INTERNATIONAL MULTI DISCIPLINARY JOURNAL FOR RESEARCH & DEVELOPMENT
eISSN 2394-6334 Impact factor: 8.854 Volume 13, issue 06 (2026)

UDK: 615.37:616.98:577.21

IMMUNOLOGICAL AND BIOTECHNOLOGICAL STAGES OF ANTITOXIN PRODUCTION AGAINST BOTULISM

Isroilov Umidjon Islomjon o‘g‘li
1-son Davolash fakulteti 2-kurs talabasi,
2- TDTU, Toshkent, O'zbekiston, izlanuvchi,
iumidjon685@gmail.com
https://orcid.org/0009-0004-2211-4890

Mirzayev Jahongir O‘ktam og‘li
1-son Davolash fakulteti 2-kurs talabasi,
2-TDTU, Toshkent, O'zbekiston, izlanuvchi,
ibodullayevsardor777@gmail.com
https://orcid.org/0009-0004-4485-083X

Boltayeva Gulirano Shotursunovna
Mikrobiologiya virusologiya va immunologiya kafedra assistenti,
TDTU, Toshkent, O'zbekiston,

Abstract

Introduction. Botulism is a rare but extremely dangerous toxicoinfectious disease caused by botulinum neurotoxins produced by Clostridium botulinum. It is manifested by blurred vision, swallowing and speech disorders, muscle weakness, and descending flaccid paralysis. In severe cases, paralysis of the respiratory muscles leads to acute respiratory failure.

Aim. The aim of this review is to analyze the traditional immunological and modern biotechnological stages of antitoxin production against botulism, as well as to highlight existing challenges and promising solutions.

BOTULIZMGA QARSHI ANTITOKSIN ISHLAB CHIQARISHNING IMMUNOLOGIK VA BIOTEXNOLOGIK BOSQICHLARI O'ZGARTIRASIZ

Annotatsiya

Kirish. Botulizm — Clostridium botulinum ishlab chiqaradigan botulinum neyrotoksinlari sababli yuzaga keladigan kam uchraydigan, ammo o‘ta xavfli toksik-infeksion kasallikdir. U ko‘rish xiralashishi, yutish va nutq buzilishi, mushaklar holsizligi hamda pastga yo‘naluvchi bo‘shashgan falaj bilan namoyon bo‘ladi. Og‘ir holatlarda nafas mushaklari falajlanib, o‘tkir nafas yetishmovchiligiga olib keladi.

Maqsad. Ushbu sharhning maqsadi botulizmga qarshi antitoksin ishlab chiqarishning an’anaviy immunologik va zamonaviy biotexnologik bosqichlarini tahlil qilish, mavjud muammolar hamda istiqbolli yechimlarni yoritishdan iborat.

Material va usullar. Sharhda botulizm antitoksinlari bo‘yicha ilmiy adabiyotlar tahlil qilindi. Ot zardobiga asoslangan poliklonal antitoksin ishlab chiqarish bosqichlari hamda rekombinant antigenlar, faj displeyi, monoklonal antitelolar, oligoklonal kombinatsiyalar va nanobodlar kabi zamonaviy yondashuvlar o‘rganildi.

Natijalar. Tahlillar antitoksin samaradorligi antitelo turi, toksinning nechta epitopini nishonga olishi va organizmda saqlanish muddatiga bog‘liq ekanini ko‘rsatdi. Ot zardobi antitoksinlari poliklonal tarkibi sababli keng neytrallovchi faollikka ega. Biroq ularning geterolog oqsil tabiati allergiya, anafilaksiya va zardob kasalligi xavfini oshiradi. $F(ab')_2$ fragmentlarining qisqa yarim yemirilish davri esa uzoq muddatli himoyani cheklaydi.

Xulosa. Botulizmga qarshi davolashda xavfsiz, standartlashgan va uzoq ta’sirli biotexnologik antitoksinlarga ehtiyoj ortmoqda. Inson monoklonal antitelolari, rekombinant antitoksinlar, oligoklonal kombinatsiyalar va nanobodlar kelajakda samarali hamda kam immunogen preparatlar yaratishda istiqbolli yo‘nalish hisoblanadi.

Kalit so‘zlar: botulizm, botulinum neyrotoksini, antitoksin, immunizatsiya, ot zardobi, rekombinant antitelo, monoklonal antitelo, oligoklonal antitelo, nanobody, BabyBIG, biotexnologiya.

Иммунологические и биотехнологические этапы производства антитоксинов против ботулизма

Аннотация

Введение. Ботулизм — редкое, но чрезвычайно опасное токсико-инфекционное заболевание, вызываемое ботулиническими нейротоксинами, продуцируемыми Clostridium botulinum. Заболевание проявляется затуманиванием зрения, нарушением глотания и речи, мышечной слабостью, а также нисходящим вялым параличом. В тяжелых случаях паралич дыхательных мышц приводит к развитию острой дыхательной недостаточности.

Цель. Целью данного обзора является анализ традиционных иммунологических и современных биотехнологических этапов производства антитоксинов против ботулизма, а также освещение существующих проблем и перспективных путей их решения.

Материалы и методы. В обзоре проведен анализ научной литературы, посвященной антитоксинам против ботулизма. Изучены этапы производства поликлональных антитоксинов на основе лошадиной сыворотки, а также современные подходы, включая рекомбинантные антигены, фаговый дисплей, моноклональные антитела, олигоклональные комбинации и нанотела.

Результаты. Анализ показал, что эффективность антитоксина зависит от типа антител, количества эпитопов токсина, на которые они направлены, а также от продолжительности их циркуляции в организме. Антитоксины на основе лошадиной сыворотки благодаря своему поликлональному составу обладают широкой нейтрализующей активностью. Однако их гетерологичная белковая природа повышает риск развития аллергических реакций, анафилаксии и сывороточной болезни. Короткий период полувыведения фрагментов $F(ab')_2$, в свою очередь, ограничивает длительность защитного действия.

Заключение. В лечении ботулизма возрастает потребность в безопасных, стандартизированных и длительно действующих биотехнологических антитоксинах. Человеческие моноклональные антитела, рекомбинантные антитоксины, олигоклональные комбинации и нанотела являются перспективными направлениями для создания эффективных препаратов с низкой иммуногенностью в будущем.

Ключевые слова: ботулизм, ботулинический нейротоксин, антитоксин, иммунизация, лошадиная сыворотка, рекомбинантное антитело, моноклональное антитело, олигоклональное антитело, нанотело, BabyBIG, биотехнология.

Materials and Methods. This review analyzes scientific literature on botulism antitoxins. The stages of production of horse-serum-based polyclonal antitoxins were examined, along with modern approaches, including recombinant antigens, phage display, monoclonal antibodies, oligoclonal combinations, and nanobodies.

Results. The analysis showed that antitoxin efficacy depends on the type of antibody, the number of toxin epitopes targeted, and the duration of antibody persistence in the body. Horse-serum-derived antitoxins have broad neutralizing activity due to their polyclonal composition. However, their heterologous protein nature increases the risk of allergic reactions, anaphylaxis, and serum sickness. The short half-life of $F(ab')_2$ fragments, in turn, limits the duration of protective effect.

Conclusion. There is an increasing need for safe, standardized, and long-acting biotechnological antitoxins in the treatment of botulism. Human monoclonal antibodies, recombinant antitoxins, oligoclonal combinations, and nanobodies represent promising directions for the development of effective future therapeutics with low immunogenicity.

Keywords: botulism, botulinum neurotoxin, antitoxin, immunization, horse serum, recombinant antibody, monoclonal antibody, oligoclonal antibody, nanobody, BabyBIG, biotechnology.

Introduction. Botulinum neurotoxins are among the most potent toxic substances known biologically [2]. They break down SNARE proteins that facilitate the release of acetylcholine from synaptic vesicles, thereby stopping the transmission of impulses in the neuromuscular junction [2, 9]. As a result, muscle contraction is disrupted, and peripheral neural palsy develops. There are several serological types of Botulinum neurotoxins, with mainly A, B, E, and in some cases F types being significant in human morbidity [1, 9]. The main clinical forms of botulism are food botulism, ulcerative botulism, infantile botulism, and less commonly, iatrogenic botulism due to botulinum toxin used for medical or cosmetic purposes [1, 3]. Food botulism can often be associated with improperly canned products, homemade canned goods, or smoked or salted food products[1]. In infantile botulism, the toxin is formed not in a ready-made form, but from C. botulinum spores colonized in the intestine [10]. Antitoxin therapy plays an important role in the treatment of botulism. Antitoxin serum binds botulinum toxin circulating freely in the blood and prevents it from entering nerve cells [3, 4]. However, the antitoxin cannot neutralize the toxin that has already penetrated the nerve cell, so administering the drug as early as possible increases the effectiveness of treatment [3]. It has been shown that the use of human botulism immunoglobulin - BabyBIG - is clinically effective in neonatal botulism [10]. Historically, antitoxins against botulism were primarily obtained through the hyperimmunization of horses with botulinum anatoxins [7]. Although such polyclonal antitoxins possess a wide spectrum of neutralizing activity, they are considered foreign proteins to the human body. Consequently, anaphylactic reactions, serum sickness, and other immunological complications may develop [7, 11]. Furthermore, the half-life of equine antitoxins based on F (ab') 2 fragments is relatively short, which poses a limitation in terms of long-term protection [11]. Modern biotechnological approaches are aimed at reducing these problems. Research is being conducted to create a new generation of antitoxins based on recombinant DNA technology, DNA displays, yeast displays, human monoclonal antibodies, oligoclonal antibody combinations, and nanobodes [4, 5, 6, 8]. In

particular, research conducted by Nowakowski et al. has proven that a combination of several antibodies binding to different toxin epitopes, rather than a single monoclonal antibody, exerts a potent neutralizing effect [5]. This approach will serve as a scientific basis for creating safer, more effective, and standardized antitoxin drugs in the future. From this perspective, the analysis of the immunological and biotechnological stages of producing antitoxin against botulism is of not only theoretical but also practical importance. This review highlights the stages of antidote production based on traditional horse serum, recombinant and monoclonal antibody technologies, nanobots, the BabyBIG drug, existing problems, and their promising solutions based on literature.

Review

Scientific research on the production of antitoxin serum against botulism has historically developed in two main directions: the first is traditional polyclonal antibodies obtained through animal immunization; the second is modern biotechnological approaches based on recombinant antibodies, monoclonal antibodies, oligoclonal combinations, and nanobodes [4, 5, 6]. Traditional methods have been used in clinical practice for many years and have served as an important treatment tool, especially for food botulism, wound botulism, and emergency epidemiological situations [1, 3]. However, the biological origin of such preparations, which are often obtained from horse serum, raises certain issues regarding their safety and tolerability in the body [7, 11]. As noted in the literature, botulinum neurotoxins have several serotypes, of which types A, B, E, and F are the most significant in the development of clinical botulism in humans [1, 9]. Therefore, antitoxin preparations must cover as many serotypes as possible. The use of polyvalent antitoxins in practice arose precisely from this need [3]. However, the antigenic diversity of botulinum toxins by serotypes and subtypes poses significant scientific and technological challenges in antitoxin production [2, 8]. For example, even small molecular differences within a single serotype can affect the binding strength and neutralization efficiency of an antibody [5, 8]. The main advantage of traditional antitoxins is their polyclonal nature. That is, a single serum contains many antibodies that can bind to different epitopes of the toxin [7]. This increases the ability to bind the toxin from multiple points and neutralize it. At the same time, the disadvantage of polyclonal sera is precisely related to their complex composition: the antibody composition and level of neutralizing activity may vary within each production batch [7, 12]. Therefore, the standardization, assessment of biological activity, and safety control of such drugs are of particular importance [12]. In modern literature, the immunogenicity of antitoxins prepared from horse serum is presented as a significant problem [7, 11]. When a heterologous protein is injected into the human body, an allergic reaction, anaphylaxis, or serum sickness may develop [11]. To reduce this risk, it is recommended to remove the Fc fragment of the immunoglobulin molecule through enzymatic cleavage and use drugs in the form of F (ab') fragments [7, 11]. Such a modification reduces immunogenicity, but also reduces the retention time of the drug in blood plasma [11]. Therefore, in recent years, research has intensified on the development of new-generation antitoxins based on recombinant antibody technologies, human monoclonal antibodies, oligoclonal antibody mixtures, and nanobodes [4, 5, 6, 8]. These approaches have advantages over traditional serums, such as high purity, clear molecular composition, low immunogenicity, stability between production batches, and a strong neutralizing effect targeted at specific serotypes or epitopes [4, 5]. Nevertheless, the widespread production of biotechnological drugs has not yet been uniformly implemented in all countries due to the complex infrastructure, high costs, and clinical trials required [4, 6].

Traditional immunological stages. The classic method for producing an antitoxin against botulism is based on the hyperimmunization of large animals, primarily horses, with botulinum toxins or immunized toxin antigens [7]. A strong humoral immune response is formed in the horse's body, and polyclonal antibodies that neutralize botulinum toxins accumulate in the blood

plasma [7, 12]. Subsequently, immunoglobulins are isolated from this plasma, purified, tested for safety, and prepared as a therapeutic antitoxin [7]. The first step is antigen preparation. At this stage, the toxin obtained from Clostridium botulinum strains is converted into anatoxin using formalin or other detoxifying methods [7, 12]. Anatoxin is a drug that has lost its toxic activity but retains its immunogenic properties. It is this property that allows it to be used as a safe antigen for animal immunization [12]. The quality of the antigen directly affects the antibody titer and the neutralizing activity of the antitoxin at the next stage. The second stage is the phased immunization of horses. Initially, the animal is injected with a small dose of anatoxin, and subsequently, the doses are gradually increased as the immune response intensifies [7]. This approach is called hyperimmunization. The process lasts for several months, during which high-titre antibodies against botulinum toxin are formed in the horse's body [12]. Immunization is usually administered subcutaneously or intramuscularly. After each injection, the animal's general condition, body temperature, local reaction, and immune response indicators are monitored [7]. The third stage is controlling the antibody titer. To produce an antitoxin, it is not enough to simply immunize the animal; it is necessary to determine whether the resulting antibodies actually have the ability to neutralize the toxin [12]. For this purpose, enzyme-linked immunosorbent assay (ELISA), toxin-antitoxin neutralization tests, and other biological tests are used [9, 12]. While the ELISA method indicates the degree of antibody binding to the antigen, neutralization tests allow for the assessment of its biological protective power [12]. Therefore, neutralizing activity is one of the most important criteria for antitoxin quality. The fourth stage is blood collection and plasma isolation after the formation of a high-titre antibody. Blood collection from horses is carried out in accordance with strict veterinary and biosafety requirements [7]. The resulting blood is centrifuged or separated into fractions using other methods. As a result, plasma rich in immunoglobulins is obtained [7, 12]. Plasma can contain not only antibodies to botulinum toxin but also many other proteins, enzymes, and biologically active components. Therefore, it will be necessary to thoroughly clean it in the subsequent stages [7]. The fifth stage is the isolation and purification of immunoglobulins. Traditionally, ammonium sulfate precipitation, alcohol fractionation, membrane filtration, and chromatographic methods have been used in this process [7, 12]. In modern production, the purity of the preparation is increased using methods such as ion-exchange chromatography, ultrafiltration, and sterile filtration [7]. The purpose of the purification process is to reduce excess proteins, cellular debris, pyrogenic substances, and potential allergenic components while preserving the therapeutically significant immunoglobulin fraction [11, 12]. The sixth stage is the enzymatic processing of the immunoglobulin molecule. Since the Ot IgG molecule is a foreign protein to the human body, its Fc fragment can be strongly recognized by the immune system [11]. This increases the risk of allergic reactions and serum diseases [11]. Therefore, the pepsin enzyme breaks down the IgG molecule and produces F (ab') 2 fragments capable of binding the toxin [7, 11]. F (ab') fragments possess relatively low immunogenicity due to the absence of the Fc part. This increases the clinical safety of the antitoxin [11]. However, there is an important limitation of the F (ab') fragments. Due to the absence of the Fc fragment, such antitoxins are unable to be recycled through neonatal Fc receptors and are eliminated from the body relatively quickly [11]. As a result, their half-life is shorter than that of complete IgG molecules [4, 11]. This condition may require long-term monitoring and, if necessary, additional therapeutic measures in some severe cases of botulism [3]. The seventh stage is the standardization and quality control of the finished antitoxin preparation. At this stage, the sterility, non-pyrogenicity, protein content, neutralizing activity, safety, and serotype coverage of the drug are evaluated [7, 12]. Antitoxin activity is usually expressed in international units. Neutralizing activity against each serotype must be investigated separately, as botulinum toxins

differ in antigenicity [1, 9]. The effectiveness of the antitoxin used in clinical practice depends on the correct execution of these quality control stages. The advantage of the traditional immunological method is that it is relatively well-studied, has been tested in practice, and can target multiple epitopes of the toxin due to a mix of polyclonal antibodies [7]. However, its disadvantages are also serious: the need to use animals, differences between production batches, high immunogenicity, the risk of allergic reactions, serum sickness, and a limited half-life of the drug [7, 11, 12]. These factors increase the need to create modern biotechnological antitoxins [4, 5, 6].

Biotechnological stages. The immunogenicity of traditional horse serum-based antitoxins, differences between production batches, short half-lives, and restrictions associated with animal use have intensified the need to create modern biotechnological antitoxins [4, 11, 12]. The primary goal of biotechnological approaches is the development of antibody preparations that possess high purity, standardization, low immunogenicity, clear molecular composition, and strong neutralizing effects [4, 5]. Such preparations are developed based on recombinant DNA technology, monoclonal antibody platforms, FG displays, yeast displays, oligoclonal antibody combinations, and nanobots [5, 6, 8, 13]. The first biotechnological step is the creation of safe recombinant antigens. Since the complete molecule of Botulinum neurotoxin is highly toxic, studies often use individual domains with antigenic properties but low toxicity risk rather than the entire molecule of the toxin [2, 4]. In particular, the receptor-binding Hc domain is an important target for antibody screening and immunological studies [8]. At this stage, the corresponding gene fragment of botulinum neurotoxin is amplified using PCR, inserted into the expression vector, and produced as a recombinant protein in bacterial or eukaryotic expression systems [4, 13]. The second stage is the creation and screening of antibody libraries. Antibody fragments that bind strongly to toxin epitopes are selected using phage display, yeast display, or other high-permeability molecular selection technologies [5, 13]. Such libraries can be created based on B-cells from immunized donors or using synthetic genetic constructs [4, 13]. During the screening process, scFv, Fab, or other antibody fragments with the highest affinity are isolated, which are then converted into full-length human IgG molecules or engineered [4, 5]. The third stage is the selection and optimization of monoclonal antibodies. Since the monoclonal antibody is directed at a single specific epitope, its composition is stable and has a high probability of standardization [4]. However, the diversity of botulinum toxins by serotype and subtype indicates that a single monoclonal antibody is not always sufficient [5, 8]. Therefore, the selected antibodies are thoroughly evaluated for their neutralizing activity, epitope binding properties, degree of affinity, thermostability, and potential immunogenicity in the human body [4, 5, 13]. When necessary, antibodies undergo molecular engineering steps such as "humanization" or affinity enhancement [13]. The fourth important direction is oligoclonal antibody therapy. Research indicates that combinations consisting of multiple antibodies binding to different functional epitopes of the toxin may be more effective for potent neutralization of botulinum toxin [5]. As shown by Nowakowski et al., a recombinant oligoclonal antibody mixture exhibits a much stronger neutralizing effect than a single antibody [5]. This approach allows for the binding, internalization, and functional activity of the toxin to the nerve cell to be blocked from multiple points simultaneously [5]. Therefore, oligoclonal antibody combinations are a promising direction for creating broad-spectrum and highly effective antitoxins in the future [4, 5]. The fifth promising area is nanobads, i.e., small antibody fragments of the VHH type. Nanobads are typically created based on heavy-chain domains of antitells from camel-like animals and are characterized by their small size, high stability, good solubility, and ability to penetrate complex epitopes [6, 8]. Nanobads against botulinum neurotoxins can encompass various subtypes, be relatively easily produced in recombinant systems, and be engineered into

multivalent formats [6, 8]. Nanobodes with particularly wide reactivity serve as an important scientific basis for creating universal or semi-universal antitoxins [6]. The sixth stage is the production and quality control of the antibody preparation. Selected recombinant antibodies or antibody fragments can be produced in cell cultures, such as CHO cells, bacterial systems, or yeast platforms [4, 13]. The preparation is then subjected to chromatographic purification, ultrafiltration, sterile filtration, and biological activity tests [4]. Quality control evaluates the purity, neutralizing activity, endotoxin level, sterility, stability, serotype coverage, and immunogenic risk of the drug [4, 13]. Such a standardized production system reduces the difference between batches and increases clinical safety compared to traditional horse serum [4].

Existing problems and their solutions. An analysis of the literature shows that there are a number of urgent problems in the production and use of antitoxin against botulism. The first problem is the immunogenicity of antitoxins derived from traditional horse serum [7, 11]. When a heterologous protein is injected into the human body, side effects such as allergic reactions, anaphylaxis, or serum sickness can occur [11]. A partial solution to this problem is the removal of the Fc fragment of the IgG molecule through enzymatic cleavage and the use of F (ab') fragments [7, 11]. However, as a long-term solution, it is advisable to switch to human monoclonal antibodies and recombinant antibody preparations [4, 5]. The second problem is the short-term retention of horse antitoxins based on F (ab') 2 in the body [11]. Due to the absence of the Fc fragment, such drugs do not have the ability to be recycled through neonatal Fc receptors and are released from the bloodstream relatively quickly [4, 11]. This condition may increase the risk of prolonged or recurrent intoxication in some patients [3]. The use of full-length human IgG antibodies is proposed as a solution, as they are stored in the body for longer and can exert a stable neutralizing effect [4]. The third problem is the diversity of botulinum neurotoxins by serotype and subtype [1, 8, 9]. Current antitoxins are often developed against certain major serotypes and may not cover all clinical and biological risk variants equally [3, 8]. This is especially important in terms of bioterrorism, laboratory safety, and epidemiological emergencies. As a solution, it is necessary to develop broad-spectrum oligoclonal antibody mixtures, monoclonal combinations targeting various epitopes, and interserotype reactive nanobodes [5, 6, 8]. The fourth problem is that late antitoxin administration reduces efficacy [3]. The antitoxin only neutralizes the toxin circulating freely in the blood; it cannot eliminate the toxin that has already entered the nerve cell [3, 4]. Therefore, in clinical practice, the principle of early antitoxin administration based on clinical assessment without waiting for laboratory confirmation in cases of suspected botulism is considered important [3]. As a solution, it is necessary to strengthen rapid diagnostic algorithms, epidemiological awareness, emergency antitoxin stocks, and intensive care systems for doctors [1, 3]. The fifth problem is the technological complexity and high cost of producing recombinant and monoclonal antibody preparations [4, 13]. Such drugs require specialized laboratory infrastructure, cell culture, purification systems, quality control, and clinical trials [4]. This makes their widespread use difficult in healthcare systems with limited resources. Solutions include optimizing production platforms, utilizing stable cell lines, implementing cheaper expression systems, and forming antitoxin reserves through international cooperation [4, 6, 13]. The sixth problem is the need for a specialized approach to the treatment of infantile botulism. Human botulism immunoglobulin - BabyBIG is a clinically important drug for infantile botulism [10]. It can reduce disease duration, length of hospital stay, and the need for artificial respiration [10]. However, the availability of such a drug is not uniform across all regions. Therefore, early detection of pediatric botulism, improved access to specific immunoglobulin preparations, and increased clinical awareness of the disease among pediatricians are necessary [10, 14].

Conclusions. Botulism is a rare but highly lethal neuroparalythic disease, the effective management of which is based on early diagnosis, timely antitoxin therapy, and respiratory support. Although polyclonal antitoxins obtained from horse serum retain their practical significance, their immunogenicity, inter-batch biological variability, and short half-life limit the possibilities of their application. Therefore, recombinant antibodies, human monoclonal antibodies, oligoclonal combinations, and antitoxins based on nanobads are promising directions for creating safe, standardized, and broad-spectrum therapeutic agents. In the future, the development of effective, affordable, and fast-delivery antitoxin platforms for resource-limited regions will remain a pressing scientific and practical task.

References

1. Zhang S., et al. Botulism: A review of clinical and public health aspects. Toxins. 2021;13(4):275.
2. Rasetti-Escargueil C., Popoff M. R. Antibodies and vaccines against botulinum toxins: available and prospective strategies. Toxins. 2019;11(9):528.
3. Centers for Disease Control and Prevention. Clinical Guidelines for Diagnosis and Treatment of Botulism, 2021. MMWR Recommendations and Reports. 2021;70(2):1–30.
4. Webb R. P., Smith L. A. Development of recombinant antibody-based therapies for botulism. BioDrugs. 2013;27(3):225–236.
5. Nowakowski A., Wang C., Powers D. B., Amersdorfer P., Smith T. J., Montgomery V. A., Sheridan R., Blake R., Smith L. A., Marks J. D. Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(17):11346–11350.
6. Inglis G. D., et al. Nanobody-based therapeutics for botulism. Microorganisms. 2022;10(8):1637.
7. Panova E. N., Shcherbakov D. N. Производство и стандартизация противоботулинических сывороток: исторические и современные аспекты. Биофармацевтический журнал. 2023;15(3):12–23.
8. Rasetti-Escargueil C., et al. Broadly neutralizing nanobodies against botulinum neurotoxin A. Toxins. 2021;13(1):18.
9. Kalb S. R., et al. Detection of botulinum neurotoxins A, B, E, and F in clinical specimens by mass spectrometry. Analytical Chemistry. 2009;81(13):5613–5620.
10. Arnon S. S. Creation and development of the human botulism immune globulin intravenous — BIG-IV, BabyBIG®. Pediatrics. 2013;131(5):e1483–e1488.
11. Kilsby A. J., et al. Immunogenicity of therapeutic equine antisera: clinical implications. Clinical Immunology. 2022;234:108909.
12. Mukovozova L. A., et al. Immunoprophylaxis of botulism: current status and future directions. Problems of Particularly Dangerous Infections. 2019;1:76–82.
13. Shcherbakov D. N., et al. Recombinant antibodies to botulinum toxin: generation and preclinical evaluation. Russian Journal of Bioorganic Chemistry. 2020;46(2):195–206.
14. Sobirova G. N., Axmedova D. R. Bolalarda botulizmning klinik kechishi va davolash tamoyillari. Pediatriya. 2021;2:89–95.

15. Giyosova N. S. Oziq-ovqat bilan bog‘liq botulizm kasalligining oldini olish va antitoksin terapiyasining ahamiyati. Pediatriya va yuqumli kasalliklar. 2021;3:67–72.
16. Atabekov I., Talipova M. O‘zbekistonda botulizmning epidemiologik xususiyatlari va profilaktikasi. Infeksion kasalliklar muammolari. 2020;4:23–29.
17. Abdullaeva M. A., Yusupova N. T. Botulizmga qarshi antitoksin zardobini ishlab chiqarishning biotexnologik usullari. O‘zbekiston farmatsevtik xabarnomasi. 2023;1:34–40.
18. Karimov X. Ya. Klinik immunologiya va allergologiya. Toshkent: Fan nashriyoti; 2019.
19. Lévy J., et al. Botulism as a potential bioterrorism agent. Clinical Microbiology and Infection. 2006;12(5):397–404.
20. Muxammadiev A. A., Kamilov R. S. Botulizm: zamonaviy diagnostika va davolash tamoyillari. O‘zbekiston Tibbiyot Jurnali. 2022;2:45–51.