TRANSLATED FROM RUSSIAN

RESEARCH OF THE HEMOLYTIC ACTION OF THE MATERIAL

1. The hemolytic action of extracts from the material was studied in accordance with GOST R ISO 10993.4-99 “Medical devices. Study of biological action of medical devices. Part 4. Study of devices interacting with blood. Supplement C. The ‘in vitro’ methods. Study of hemolytic action of medical devices on isolated rabbit red corpuscles.

The experimental animals were kept in accordance with GOST R ISO 10993.2-99 “Medical devices. Study of biological action of medical devices. Part 2. Regulations on protection of animals”.

Samples
The material is submitted in a sterile form. “ARGIFORM” is filled in disposable injection plastic syringes, which are tipped and vacuum-sealed in individual blister packaging. Each set consisting of a blister packaging with a syringe and an injection cannula which are packed into a separate branded carton box. The marking, description and the trademark are printed on the boxes.

2. Description and results of the study

The hemolytic action of material extracts was studied ‘in vitro’, according to the standard methods, by 100% hemolysis, on isolated rabbit erythrocytes.
The permissible parameters: a device is considered free from hemolytically active substances if the hemolysis rate in 3 replications (3 erythrocyte suspensions taken from 3 rabbits), is less than 2%.

The blood taken from puncture of ventricle of rabbits’ hearts was put into test-tubes treated with natrium citrate. 5 ml of citrated blood was centrifuged for 10 min at 900 RPM. The liquid above the precipitate was poured off; 8.0 ml of physiological sodium chloride solution was added to residue of erythrocytes. Then contents of test-tube were thoroughly mixed and centrifuged. The supernatant fluid was poured off again.

The following samples were prepared:
a) a sample with 100 % hemolysis (5.0 ml of distilled water plus 0.5 ml of washed red blood corpuscles);
b) a control sample (5.0 ml of physiological salt solution plus 0.5 ml of washed red blood corpuscles);
c) an experimental sample (5.0 ml of extract from the tested material plus 0.5 ml of washed red blood corpuscles).

The results are shown in table #1

Sample with 100% hemolysis	Control sample	Experimental sample
(In 3 replications)		
5 ml of aqua destillata 0.5 ml of red blood cells	5 ml of 0.9% NaCl 0.5 ml of red blood cells	5 ml of extract 0.5 ml of red blood cells
Test-tubes were put into a thermostat for 1 h at 37 °C		
Suspension centrifuged at 2000 RPM for 20 min		
The supernatant fluid was poured off to separate test-tubes to measure the optical density *E* on a spectrophotometer at a wave-length of 540 nm against water, a flask is 1 cm size		

The calculation of hemolysis for each of 3 replications was done according to the formula:

$$\frac{E_{experimental\ sample} - E_{control\ sample}}{E_{100\%\ hemolysis\ sample}} \cdot 100\%,$$

where $E_{exp.sample}$ is an optical density of the extract with suspension of red corpuscles;

$E_{contr.sample}$ is an optical density of 0,9% NaCl solution with suspension of red corpuscles;

$E_{with\ a\ 100\%\ hemolysis}$ is an optical density of pure water with suspension of red corpuscles.

Table #1

# of rabbit	Extract #1	Extract #2	Control	H_2O 100% hemolysis
	Optical % of hemolytic density	Optical % of hemolytic density		
1	0.012 – 0.19	0.007 – 0	0.009	1.593
2	0.018 – 0.34	0.011 – 0	0.011	2.101
3	0.021 – 0.48	0.014 – 0.14	0.011	2.101
M	0.336	0.046		

3. Conclusion on hemolytic action

The extracts from all tested samples of material did not display any hemolytic action at ‘in vitro’ tests with isolated rabbits’ erythrocytes: the hemolysis amounted from 0.05 to 0.34%, when permissible value of the index is less than 2%.

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