration
- samples – quantity: 15pcs, batch number 1698, manufactured (date of sterilization) 02.07.2015, expiration date 02.07.2017/
- TU 9398-001-52820385-2015

3. Medical device testing laboratory performed testing of Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015 in accordance with approved program (see Annex 2 to this report).

Evaluation of documentation	*Documentation is supplied as required and in full*
Intended use of device	*Medical device is intended for: - soft tissue correction and mammal glands plasty by the method of Lukomskiy G.I.; - for soft tissue correction in urology at treatment of urinary stress incontinence and reflux; - as a substitute for synovial fluid at treatment of patients with degenerative joint diseases.*
Type and time of the contact with human body	*The device has long term contact with internal environment of the body (full resorption period is from 6 month to 2 years)*
Potential risk class	*3 class*
Name of the materials used or composition of device	*Material is homogenous, jelly-like substance, color varies from transparent to light yellow (presence of few bubbles and different coloration are acceptable)* *Material and aqueous extract from it: dry residue, % 4,0 ± 1,5, pH from 4,0 to 8,5* *Material is a jelly-like polymer based on acrylamide and N,N' – methilen-bis-acrylamide, produced from raw materials: Acrylamide – chem.purity not less than 98%, N,N' – methilen-bis-acrylamide – chem.purity not less than 98%, ammonium persulfate - chem.purity not less than 98%, phosphate buffer - chem.purity not less than 98%, purified water containing silver ions (colloidal silver), or Poviargolum) – FS 42-0324-49* *Concentration of silver ions from 1.0 to 25.0 mcg/g VFS 42-2845-97* *Composition of final product:* *3-dimensional polyacrylamide, %: 4,0 ± 1,5;* *purified water, %: 96,0 ± 1,5;* *silver ions, %: 0,0001 - 0,0025.*
Type of sterilization	*Steam sterilization. Sterilization of packaged syringes is carried out in the sterilizer UNISTERI 336-1*

4. Brief description of test results

-- Sanitary-chemical testing of aqueous extracts (physic-chemical properties):

- content of reducing impurities was 0.02 ml, the max permissible value not more than 1.0 ml;
- changes of pH was 0.81 units, the max permissible change not more than 1.0 unit
- ultraviolet absorption (220-360 nm) was 0.179 units, the max permissible value not more than 0.3 units
- Cu content was less than 0.001 mg/l, the max permissible value less than 1.0 mg/l
- Pb content was less than 0.001 mg/l, the max permissible value less than 0/03 mg/l
- Cr content was less than 0.001 mg/l, the max permissible value less than 0.1 mg/l
- Cd content was less than 0.0001 mg/l, the max permissible value less than 0.001 mg/l
- Ba content was less than 0.001 mg/l, the max permissible value less than 0.1 mg/l
- Sn content was less than 0.001 mg/l, the max permissible value less than 0.1 mg/l
- Zn content was less than 0.001 mg/l, the max permissible value less than 1.0 mg/l

- acrylamide content was less than 0.001 mg/l, the max permissible value less than 0.01mg/l
- acrylonitril content was 0.005 mg/l, the max permissible value less than 0.02mg/l
- benzol content was less than 0.001 mg/l, the max permissible value less than 0.01 mg/l
- butyl acrylate content was less than 0.001 mg/l, the max permissible value less than 0.01 mg/l
- HMDA content was less than 0.001 mg/l, the max permissible value less than 0.01 mg/l
- methyl acrylate content was less than 0.001 mg/l, the max permissible value 0.02 mg/l
- methyl methacrylate content was less than 0.001 mg/l, the max permissible value less than 0.25 mg/l
- methyl alcohol content was less than 0.002 mg/l, the max permissible value less than 0.2 mg/l
- phenol content was 0.002 mg/l, the max permissible value less than 0.05 mg/l.
- epsilone-caprolactone content was less than 0.001 mg/l, the max permissible value less than 0.5mg/l
- formaldehyde content was less than 0.010 mg/l, the max permissible value less than 0.1mg/l.

-- Toxicological testing (biological properties):

- In the acute experiment with white outbred mice (males) at the intraperitoneal administration of the aqueous extracts in the amount 50 ml per 1 kg of the body mass there were no deaths. Laboratory animals did not reveal any clinical signs of intoxication: overall condition, behavioral response, condition of hair coat, feed eating in the experimental group did not differ from the control. At preparation in the place of administration of aqueous extracts, the regional lymphatic nodes, internal organs of animals did not show the signs of pathology. Weight coefficients of internal organs (kidneys, liver, spleen) did not have significant difference from those of the control group.
- In *in vitro* experiment with isolated and washed erythrocytes of rabbit hemolytic effect of aqueous extracts was not observed.
- In the experiment with rats and rabbits it was seen that the extracts had no local irritating effect on mucosa.
- Sterility testing – the samples are sterile.
- Pyrogenicity testing – the samples are apyrogenic.
- Toxicity index was 73.8%, the permissible range of 70 – 120%.

5. Conclusions of the tests performed

In the experimental testing the samples showed sufficient chemical stability, the aqueous extracts from them did not show negative effects on the biological objects.

6. Conclusions regarding the compliance of device with technical documentation

Tested samples comply with the requirements of technical documentation and current national standards.

7. Evaluation of toxicology test results and recommendation on usage

The test results allow for further clinical testing of the device.

CONCLUSION

Medical device *Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015: Material hydrous biopolymer with silver ions, sterile ARGIFORM (as a substitute of synovial fluid at treatment of patients with degenerative joint diseases), under the trademark NOLTREX™*

Product design: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor

Manufacturer: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor

Address of manufacture: 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor, Russia

COMPLIES with the requirements for safe use.

Annex 1. Toxicology testing protocol № 89\2.015P dated 10th of November 2015
Annex 2. Toxicology testing program *(not presented in this translation – RC BIOFORM)*
Annex 3. Sterility and pirogenicity testing protocol № 89\2.015SP dated 10th of November 2015

Signature: (signature) A.A. Laptanovich

9. Tested parameters and devices used for testing and measurement

№	Parameter	devices and laboratory animals used in test
9.1	**Reducing impurities**	1 Electronic Scales AP210 OHAUS, Switzerland 2. Stopwatch INTEGRAL C-01, factory "ZAVOD", Republic of Belarus 3. Titration buret 4. Measuring utensils 5 Reagents according to methodology
9.2	**Change in pH**	1. pH-meter pH-213 "HANNA instruments", Germany
9.3	**Ultraviolet absorption**	1. Spectrophotometer AGILENT HP 8453, Alient Technologies, Germany 2. quartz cell L=1 cm
9.4	**Toxicological characteristics** ***Irritating effect on the skin and mucous membrane of animals (in points)*** **Erythema and scrub formation** no erythema …….. (0) weak erythema ……. (1) clear erythema …….. (2) moderate erythema …… (3) severe erythema (beetroot red) ….. (4) **Swelling** no swelling ……. (0) mild swelling …….. (1) marked swelling ……. (2) moderate swelling (up to 1mm high)…….. (3) severe swelling (over 1mm high) .. (4) **Effect on conjunctiva of rabbit** no reaction ……..... (0) slight redness of the conjunctiva …. (1) conjunctiva redness and sclera partially…… …..(2) sudden redness of conjunctiva and a whole sclera, purulent	1. Rabbits 2. White rats 3. White mice 4. Eyedroppers 5. Glass spreader 6. Electronic Scales AP210 OHAUS, Switzerland
9.5	**Determination of acute toxicity in white mice**	1. White mice 2. 1ml syringe 3. Electronic Scales AP210 OHAUS, Switzerland

9.6	**Determination of hemolytic activity**	1. Rabbits 2. Centrifuge 1. Spectrophotometer AGILENT HP 8453, Alient Technologies, Germany 4. Measuring utensils 5. Reagents according to methodology
9.7	**Determination of toxicity index**	1. frozen sperm of cattle 2. Toxicity analyzer AT-05, BMK-Invest, Russia 3. Thermostat 4. Reagents according to methodology
9.8	**Determination of metal contents**	1. atomic absorption spectrophotometer "KVANT –Z.ЭТА", OOO Kortek, Russia 2. Reagents according to methodology
9.9	**Determination of chemicals contents**	1. Chromatograph for liquids AGLIENT 1100, "Aglient Technologies", Germany 2. Chromatograph for gases AGLIENT 7890N, "Aglient Technologies", Germany 3. Measuring utensils 4. Reagents according to methodology
9.10	**Determination of sterility and pirogenicity**	1. Isolated containers 2. Laminar units 3. Thermostat 4. Culture medium 5. Microscope with image analyzing function 6. LAL-test 7. Measuring utensils 8. Reagents according to methodology
Environmental conditions during testing complied with normative documentation		

10. Test results: sample code: ОИ17-1; testing dates: 26.10.2015 – 09.11.2015

Parameter	Permissible values	Results	Conclusion
Oxidizable impurities	less than 1,0ml. (0,02H sodium thiosulfate solution)	0.02	complies
Change in pH	less than ± 1,0	0,81	complies
Ultraviolet absorption	less than 0,3 units (range 230-260nm.)	0,179 units	complies
Toxicological test results			
Irritating effect on the skin and mucous membrane of animals in points			
Skin	0	0	complies
Conjunctiva of rabbit	0	0	complies
Acute toxicity in white mice at intra-abdominal administration			
Deaths	none	none	complies
Clinical symptoms of intoxication	none	none	complies
Macroscopic changes of organs and tissues	none	none	complies

Weight coefficient of internal organs (the presence of significant changes)	none	none	complies
Hemolytic activity	less than 2 %	0.24%	complies
Toxicity index	70 - 120 %	73.8%	complies
Metal concentrations in the aqueous extract			
Copper (Cu)	less than 1,0 mg/l	less than 0,001	complies
Lead (Pb)	less than 0,03 mg/l	less than 0,001	complies
Chromium (Cr)	less than 0,1 mg/l	less than 0,001	complies
Cadmium (Cd)	less than 0,001 mg/l	less than 0,0001	complies
Barium (Ba)	less than 0,1 mg/l	less than 0,001	complies
Tin (Sn)	less than 0,1 mg/l	less than 0,001	complies
Zinc (Zn)	less than 0,1 mg/l	less than 0,001	complies
Concentration of chemicals in the aqueous extract			
Acrylamide	less than 0.01 mg/l	less than 0.001	complies
acrylonitril	less than 0.02 mg/l	0.005	complies
Benzol	less than 0.01 mg/l	less than 0.001	complies
Butyl acrylate	less than 0.01 mg/l	less than 0.001	complies
HMDA	less than 0.01 mg/l	less than 0.001	complies
methyl acrylate	less than 0.02 mg/l	less than 0.001	complies
Methyl methacrylate	less than 0.25 mg/l	less than 0.001	complies
Methyl alcohol	less than 0.2 mg/l	0.002	complies
phenol	less than 0.05 mg/l	0.002	complies
Formaldehyde	less than 0.1 ml/l	0.010	complies
Epsilone-caprolactone	less than 0.5 mg/l	less than 0.001	complies
Sterility and pyrogenicity of samples			
Sterility	sterile	sterile	complies
Pyrogenicity	non-pyrogenic	non-pyrogenic	complies

11. CONCLUSION

The samples presented for testing: Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015:
Material hydrous biopolymer with silver ions, sterile ARGIFORM (as a substitute of synovial fluid at treatment of patients with degenerative joint diseases), under the trademark NOLTREX™
Product design: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor. Manufacturer: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor. Address of manufacture: 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor, Russia
are non-toxic, comply with the requirements of normative documentation

12. Signatures

Protocol prepared by *(signature)* *E.V. Grekova*
Responsible *(signature)* *A.A. Laptanovich*

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"APPROVED" (round seal)
The head of Medical Devices Testing Center of FRCC PCM, Candidate of medical science
_____ Martynov A.K.

PROTOCOL № 89\2.015SP dated 10 November 2015

Testing of medical devices (materials) for sterility and pyrogenicity

1. Name of the device (material), type, model:

Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015:
Material hydrous biopolymer with silver ions, sterile ARGIFORM (as a substitute of synovial fluid at treatment of patients with degenerative joint diseases), under the trademark NOLTREX™

2. Name and address of the manufacturer:

Product design: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor. Manufacturer: RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor. Address of manufacture: 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor, Russia

3. Name and address of the company ordering the testing:

RESEARCH CENTER BIOFORM, Limited Liability Company, 142784, Moscow, 22nd km of Kievskoe Hwy, s. Moskovskiy, housing estate 4, bldg 2, block G, 5th floor.

4. Sterilization method for the device:

Steam sterilization. Sterilization of packaged syringes is carried out in the sterilizer UNISTERI 336-1

5. The testing is conducted in accordance with:

GOST EN 556-1-2011 Sterilization of medical devices. The requirements to medical device of sterile category. Part 1. Requirements to medical devices subject to finish sterilization.
GOST ISO 11737-2-2011 Sterilization of medical devices. Microbiological methods. Part 2. Sterility testing conducted at validation of sterilization process, Annex A.
GOST ISO 10993-1-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 1. Evaluation and testing."
GOST ISO 10993-11-2011 "Medical devices. Evaluation of biological effect of medical devices. Part 11. The study of general toxic effect."
GOST 31214-2003 Medical devices. The requirements to samples and documentation submitted for toxicological, sanitary-chemical testing, sterility testing and pyrogenicity testing; Annex B
OFS 42-0062-07 Bacterial endotoxins

6. The place of testing:

Medical Devices Testing Center of FRCC PCM. Address: 119435, Russia, Moscow, Malaya Pirogovskaya street, house 1A, building 3, 4th floor, room №№ 424, 425, 428.

7. Dates of testing:

From 26th of October 2015 to 09th of November 2015

8. Parameters tested and measuring devices used:

№	Parameter tested	Equipment used for testing
8.1	Sterility	- device for purification and sterilization of air УЦС-99-01-САМПО, ОМ-22, Sampo, Russia - biological laminar unit with the function for purification and sterilization of air ГЛ-УОС-99-01 САМПО, Sampo, Russia - dry-air thermostat ТСО-180 СПУ, Russia

		- refrigerator with freezer, STINOL, Russia - electronic thermohygrometer CENTER, model CENTER 311, Center Technology Corp, Taiwan
8.2	Pyrogenicity	- solid thermostat PyroTherm Opulus, model PT-1/240, Hungary - apyrogenic vessels and handpieces - automatic pipette with volume 100 – 1000 mcl - apyrogenic measuring utensils - LAL-water - reagent Charles River Endosafe, USA (limulus amebocyte lysate; endotoxin standard – 20IU/ml)
Environmental conditions during testing complied with normative documentation		

Annex 3. Page 2. Translated from Russian

9. Test results: sample code: ОИ1375-1; testing dates: 15.01.2016 – 29.01.2016

Parameter tested	Permissible values	Results	Conclusion
Inoculation of medium: trypticase-soy broth	No growth of microorganisms	No growth	complies
Inoculation of medium: beef-extract broth with glucose	No growth of microorganisms	No growth	complies
Inoculation of medium: Sabouraud dextrose broth	No growth of microorganisms	No growth	complies
Overall endotoxin content	< 20 EU	< 20 EU	complies

10. CONCLUSION

The samples presented for testing: Material Hydrous biopolymer with Silver Ions, sterile ARGIFORM, TU 9398-001-52820385-2015:
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comply with the normative documentation requirements in regards to Sterility and Pyrogenicity

11. Signatures

Responsible (signature) A A Laptanovich