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Method of obtaining and storing hyperimmune anthrax serum

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SUMMARY
Anthrax is a highly dangerous disease of animals and humans caused by the spore-forming bacterium *Bacillus anthracis*. Currently, the disease is widespread in many countries of the world. Many regions of the Russian Federation are anthrax-endemic. A large number of anthrax treatment, diagnosis and prevention tools are developed using hyperimmune serum. Currently known commercial hyperimmune sera are produced by 2-month long immunization of horses, which is a long and expensive process. This suggests the need to develop faster and cheaper ways to produce anti-anthrax hyperimmune sera; such possible ways became the objective of this study. A live culture of *Bacillus anthracis* 55-VNIIVViM vaccine strain, used to produce live vaccines against animal anthrax, was used in the experiments. Rabbits were used as animal models. Based on the findings the method of rabbit immunization was selected. The optimal method included intravenous injection of the antigen in increasing amounts according to the following scheme: injection I – 0.5 cm^3; injection II – 1 cm^3; injection III – 2 cm^3 at a dose of 100 million mc/animal in 1 cm^3, with 4-day interval between injections. This scheme made it possible to produce the serum with a high antibody titer equal to 14 log_2. For long-term storage of the serum produced, the freeze-drying modes were optimized, giving 2% residual moisture content of the finished product. The analysis of the freeze-dried serum storage terms showed that the initial activity and physico-chemical properties of the product are maintained for 30 months.

Keywords: *Bacillus anthracis*, immunization, serum, antigen, anthrax, antibodies

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Способ получения и хранения гипериммунной сибиреязвенной сыворотки

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РЕЗЮМЕ
Сибирская язва – особо опасная инфекционная болезнь животных и человека, вызываемая спорообразующей бактерией *Bacillus anthracis*. В настоящее время данное заболевание широко распространено во многих странах мира. Некоторые регионы Российской Федерации являются эндемичными по сибирской язве. Большое число средств терапии, диагностики и профилактики сибиреязвенной инфекции разрабатываются на основе гипериммунных сывороток крови. Известные в настоящее время коммерческие сыворотки крови получают путем гипериммунизации лошадей, длящейся в течение 2 мес. и представляющей длительный и дорогостоящий процесс. Данный факт свидетельствует о необходимости разработки более быстрых и дешевых способов получения гипериммунных противосибиреязвенных сывороток крови, что и явилось целью работы. В опыте использовали живую культуру вакцинного штамма 55-ВНИИВВиМ *Bacillus anthracis*, который применяется в России для создания живых лекарственных препаратов против сибирской язвы животных. В качестве модели для получения сывороток крови были выбраны кролики. В результате проведенной работы подобран способ гипериммунизации кроликов, включающий внутривенное введение антигена в нарастающем объеме по схеме: I инъекция – 0,5 $см^3$; II инъекция – 1 $см^3$; III инъекция – 2 $см^3$ в дозе 100 млн м. к./гол. в 1 $см^3$ с интервалом между введениями 4 сут. Указанная схема дала возможность получить сыворотку крови с высоким титром антител, равным 14 log_2. Для долгосрочного хранения полученной сыворотки отработан режим ее лиофилизации, позволивший достичь остаточной влажности готового препарата в 2%. При изучении длительности хранения лиофилизированной сыворотки было установлено, что исходная активность и физико-химические свойства препарата сохраняются в течение 30 мес.

Ключевые слова: *Bacillus anthracis*, гипериммунизация, сыворотка, антиген, сибирская язва, антитела

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INTRODUCTION

Anthrax is a deadly infectious disease caused by the spore-forming bacterium *Bacillus anthracis* [1, 2]. To date, outbreaks of this infection continue to be registered both among animals and among people in many countries of the world [3–5]. In addition, there is a constant risk of the disease introduction from other countries [6]. According to the World Organization for Animal Health, in 2019, anthrax was registered in 46 countries of the world, in 2020 – in 23, in 2021 – in 22, in 2022 in 16 countries [7]. Many regions of the Russian Federation are endemic for anthrax [8, 9]. In most cases, the disease is sporadic, involving a small number of animals. However, the anthrax outbreak that occurred in Yamal demonstrates the risk of complicated situation [10, 11]. This fact makes the development of diagnostic, prevention and therapeutic tools to control this highly dangerous disease an urgent matter.

The test kit for determination of antibody titers in sera of anthrax-vaccinated animals by indirect immunohemagglutination test (IHA) [12] shall be standardized in the process of its development, including determination of the antigen activity. Moreover, in addition to determination of antibody levels in vaccinated animals, a confirmation test using known positive control serum is required. Subsequently, this serum will be included as the reagent for the developed test kit.

Currently known commercial hyperimmune sera are obtained by long-term (for 2 months) immunization of horses [13]. This suggests the need to search for faster and cheaper ways of hyperimmune anthrax serum production. The novelty of this study consists in the proposal of a rabbit immunization scheme, which allows obtaining of highly active anthrax hyperimmune serum in a short time.

In this regard, the goal was to develop a method for hyperimmune anthrax serum production, which will serve as a control for serological testing to determine the antibody level in animals vaccinated against anthrax.

MATERIALS AND METHODS

Strain. *B. anthracis* 55-VNIIVViM (pX01+/pX02–) vaccine strain was used as an immunizing antigen.

Nutrient media. For the cultivation of *B. anthracis*, meat-peptone agar (MPA) and meat-peptone broth (MPB), 5% blood agar, 12% gelatin, skimmed milk and Hottinger broth produced by the FSBSI "FCTRBS-ARRVI" (Russia) were used.

Laboratory animals. In order to obtain hyperimmune anthrax serum, 2.5–3.0 kg chinchilla rabbits after a 30-day quarantine, were used. Fifteen animals in total were divided into three groups (5 animals per each).

Experiments on animals were conducted in compliance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Reagents and test kits. 0.9% sodium chloride solution (Grotex LLC, Russia) was used to harvest the culture from the agar surface, as well as a diluent for IHA test.

For IHA test, the anthrax antigenic erythrocyte diagnosticum was used (FSBSI "FCTRBS-ARRVI", Russia).

Methods. The strain biological properties were tested according to MG 4.2.2413-08 "Laboratory diagnostics and detection of the anthrax causative agent"[1].

Suspension for immunization of laboratory animals with concentrations of 100 and 500 million mc per 1 cm^3, depending on the scheme, was prepared from a strain grown on MPA at 37 °C for 2 days.

The antibody titer increase was monitored by IHA test every 3 days in U-bottom plates (OOO "MiniMed", Russia) before the next administration of the antigen, according to the manufacturer instructions for the diagnosticum used. 0.2 cm^3 of 0.9% sodium chloride solution was added to all the wells of the plate using a multichannel pipette. 0.2 cm^3 of the obtained and control (negative) serum were added to the first wells of the rows, and then two-fold serial dilutions were performed. After preparation of the corresponding serum dilutions, 0.05 cm^3 (50 μL) suspensions of anthrax antigenic erythrocyte diagnosticum were added into all wells. The plates were carefully shaken to mix the reaction components and left at 10–20 °C for 1.5–2.0 hours. After exposure, the results were read.

Blood was collected from producing animals totally from the heart. Blood was collected into sterile glass

[1] MG 4.2.2413-08 Laboratory diagnostics and detection of the anthrax causative agent: methodical guidelines. Moscow: Rospotrebnadzor Federal Center of Hygiene and Epidemiology; 2009. 69 p. Available at: https://files.stroyinf.ru/Data2/1/4293752/4293752010.pdf.

cylinders with 0.9% sodium chloride solution pre-moistened walls, placed in a thermostat for coagulation for 45–60 minutes, then the clot was separated from the cylinder walls by circular movements using a glass sterile stirring rods and put in the refrigerator at 4 °C for 24 hours. The separated serum was decanted from the clot using a sterile pipette after activity testing.

The obtained sera were freeze-dried using LZ-9.2 freeze-dryer (Frigera, Czech Republic).

The obtained serum was evaluated for the following parameters: appearance, colour, impurities, solubility, activity after during long-term storage, moisture content of the freeze-dried product.

The appearance, colour, impurities were checked visually.

To determine the solubility, 1 cm^3 of 0.9% sodium chloride solution was added to the bottles with serum. After that, the bottles were shaken and dry mass dissolution process was monitored.

The activity of the obtained freeze-dried serum during long-term storage at 4 °C was determined after 3, 6, 9, 12, 15, 18, 24, 30, 36 and 42 months by IHA test using erythrocyte antigenic anthrax diagnosticum. Serum stability values were calculated in $\log_2$.

Determination of moisture content of lyophilized serum was performed according to GOST 24061-2012[2].

The obtained data were statistically processed using Mann – Whitney U-test. The test results are presented as $M \pm S_D$, where M is the average value, S_D is the standard deviation. Differences were considered statistically significant at $p < 0.01$ (after adjustment for the number of comparisons) [14].

TEST RESULTS

The aim of first working stage was to study the basic biological properties of *B. anthracis* 55-VNIIVViM strain.

After inoculation of the strain on MPA and MPB and cultivation for 24 hours, cultural, morphological, tinctorial properties were studied, motility was recorded.

Flat, dull greyish, rough (R-form) colonies were observed on MPA (Fig. 1A) with darkened centre and fringed edge with put out curled protrusions (Fig. 1B).

24 hours after inoculation in MPB, the medium remained transparent, a loose cotton wool-like sediment formed at the bottom (Fig. 1C). When shaking the test tube, the broth did not become cloudy, the sediment was hardly broken into small flakes.

The broth culture was smeared, Gram-stained and microscoped. Typical chains consisting of anthrax gram-positive rods were observed in the smears (Fig. 1D).

The analysis of the basic biological properties of *B. anthracis* 55-VNIIVViM strain showed that its properties are typical for its species (Table 1).

The aim of the next working stage was the search for optimal immunization scheme for laboratory animals to obtain active hyperimmune anthrax serum. The prepared antigen was administered using three schemes: 1) in increasing volumes intravenously: I injection – 0.5 cm^3; II injection – 1.0 cm^3; III injection – 2.0 cm^3 at a dose of 100 million mc/animal per 1 cm^3 with 4 day-interval between injections; 2) once at a dose of 500 million mc/animal per 1 cm^3 intravenously; 3) twice at a dose of 100 million mc/animal per 1 cm^3 intradermally along the spinal column into five points on each side with a 5 day-interval between injections.

In the process of rabbit immunization, the dynamics of the antibody titer growth was monitored by blood collection and IHA test every 3 days. The use of 55-VNIIVViM vaccine strain as an antigen made it possible to obtain an active immune anthrax serum. The results of rabbit hyperimmune serum activity testing after using three schemes of hyperimmunization are shown in Figure 2.

During the entire period of laboratory animal hyperimmunization, an increase in the antibody titer was observed.

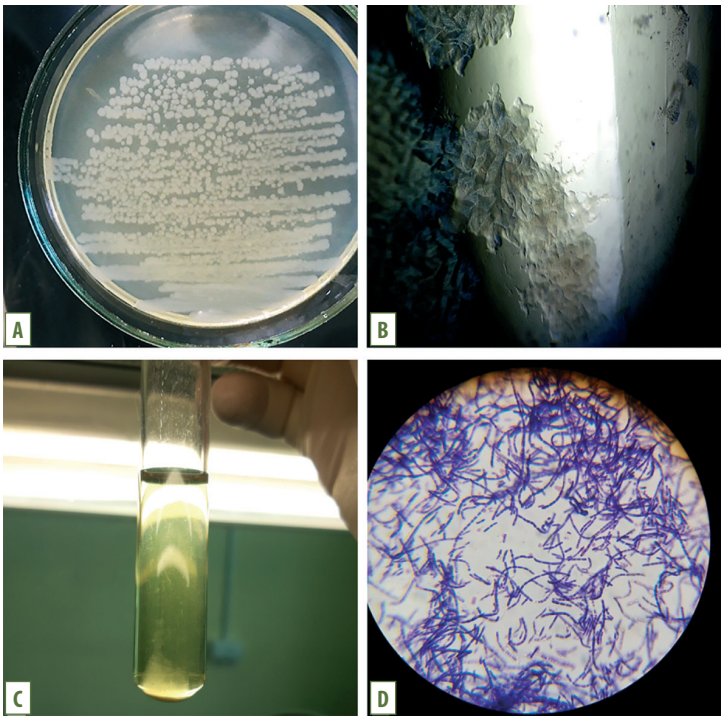

Fig. 1. B. anthracis 55-VNIIViM morphology:
A – R-shaped colonies on MPA;
B – B. anthracis 'curly hair' colonies (8 × 40);
C – B. anthracis typical 'cotton wool'-like growth in MPB;
D – morphology of Gram-stained B. anthracis cells

Table 1
Major biological properties of *B. anthracis* 55-VNIIVViM strain

No.	Indicator (property)	*B. anthracis* 55-VNIIVViM properties	*B. anthracis* 55-VNIIVViM according to Bergey's manual
1	Motility	–	–
2	Hemolytic properties	–	–
3	Proteolytic properties: 12% gelatin skimmed milk	 + +	 + +
4	Capsule formation	–	–
5	Susceptibility to penicillin	+	+
6	Spore formation	+	+

"+" – yes; "–" – no.

[2] GOST 24061-2012 Medicine remedies biological lyophilized for veterinary use. Method for determination mass moisture. Available at: https://docs.cntd.ru/document/1200103299.

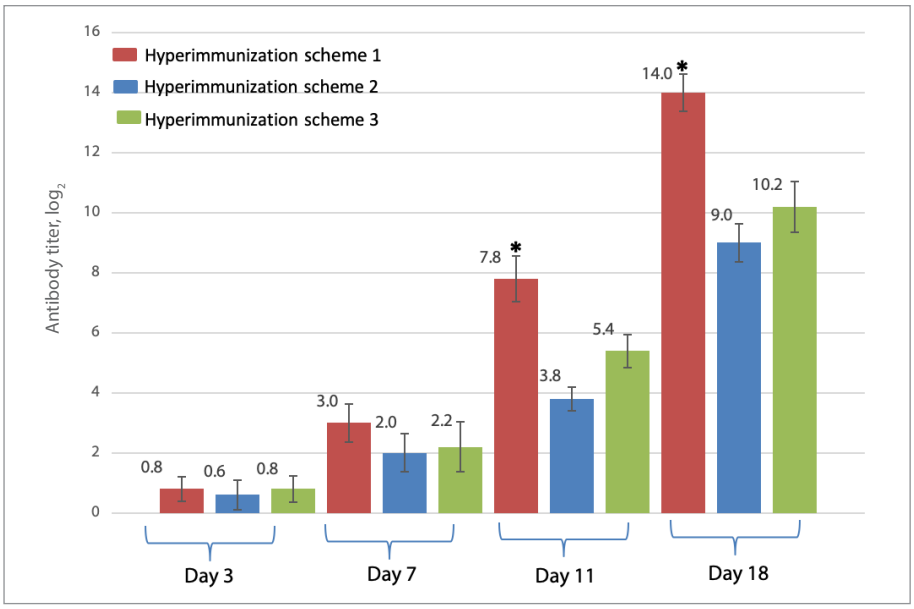

Fig. 2. Specific antibody titer dynamics in sera of hyperimmunized rabbits (statistically significant difference, $p < 0.01$)*

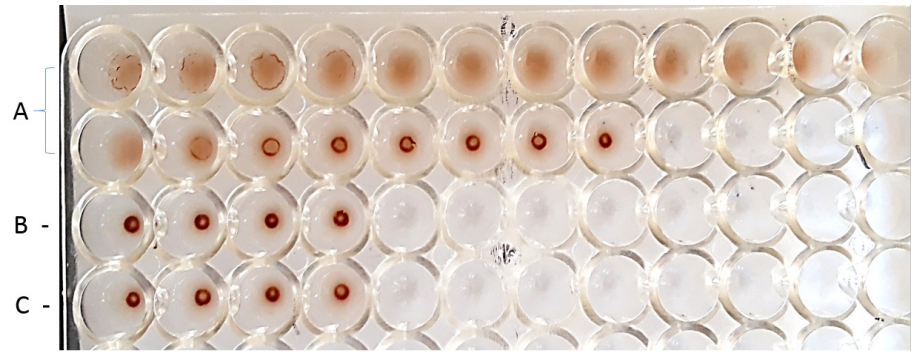

Fig. 3. Activity of produced hyperimmune rabbit sera tested by IHA test using RBC anthrax antigen: A – reaction to produced hyperimmune serum (antibody titer 14 $\log_2$); B – reaction to normal serum (serum of non-immunized animal); C – reaction to saline solution

The highest level of specific antibodies in rabbit sera was observed after the first scheme immunization: after antigen injection by day 18, the titer reached the value of 14 $\log_2$, which is equivalent to 1:16384 serum dilution (Fig. 3). Therefore, this scheme was used for the development of diagnostic products.

Sera used as components of diagnostic test kits remain active better and are more easily transported when freeze-dried ones [15]. Therefore, tests were conducted to choose the optimal lyophilization conditions. For this purpose, sera was freeze-dried using two different modes (Table 2).

When two freeze-drying modes were compared, it was found that mode 1 makes the serum homogeneous of creamy white colour (Fig. 4A). Mode 2, in which freeze-drying process took a longer time, made the product excessively dry (Fig. 4B).

Table 2
Different modes of serum freeze-drying

Days	Mode 1		Mode 2	
	time	stage	time	stage
1	11:30	1. Turning on the freeze-drier	8:00	1. Turning on the freeze-drier
	12:00	2. Loading of sera into the freeze-drier at –35 °C	8:30	2. Loading of sera into the freeze-drier at –35 °C
	16:00	3. Turning off the freezing at –37 °C	16:00	3. Turning off the freezing at –36 °C
2	8:00	1. Turning on the heating (t in the chamber +10 °C)	8:00	1. Turning on the heating (t in the chamber +10 °C)
	11:00	2. Turning off the freeze-drier (t in the chamber +35 °C)	11:00	2. Turning off the freeze-drier (t in the chamber +35 °C)

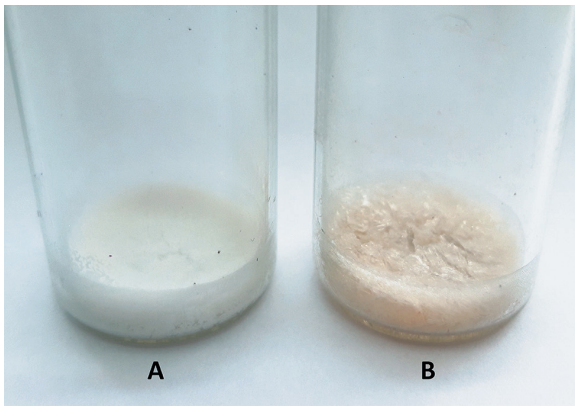

Fig. 4. Visual evaluation of freeze-dried hyperimmune serum: A – freeze-drying mode 1; B – freeze-drying mode 2

Table 3
Physico-chemical and biological parameters of freeze-dried serum

Parameter	Serum characteristics
Appearance	Dry solid matter in the form of a tablet
Colour	Creamy white colour
Solubility	When 1 cm^3 of 0.9% NaCl solution was added, it dissolved within 1–3 minutes
Activity	Agglutinates antigenic erythrocyte anthrax diagnosticum in 1:16384 dilution
Moisture content, %	2.0

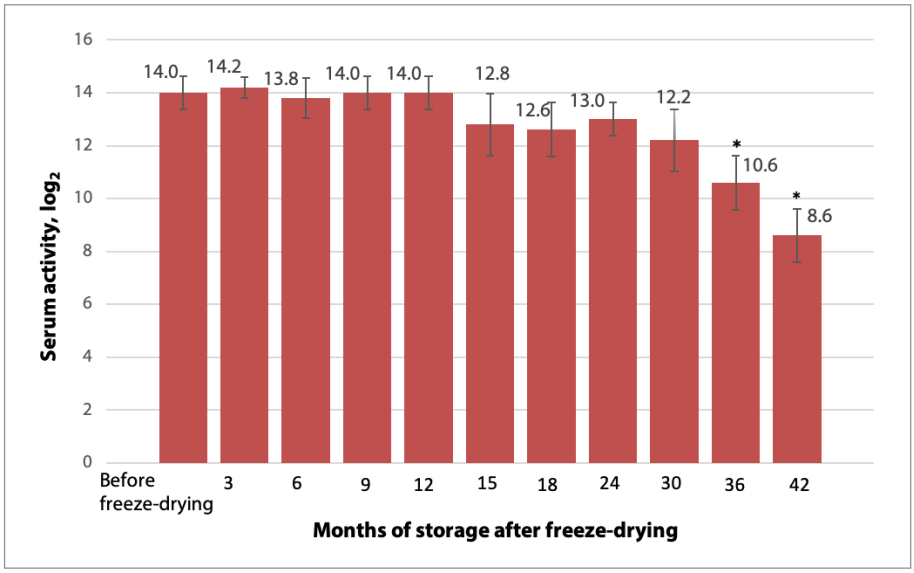

Fig. 5. Activity of freeze-dried hyperimmune rabbit serum during long-term storage at 4 °C (statistically significant difference, $p < 0.01$)*

After lyophilization, the quality control of the obtained serum was performed. The physico-chemical and biological parameters of the serum are given in Table 3.

The stability testing of the obtained freeze-dried serum during long-term storage at 4 °C revealed that the initial titer persists for at least 30 months, followed by a decrease in activity to 10 $\log_2$ 36 months after lyophilization (Fig. 5). Consequently, freeze-drying of rabbit hyperimmune serum makes it possible to maintain high activity of the product for a long time.

DISCUSSION

During the study, rabbit anti-anthrax serum with a high antibody titer was obtained. The search for manufacturing methods of such freeze-dried products is associated with the need to use it as a control of the developed anthrax diagnosticum. Many researchers have demonstrated the effectiveness of using mice, guinea pigs, goats and horses to produce anti-anthrax sera [16–21]. In our experiment, rabbits served as models. This made it possible to obtain a larger volume of serum compared to using of mice or guinea pigs (the amount of serum from one mouse varies within 0.5 mL). Whereas larger animals, such as goats and horses, require higher costs for the keeping infrastructure.

The preparation of mono- and polyclonal globulins and sera against *B. anthracis* antigens is widely described in the scientific literature [22–26]. However, the production of polyclonal sera to the antigens of *B. anthracis* live cells is not discussed in detail. M. Caldwell et al. described the production of equine serum after hyperimmunization with *B. anthracis* Sterne (pX01+/pX02–) strain [21]. In the course of our experiment, we also produced serum against live bacterial cells of *B. anthracis* 55-VNIIVViM (pX01+/pX02–) strain, since it is to be used primarily as a positive diagnostic control and reproduce the properties of sera of immunized animals. The use of live bacterial cells of the 55-VNIIVViM strain is justified in our case, since it is used to develop anthrax vaccines in Russia [10]. In a recent study, it was demonstrated that live anthrax vaccines ensure the development of strong and lasting immunity to the major antigens of this pathogen [27].

Using *B. anthracis* 55-VNIIVViM (pX01+/pX02–) strain cells, three immunization schemes were tested in the experiment. The antibodies were developed best after intravenous sequential administration of the antigen every

4 days: 0.5 cm^3 with the first administration, 1.0 cm^3 with the second one and 2.0 cm^3 with the third administration at 100 million spores per 1 cm^3 concentration. After hyperimmunization the serum with an antibody level equal to 14 log_2 was obtained, which corresponds to 1:16384 titer. In similar studies, M. Caldwell et al. hyperimmunized horses 12 times with 1.0 cm^3 of spore vaccine based on *B. anthracis* Sterne strain once a month, which allowed to obtain an antibody titer of 16.25 log_2 [21]. In their experiment, C. D. Kelly et al. immunized goats with recombinant *B. anthracis* protective antigen (PA83), covalently coupled to a novel non-toxic muramyl dipeptide (NT-MDP) derivative at a dose of 100 μg. The antigen was administered on days 1, 14, 28 and 56. As a result, the authors managed to obtain the serum with an anti-PA antibody titer equal to 1:16000 [16]. Thus, the scheme we used made it possible to obtain a high level of antibodies in a shorter time.

Lyophilization is recognized as one of the best ways to preserve and store sera [15]. Therefore, two freeze-drying modes were tested to preserve the obtained serum. It was found that mode 1 produced a homogeneous creamy white serum, while mode 2 made the product excessively dry. In the process of serum freeze-drying, no protectants were used. R. Brogna et al. also demonstrated that serum lyophilization using protectants or without them does not affect the viability of immunoglobulins [28]. The antibody titer in the lyophilized serum remained at the original level for 30 months.

The developed method for serum production using rabbits hyperimmunized against anthrax has a number of advantages (low cost, ease of production, high antibody titer) and is a worthy alternative to expensive methods of hyperimmune equine sera production. The proposed technology for serum production can be used for the development of specific diagnostic products.

CONCLUSION

The studies performed allowed to elaborate the method for production of highly active hyperimmune anti-anthrax rabbit serum with an antibody titer equal to 14 log_2 was developed. The mode of the obtained serum lyophilization was optimized, which made it possible to achieve 2% residual moisture of the finished product. It was established that storage time of the lyophilized serum without loss of its original activity and physico-chemical properties is 30 months.

REFERENCES

1. Seid K., Shiferaw A. M., Yesuf N. N., Derso T., Sisay M. Livestock owners' anthrax prevention practices and its associated factors in Sekota Zuria district, Northeast Ethiopia. *BMC Vet. Res*. 2020; 16 (1):39. DOI: 10.1186/s12917-020-2267-0.

2. Ivanova S. V., Rodionov A. P., Melnikova L. A. Monitoring the potential hazards of anthrax outbreaks. *Hippology and veterinary*. 2021; 1 (39): 93–100. EDN: RBOFZY. (in Russ.)

3. Cossaboom C. M., Khaiseb S., Haufiku B., Katjiuanjo P., Kannyinga A., Mbai K., et al. Anthrax epizootic in wildlife, Bwabwata National Park, Namibia, 2017. *Emerg. Infect. Dis*. 2019; 25 (5): 947–950. DOI: 10.3201/eid2505.180867.

4. Muturi M., Gachohi J., Mwatondo A., Lekolool I., Gakuya F., Bett A., et al. Recurrent anthrax outbreaks in humans, livestock, and wildlife in the same locality, Kenya, 2014–2017. *Am. J. Trop. Med. Hyg*. 2018; 99 (4): 833–839. DOI: 10.4269/ajtmh.18-0224.

5. Mwakapeje E. R., Høgset S., Fyumagwa R., Nonga H. E., Mdegela R. H., Skjerve E. Anthrax outbreaks in the humans – livestock and wildlife interface areas of Northern Tanzania: a retrospective record review 2006–2016. *BMC Public Health*. 2018; 18:106. DOI: 10.1186/s12889-017-5007-z.

6. Noordhuizen J., Surborg H., Smulders F. J. On the efficacy of current biosecurity measures at EU borders to prevent the transfer of zoonotic and livestock diseases by travellers. *Vet. Q*. 2013; 33 (3): 161–171. DOI: 10.1080/01652176.2013.826883.

7. WAHIS: World Animal Health Information System. Available at: https://wahis.woah.org.

8. Ivanova S. V., Melnikova L. A., Rodionov A. P., Makaev Kh. N., Safina G. M., Murtazina G. Kh., et al. Analysis of the epizootic situation and improvement of the scheme for the specific prevention of anthrax. *Int. J. Res. Pharm. Sci*. 2020; 11 (1): 949–952. DOI: 10.26452/ijrps.v11i1.1919.

9. Pisarenko S. V., Eremenko E. I., Ryazanova A. G., Kovalev D. A., Buravtseva N. P., Aksenova L. Yu., et al. Genotyping and phylogenetic location of one clinical isolate of *Bacillus anthracis* isolated from a human in Russia. *BMC Microbiol*. 2019; 19:165. DOI: 10.1186/s12866-019-1542-3.

10. Liskova E. A., Egorova I. Y., Selyaninov Y. O., Razheva I. V., Gladkova N. A., Toropova N. N., et al. Reindeer anthrax in the Russian Arctic, 2016: climatic determinants of the outbreak and vaccination effectiveness. *Front. Vet. Sci*. 2021; 8:668420. DOI: 10.3389/fvets.2021.668420.

11. Ezhova E., Orlov D., Suhonen E., Kaverin D., Mahura A., Gennadinik V., et al. Climatic factors influencing the anthrax outbreak of 2016 in Siberia, Russia. *Ecohealth*. 2021; 18 (2): 217–228. DOI: 10.1007/s10393-021-01549-5.

12. Ivanov A. V., Makaev Kh. N., Melnikova L. A., Barbarova L. A., Murtazina G. Kh., Ivanova S. V., Khisamutdinov A. G. Method of obtaining erythrocyte antigen of anthrax antigen, method of obtaining control positive serum for kit of detection of antibodies in the blood serum of animals vaccinated against anthrax, in the reaction of indirect hemagglutination and kit for detection of antibodies. Patent No. 2599035 Russian Federation, Int. Cl. C12N 1/20 (2006.01), G01N 33/531 (2006.01). FSBSI "FCTRBS-ARRVI". Application: 2015128403/15. Date of filing: 13.07.2015. Date of publication: 10.10.2016. Bull. No. 28. (in Russ.)

13. Romanov G. I., Manichev A. A., Salenko L. S., Stepanova V. V., Zakharov D. G., Komelina L. I., et al. Method for producing serum against anthrax. Patent No. 1347224 Russian Federation, Int. Cl. 61 K39/40. Vsesojuznyj gosudarstvennyj nauchnokontrol'nyj institut vetpreparatov. Application: 4066843/13. Date of filing: 08.05.1986. Date of publication: 20.11.1995. (in Russ.)

14. Bavrina A. P. Modern rules for the use of parametric and nonparametric tools in the statistical analysis of biomedical data. *Medical Almanac*. 2021; 1 (66): 64–73. EDN: IZXMBZ. (in Russ.)

15. Fissore D., McCoy T. Editorial: freeze-drying and process analytical technology for pharmaceuticals. *Front. Chem*. 2018; 6:622. DOI: 10.3389/fchem.2018.00622.

16. Kelly C. D., O'Loughlin C., Gelder F. B., Peterson J. W., Sower L. E., Cirino N. M. Rapid generation of an anthrax immunotherapeutic from goats using a novel non-toxic

muramyl dipeptide adjuvant. *J. Immune Based Ther. Vaccines*. 2007; 5:11. DOI: 10.1186/1476-8518-5-11.

17. Beedham R. J., Turnbull P. C., Williamson E. D. Passive transfer of protection against *Bacillus anthracis* infection in a murine model. *Vaccine*. 2001; 19 (31): 4409–4416. DOI: 10.1016/s0264-410x(01)00197-9.

18. Kobiler D., Gozes Y., Rosenberg H., Marcus D., Reuveny S., Altboum Z. Efficiency of protection of guinea pigs against infection with *Bacillus anthracis* spores by passive immunization. *Infect. Immun*. 2002; 70 (2): 544–560. DOI: 10.1128/IAI.70.2.544-550.2002.

19. Herrmann J. E., Wang S., Zhang C., Panchal R. G., Bavari S., Lyons C. R., et al. Passive immunotherapy of *Bacillus anthracis* pulmonary infection in mice with antisera produced by DNA immunization. *Vaccine*. 2006; 24 (31–32): 5872–5880. DOI: 10.1016/j.vaccine.2006.04.065.

20. Reuveny S., White M. D., Adar Y. Y., Kafri Y., Altboum Z., Gozes Y., et al. Search for correlates of protective immunity conferred by anthrax vaccine. *Infect. Immun*. 2001; 69 (5): 2888–2893. DOI: 10.1128/IAI.69.5.2888-2893.2001.

21. Caldwell M., Hathcock T., Brock K. V. Passive protection against anthrax in mice with plasma derived from horses hyper-immunized against *Bacillus anthracis* Sterne strain. *PeerJ*. 2017; 5:e3907. DOI: 10.7717/peerj.3907.

22. Plotkin S., Grabenstein J. D. Countering anthrax: vaccines and immunoglobulins. *Clin. Infect. Dis*. 2008; 46 (1): 129–136. DOI: 10.1086/523578.

23. Kummerfeldt C. E. Raxibacumab: potential role in the treatment of inhalational anthrax. *Infect. Drug. Resist*. 2014; 7: 101–109. DOI: 10.2147/IDR.S47305.

24. Chitlaru T., Altboum Z., Reuveny S., Shafferman A. Progress and novel strategies in vaccine development and treatment of anthrax. *Immunol. Rev*. 2011; 239 (1): 221–236. DOI: 10.1111/j.1600-065X.2010.00969.x.

25. Schneemann A., Manchester M. Anti-toxin antibodies in prophylaxis and treatment of inhalation anthrax. *Future Microbiol*. 2009; 4 (1): 35–43. DOI: 10.2217/17460913.4.1.35.

26. Huang E., Pillai S. K., Bower W. A., Hendricks K. A., Guarnizo J. T., Hoyle J. D., et al. Antitoxin treatment of inhalation anthrax: a systematic review. *Health Secur*. 2015; 13 (6): 365–377. DOI: 10.1089/hs.2015.0032.

27. Firstova V. V., Shakhova A. S., Riabko A. K., Silkina M. V., Zeninskaya N. A., Romanenko Y. O., et al. Characterization of the adaptive immune response of donors receiving live anthrax vaccine. *PLoS One*. 2021; 16 (12):e0260202. DOI: 10.1371/journal.pone.0260202.

28. Brogna R., Oldenhof H., Sieme H., Figueiredo C., Kerrinnes T., Wolkers W. F. Increasing storage stability of freeze-dried plasma using trehalose. *PLoS One*. 2020; 15 (6):e0234502. DOI: 10.1371/journal.pone.0234502.

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