https://doi.org/10.31489/2025BMG1/36-43

UDC 57.083.3:578.824.1 Received: 9.09.2024 | Accepted: 22.11.2024

A.B. Nurmukhambetova, Zh.M. Batanova, G.A. Bayandy, Y.A. Krykbayev*, E.Zh. Bitanova, N.N. Akhmetsadykov, D.S. Amankeldi

Research and Production Enterprise "Antigen" LLP, Almaty, Kazakhstan
**Corresponding author's e-mail: krykbaev_e@mail.ru*

Modes for chromatographic purification of immunoglobulin G (IgG) from the serum of horses hyperimmunized with rabies antigen

Immunoglobulin G (IgG) plays a key role in the body's immune response, its high specificity to antigens and effectiveness in neutralizing pathogens make it a valuable tool in medical and scientific research. Horses are a source of significant amounts of IgG, but their purification to a level suitable for clinical or scientific use requires specialized methods. Development of chromatographic purification modes for immunoglobulin G (IgG) from the serum of horses hyperimmunized with rabies antigen will allow standardizing the technology in accordance with international quality and safety standards for the production and control of immunobiological medicines, vaccines and diagnostic test systems. Development of step-by-step modes and procedures for chromatographic purification of the serum of horses hyperimmunized with rabies antigen from the CVS-11 strain, with an assessment of the efficiency of the purification technology, protein yield and purity. The study used serological methods for obtaining and preparing serum from hyperimmunized horses, as well as biotechnological methods of gel filtration, ion exchange chromatography and subsequent electrophoresis. The practical significance of the study may allow developing technologies for obtaining immunobiological preparations based on immunoglobulins, and can be used in the fields of medicine, biotechnology and scientific research that require the use of highly purified immunoglobulins. According to the presented purification technology, immunoglobulins weighing 150 kDa were obtained, which under reducing conditions are divided into 2 light chains (28 kDa) and 2 heavy chains (55 kDa).

Keywords: immunoglobulin, purification technology, chromatography, rabies, serum, rabies antigen, hyperimmunization, antibodies.

Introduction

Rabies virus (RABV) belongs to phylogroup I of the genus Lyssavirus of the family Rhabdoviridae of the order Mononegavirales [1]. It is a zoonotic virus that is almost ubiquitous worldwide in various reservoir animals, including domestic and wild dogs and bats. Despite considerable efforts, most countries face serious difficulties in controlling RABV [2, 3], and in fact the virus has only been eradicated in a few developed countries by mass vaccination of wild and domestic dogs [4].

Today, about 3 billion people live at risk of contracting rabies from bites of infected animals, mainly in Asia and Africa, where half of the victims are children under 15 years of age [5, 6]. Nevertheless, 19–50 million people receive post-exposure prophylaxis (PEP) each year.

Following a bite from a potentially infected animal, immediate administration of three doses of vaccine within the first week and one dose of rabies immunoglobulin (RIG) is recommended to kill the virus before it enters the nervous system [7, 8].

After the onset of rabies symptoms, the mortality rate reaches almost 100 %. It has been reported that about 59 000 people die from rabies each year worldwide, most of them in developing countries [9].

Although inactivated RABV vaccines are safe, the immunogenicity of inactivated vaccines is relatively lower than that of live attenuated vaccines [10, 11]. For post-exposure prophylaxis, four to five vaccinations of inactivated vaccine are required to achieve sufficient protection, resulting in a relatively long period and high immunization cost. Thus, there is still a need to improve the efficacy of other methods against rabies.

The rabies virus spreads within the wound itself before entering the motor nerve and infecting the central nervous system [12]. Vaccine-induced active immunity develops approximately 7–10 days after the first dose of vaccine; administration of antirabies immunoglobulin provides passive immunity to protect the patient during this hiatus. In cases with a detectable site of exposure, such as a bite, wound washing and infiltration with antirabic immunoglobulin are critical for local neutralization of the viral inoculum [13]. Cases of fatal failure of PCP treatment often result from deviating from recommendations with inadequate or missed wound infiltration with antirabic immunoglobulin [14, 15].

Purification of equine IgG is challenging because the product must be thoroughly purified in large enough quantities in an economical manner. Any clinically used antibody must be pure, as contaminating serum proteins can cause various adverse reactions. Horse IgG is widely used in developing countries where the cost of the product can be a major limiting factor [16].

Classical protocols for the isolation and purification of IgG antibodies often did not result in a high degree of purity. Currently, chromatographic methods are used to purify proteins to a high degree. The separation and extraction of proteins by chromatographic methods are influenced by factors such as buffer type and pH, gradient length, mobile phase flow rate, ionic strength, and protein characterization. Selecting the ideal conditions for protein purification involves controlling and varying these parameters.

In the present study, we evaluated the efficacy of anion-exchange and exclusion separation as a single-step method or combined protocol for purification of antirabic IgG from equine serum. We also investigated ammonium sulfate precipitation and n-hexane delipidation of serum prior to chromatographic separation with respect to the final purity of polyclonal antibodies. Based on these results, we propose a strategy for purification of polyclonal anti-rabies IgG antibodies from horse serum by high-resolution ion-exchange chromatography.

Studies of anti-rabies immunoglobulin (RIG) have revealed differences in antibody titers, with the neutralization test in mice showing higher levels than the rapid fluorescence focus inhibition test [17]. This discrepancy affects the efficacy of anti-rabies immunoglobulin in post-exposure treatment with rabies vaccine. Studies in dogs have shown that the immunoglobulin response to rabies virus immunization is characterized by an initial increase in IgM and a subsequent increase in IgG [18]. In search of alternatives to human and equine antirabic immunoglobulin, rabbit antirabic immunoglobulin was developed and found to be safe and effective for post-exposure prophylaxis. Hyperimmunization has been identified as a method of inducing desired antibody titers against rabies in potential plasma donors [19].

Antirabies immunoglobulin, especially human antirabies immunoglobulin (HRIG), has been shown to be safe and effective in pediatric patients with suspected exposure to the virus [20]. However, there are significant gaps in the market availability of rabies biologics including immunoglobulins in India, which may hinder the prevention and elimination of rabies in humans [21]. The development of monoclonal antibodies for rabies post-exposure prophylaxis is a promising area, with the first anti-rabies monoclonal antibody recently receiving regulatory approval in India [22]. It is important to note that the diagnosis of rabies in patients who have recently received intravenous immunoglobulin (IVIG) may be difficult because these patients may have serum RLNA despite not having been vaccinated against rabies [23].

Experimental

The studies were performed in the laboratory "Virology" of Research and Production Enterprise "Antigen" LLP in the period from July 2023 to March 2024.

Strain

Strain CVS-11 (Anses, France) is a fixed strain of rabies virus used in laboratory and production research. It was isolated in 1931 from the brain of an infected dog in New York City. The CVS-11 strain is adapted to growth in cell cultures and is widely used for rabies virus research and vaccine development.

Horses are producers

Producing horses were hyperimmunized with increasing doses of inactivated rabies virus CVS-11 strain antigen, ranging from a concentration of 3.0-3.5 lg TDC /cm_{50}^3, to a concentration of 6.0-6.5 lg TDC /cm_{50}^3.

Assembly of hyperimmunized serum

Hyperimmunized serum was prepared in the conventional manner, clarified by centrifugation (1000 g, 15 min) and diluted 1:1 with sodium phosphate buffer at pH 7.2 before preparation. The presence of polyclonal antibodies (pAb) against rabies virus was serologically confirmed by AGID radial immunodiffusion or ELISA.

Ammonium sulfate precipitation

The precipitation was carried out at +4 °C. To 100 ml of serum, 50 ml of saturated ammonium sulfate solution was added. The next day, this material was centrifuged (10,000 g for 20 minutes) and washed twice with 50 % saturated ammonium sulfate solution. The precipitate was dissolved in distilled water. To remove salts and low molecular weight compounds, serum was dialyzed against 0.05MNaP++0.015 M NaCl buffer 20 times the sample volume with agitation, at 4 °C in a Spectra Por dialysis bag (Spectrum Laboratories), with a molecular weight capacity of 12–14 kDa. The precipitate solution was mixed with an equal volume of

n-hexane and centrifuged (20000 g, 40 °C for 30 min) to remove lipids. The final solution was filtered through a Millipore filter (0.22 μm) and the clear supernatant was loaded onto the column.

Chromotographic procedures

Gel filtration was performed on XK 26/100 Sephacryl S-200 HR columns (GE Healthcare, Sweden). The column was equilibrated with five volumes of 0.05 M Na-phosphate buffer + 0.015 M NaCl, adjusted to pH 7.0. Samples were subjected to chromatography at flow rates of 0.8 mL/min. Molecular mass calibration curves were constructed to determine the molecular mass of all sample components.

Ion-exchange chromotography procedure

Ion-exchange chromatography was performed on an XK 16*40 anion-exchange column, DEAE Sephacel, (GE Healthcare, Sweden) with starting buffer A, 20 mMNaP+(pH 7.0) and elution buffer B, 1 M NaCl. The gradient was generated for 20 minutes at a flow rate of 1.2 mL/min. Sample loading was 5 mL of sample after injection using a 0.1-2.0 mL sample loop. The appearance of protein in the fractions was monitored using a spectrophotometer by measuring the OD at 280 nm. 100 μl of each fraction was precipitated with ethanol and analyzed by SDS-PAGE.

Electrophoresis

The purity of the different IgG preparations was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of recovered and unrecovered samples. The total concentration of polyacrylamide in the separating gel was 8 or 12 %. Coomassie brilliant blue R-250 was used to visualize protein bands. Low molecular weight marker from Thermo scientific (Vilnius, Lithuania) was used as a standard.

Results and Discussion

Ammonium sulfate precipitation.

Equine hyperimmune serum against rabies was used as the study material. A preliminary experiment was conducted to study the efficiency of IgG precipitation by different concentrations of ammonium sulfate, ethanol and isopropanol (Fig. 1).

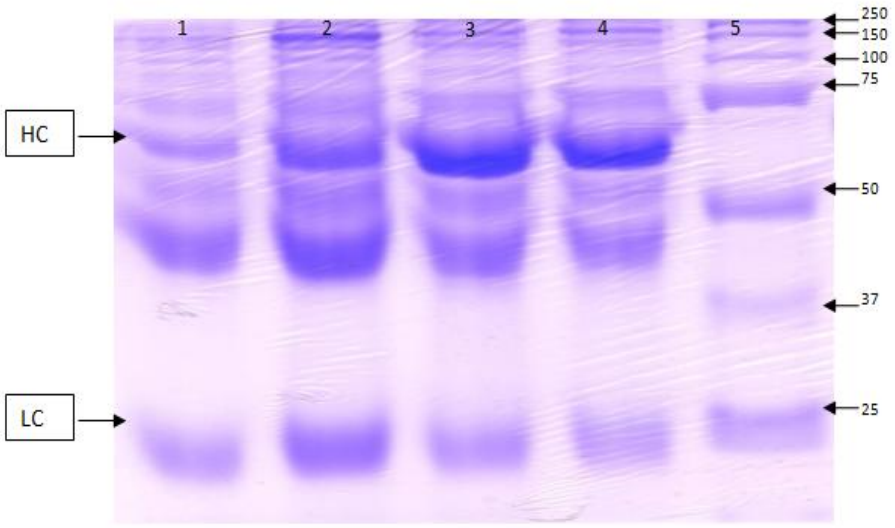

Figure 1. Electrophoregram comparing the precipitation of IgG from hyperimmune serum by different methods. 1 — Precipitate after precipitation with Ammonium Sulfate (40 % saturated), 2 — Precipitate after precipitation with Ammonium Sulfate (50 % saturated), 3 — Precipitate after precipitation with Isopropanol (1:1), 4 — Precipitate after precipitation with 96 % Ethanol (2:1), 5 — Standard molecular weight marker. The corresponding molecular mass of each band is indicated in kDa.

Analysis of the protein by SDS-PAGE under reducing conditions showed that the basis for separation is preferential precipitation of IgG at high concentrations of ammonium sulfate. At lower salt concentrations in this range, the precipitate consists mainly of IgG, but a significant amount of antibody still remains in solution and hence the recovery rate is very low. On the other hand, at higher salt concentrations the recovery of

IgG is very high but also precipitates a significant amount of impurities, primarily serum albumin, which results in very low purity and requires further purification [24] Also from the SDS-PAGE results it can be analyzed that precipitation with isopropanol and 96 % ethanol can be used for the primary precipitation and concentration of immunoglobulins.

Gel-filtration chromotography procedure

In the first stage of the experiments, horse serum containing IgG against rabies virus was loaded onto a chromatograph column. Prior to chromatography, ammonium sulfate precipitation and n-hexane delipidation were used as a general purification step.

Gel filtration chromatography on a 26/70 Sephacryl S-200 HR XC column was used to obtain high purity IgG fractions. The column was loaded with 5 ml of post-dialysis serum and the best performance was achieved at a flow rate of 0.8 ml/min. This step allowed the separation of immunoglobulin with a molecular weight (MW) of 150,000 kDa from most of the albumin fraction and other serum proteins. The chromatogram plot of the primary purified IgG fraction on the Sephacryl S-200 NR column is shown in Figure 2.

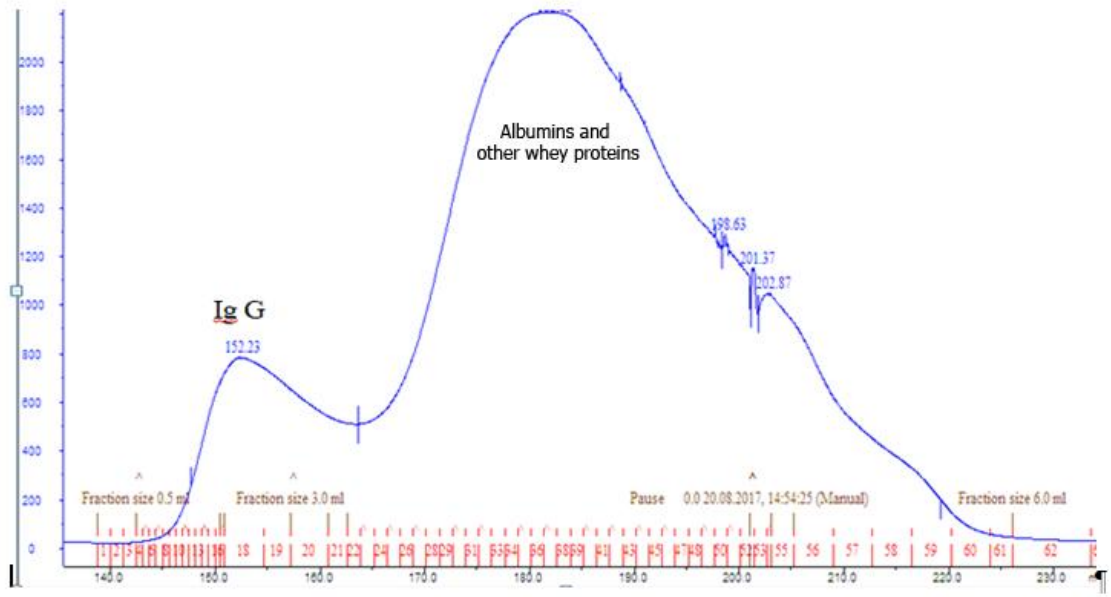

Figure 2**.** Chromatogram of anti-rabies IgG obtained on a 26/70 Sephacryl S-200 NR XC column

The first peak containing the target immunoglobulin fraction was analyzed by SDS-PAGE (Fig. 3).

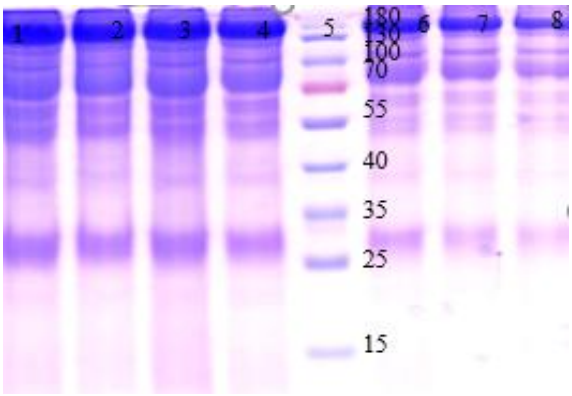

Figure 3. SDS-PAGE of immunoglobulins obtained by gel filtration chromatography.
1,2,3,4,6,7,8- immunoglobulin fractions obtained by gel filtration chromatography, 5- Standard molecular weight marker. The corresponding molecular mass of each band is indicated in kDa.

From the results of SDS-PAGE under reducing conditions, it can be seen that the chromatographic fraction of the first peak contains not only IgG but also traces of other serum proteins. The target fractions containing IgG were further purified by ion exchange chromatography.

Ion exchange chromatography procedure

The second step of purification of anti-rabies IgG consisted of anion-exchange chromatography on an XK 16*40 column, DEAE Sephacel, GE Healthcare. This column was used for secondary purification of the IgG peak obtained from the gel filtration column. The best results were obtained when protein elution was performed with 20 mM sodium-phosphate buffer at pH starting at 8.5 and ending at 7.5, using a linear salt gradient from 0 to 1 M NaCl concentration for 20 min at a flow rate of 1.2 mL/min. The resulting chromatogram was shown in Figure 4. The elution profile gave at least two major peaks. The first peak in the chromatogram is the fraction of positively charged IgG that did not bind to the positively charged sorbent. The second major peak is the remaining serum proteins firmly bound to the sorbent and then eluted at high salt concentration. Thus, we were able to purify the anti-rabies IgG from the remaining serum proteins. The purity verification of the obtained IgG is shown in Figure 5.

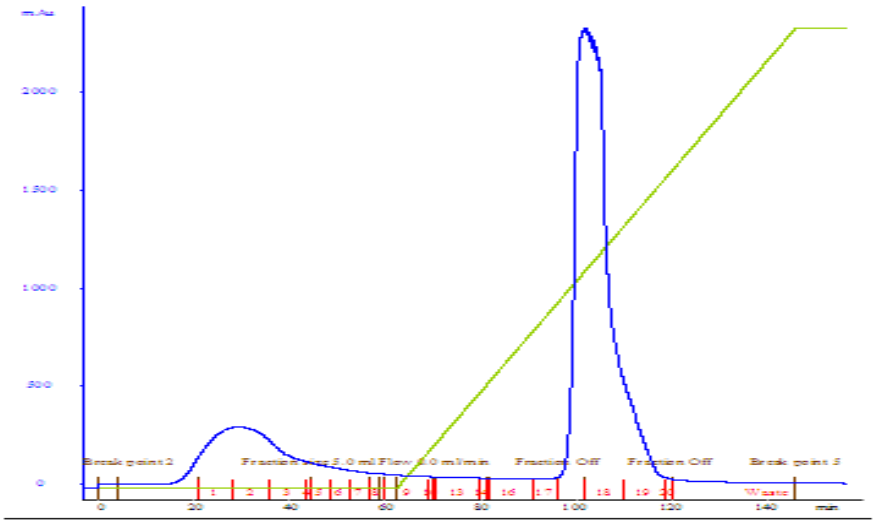

Figure 4. Chromatogram of the receipt of anti-rabies IgG obtained on XK 16*40 column, DEAE Sephacel

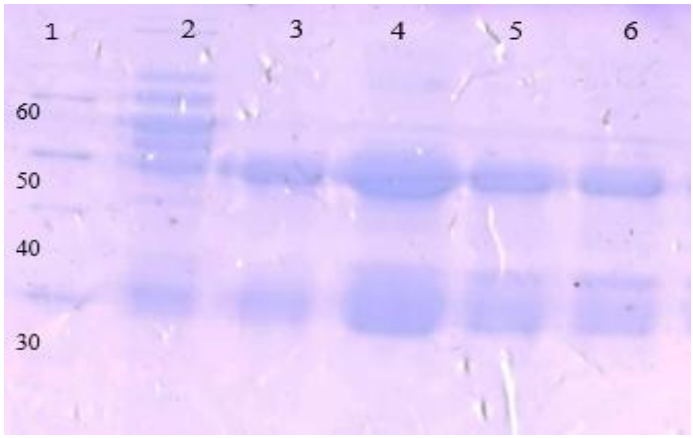

Figure 5**.** SDS-PAGE of immunoglobulins obtained by ion exchange chromatography.
1- Standard molecular mass marker. The corresponding molecular mass of each band is indicated in kDa.
2,3,4,6,-fractions of immunoglobulins obtained by ion exchange chromatography

The purity of immunoglobulin purified on the ion exchanger can be seen by SDS-PAGE under reducing conditions. IgG with a molecular mass of 150 kDa. Under reducing conditions, separated into 2 light chains (28kDa) and 2 heavy chains (55kDa).

Conclusion

According to the results obtained, chromatographic purification is a modern and effective method for obtaining highly purified anti-rabies immunoglobulins. According to the presented purification technology, immunoglobulins with the mass of 150 kDa were obtained, which are separated into 2 light chains (28 kDa) and 2 heavy chains (55 kDa) under reducing conditions. The development of domestic technology for purification of immunoglobulins will make it possible to obtain not only antirabicimmunobiologic preparations, but also preparations against other infectious diseases. The findings of the study emphasize the importance of proper selection and optimization of IgG purification methods to ensure high purity and product yield. These results have practical implications for the production of IgG-based drugs, which are widely used in medical practice, including for the prevention and treatment of rabies as well as other infections. Further research in this area may contribute to the improvement of production technologies and the quality of immunoglobulins.

Funding

The research was carried out within the framework of realization of the IRN project № AP19680178 of the competition for grant financing on scientific and (or) scientific-technical projects for 2023–2025 years (Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan).

References

1 Schnell, M.J., McGettigan, J.P., Wirblich, C., & Papaneri, A. (2010). The cell biology of rabies virus: using stealth to reach the brain. *Nature reviews microbiology*, *8(*1), 51–61.

2 Bourhy, H., Dautry-Varsat, A., Hotez, P. J., & Salomon, J. (2010). Rabies, still neglected after 125 years of vaccination. *PLoS neglected tropical diseases, 4*(11), e839.

3 Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., ... & Dushoff, J. Global Alliance for Rabies Control Partners for Rabies Prevention. 2015. Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis, 9*(4), e0003709.

4 Fooks, A.R., Banyard, A.C., Horton, D.L., Johnson, N., McElhinney, L.M., & Jackson, A.C. (2014). Current status of rabies and prospects for elimination. *The Lancet, 384*(9951), 1389–1399.

5 Knobel, D.L., Cleaveland, S., Coleman, P.G., Fèvre, E.M., Meltzer, M.I., Miranda, M.E.G., ... & Meslin, F.X. (2005). Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World health Organization, 83*, 360–368.

6 Suraweera, W., Morris, S.K., Kumar, R., Warrell, D.A., Warrell, M.J., Jha, P., & Million Death Study Collaborators. (2012). Deaths from symptomatically identifiable furious rabies in India: a nationally representative mortality survey. *PloS: Neglected Tropical Diseases,* e0001847. https://doi.org/10.1371/journal.pntd.0001847

7 Cantaert, T., Borand, L., Kergoat, L., Leng, C., Ung, S., In, S., ... & Tarantola, A. (2019). A 1-week intradermal dose-sparing regimen for rabies post-exposure prophylaxis (RESIST-2): an observational cohort study. *The Lancet Infectious Diseases, 19*(12), 1355–1362.

8 (2018). WHO. Rabies vaccines: WHO position paper. Retrieved from http://www.who.int/rabies/resources/who_wer9316/en/

9 Mondiale de la Santé, O., & World Health Organization. (2017). Human rabies: 2016 updates and call for data. *Weekly Epidemiologic Record= Relevé épidémiologique hebdomadaire, 92*(7), 77–86.

10 McGettigan, J.P., David, F., Figueiredo, M.D., Minke, J., Mebatsion, T. & Schnell, M.J. (2014). Safety and serological response to a matrix gene-deleted rabies virus-based vaccine vector in dogs. *Vaccine, 32*(15), 1716–1719.

11 Huang, F., Ahmad, W., Duan, M., Liu, Z., Guan, Z., Zhang, M., ... & Amjad, A.M. (2015). Efficiency of live attenuated and inactivated rabies viruses in prophylactic and post exposure vaccination against the street virus strain. *Acta Virol, 59*(2), 117–24.

12 Hemachudha, T., Ugolini, G., Wacharapluesadee, S., Sungkarat, W., Shuangshoti, S., & Laothamatas, J. (2013). Human rabies: neuropathogenesis, diagnosis, and management. *The Lancet Neurology, 12*(5), 498–513.

13 Rupprecht, C.E., Briggs, D., Brown, C.M., Franka, R., Katz, S.L., Kerr, H.D., et al. Centers for Disease Control and Prevention (CDC). (2010). Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. *MMWR Recomm Rep, 59*(RR-2), 1–9.

14 Si, H., Guo, Z.M., Hao, Y.T., Liu, Y.G., Zhang, D.M., Rao, S.Q., & Lu, J.H. (2008). Rabies trend in China (1990–2007) and post-exposure prophylaxis in the Guangdong province. *BMC Infectious Diseases, 8*, 1–10.

15 Wilde, H., Sirikawin, S., Sabcharoen, A., Kingnate, D., Tantawichien, T., Harischandra, P. A., ... & Sitprija, V. (1996). Failure of postexposure treatment of rabies in children. *Clinical infectious diseases, 22*(2), 228–232.

16 Fernandes, A., Kaundinya, J.O., Daftary, G., Saxena, L., Banerjee, S., & Pattnaik, P. (2008). Chromatographic purification of equine immunoglobulin G F(ab)2 from plasma. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 876*(1), 109–115. https://doi.org/10.1016/j.jchromb.2008.10.030

17 Glück, R., Wegmann, A., Keller, H., Hoskins, J.M., & Germanier, R. (1987). Human rabies immunoglobulin assayed by the rapid fluorescent focus inhibition test suppresses active rabies immunization. *Journal of biological standardization, 15*(2), 177–183.

18 Jayakumar, R., & Ramadass, P. (1991). Immunoglobulin response to rabies virus immunization in dogs. *Vaccine, 9*(9), 611-612. https://doi.org/10.1016/0264-410x(91)90183-7

19 Cabasso, V.J., Loofbourow, J.C., Roby, R.E., & Anuskiewicz, W. (1971). Rabies immune globulin of human origin: preparation and dosage determination in non-exposed volunteer subjects. *Bulletin of the World Health Organization, 45*(3), 303–315.

20 Hobart-Porter, N., Stein, M., Toh, N., Amega, N., Nguyen, H.B., & Linakis, J. (2021). Safety and efficacy of rabies immunoglobulin in pediatric patients with suspected exposure. *Human Vaccines & Immunotherapeutics, 17*(7), 2090–2096.

21 Sudarshan, M.K., Narayana, D.H.A., & Jayakrishnappa, M.B. (2019). Market mapping and landscape analysis of human rabies biologicals in India. *Indian Journal of Public Health, 63*(5), 37–43.

22 Sparrow, E., Torvaldsen, S., Newall, A.T., Wood, J.G., Sheikh, M., Kieny, M.P., & Abela-Ridder, B. (2019). Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis: A review of the current status of the clinical development pipeline. *Vaccine, 37*, A132–A139.

23 Vora, N.M. (2018). Potential confounding of diagnosis of rabies in patients with recent receipt of intravenous immune globulin. *Morbidity and Mortality Weekly Report*, 67.

24 Stec, J., Bicka, L.E.O.K.A.A.D.I.A. & Kuźmak, J.A.C.E.K. (2004). Isolation and purification of polyclonal IgG antibodies from bovine serum by high performance liquid chromatography. *Bulletin of the Veterinary Institute in Pulawy, 48*(3), 321–327.

А.Б. Нурмухамбетова, Ж.М. Батанова, Г.А. Баянды, Е.А. Крыкбаев,
Э.Ж. Битанова, Н.Н. Ахметсадыков, Д.С. Аманкелді

Рабикалық антигенмен гипериммундалған жылқылардың сарысуынан G (IgG) иммуноглобулинді хроматографиялық тазарту режимдері

G (IgG) иммуноглобулин ағзаның иммундық реакциясында шешуші рөл атқарады, оның антигендерге жоғары ерекшелігі және патогенді бейтараптандырудағы тиімділігі оны медициналық және ғылыми зерттеулерде құнды құралға айналдырады. Жылқы продуценттері IgG едәуір мөлшерінің көзі болып табылады, бірақ оларды клиникалық немесе ғылыми қолдануға жарамды деңгейге дейін тазарту үшін арнайы әдістер қажет. Рабикалық антигенмен гипериммундалған жылқылардың сарысуынан G (IgG) иммуноглобулин хроматографиялық тазарту режимдерін әзірлеу иммунобиологиялық препараттарды, вакциналарды және диагностикалық тест-жүйелерді өндіру және бақылау сапасымен қауіпсіздігінің халықаралық стандарттарына сәйкес технологияны стандарттауға мүмкіндік береді. CVS-11 штамынан рабикалық антиген мен гипериммундалған жылқылардың сарысуын хроматографиялық тазартудың кезеңдік режимдері мен процедураларын әзірлеу, тазарту технологиясының тиімділігіне, ақуыздың шығымдылығына және тазалық дәрежесіне бағалау жүргізілді. Зерттеуде гипериммунизацияланған жылқылардың сарысуларын алу мен дайындаудың серологиялық әдістері, сондай-ақ гельді сүзу, ион алмасу хроматографиясы және одан кейінгі электрофорездің биотехнологиялық әдістері қолданылды. Зерттеудің практикалық маңыздылығы иммуноглобулиндерге негізделген иммунобиологиялық препараттарды алу технологияларын дамытуға мүмкіндік береді және жоғары тазартылған иммуноглобулиндерді пайдалануды талап ететін медицина, биотехнология және ғылыми зерттеулер салаларында қолданылуы мүмкін. Ұсынылған тазарту технологиясына сәйкес салмағы 150 кДа иммуноглобулиндер алынды, олар қалпына келтіру жағдайында 2 жеңіл тізбекке (28 кДа) және 2 ауыр тізбекке (55 кДа) бөлінеді.

Кілт сөздер: иммуноглобулин, тазарту технологиясы, хроматография, құтыру, сарысу, рабикалық антиген, гипериммунизация, антиденелер.

А.Б. Нурмухамбетова, Ж.М. Батанова, Г.А. Баянды, Е.А. Крыкбаев,
Э.Ж. Битанова, Н.Н. Ахметсадыков, Д.С. Аманкелді

Режимы хроматографической очистки иммуноглобулина G (IgG) из сыворотки лошадей гипериммунизированных рабическим антигеном

Иммуноглобулин G (IgG) играет ключевую роль в иммунном ответе организма. Его высокая специфичность к антигенам и эффективность в нейтрализации патогенов делают его ценным инструментом в медицинских и научных исследованиях. Лошади продуценты являются источником значительного

количества IgG, но их очистка до уровня, пригодного для клинического или научного использования, требует специализированных методов. Отработка режимов хроматографической очистки иммуноглобулина G (IgG) из сыворотки лошадей, гипериммунизированных рабическим антигеном, позволит стандартизировать технологию в соответствии с международными стандартами качества и безопасности производства и контроля иммунобиологических препаратов, вакцин и диагностических тест-систем. Проведена отработка поэтапных режимов и процедур хроматографической очистки сыворотки лошадей, гипериммунизированных рабическим антигеном из штамма CVS-11, с оценкой эффективности технологии очистки, выхода белка и степень чистоты. В исследовании применялись серологические методы получения и подготовки сывороток гипериммунизированных лошадей, а также биотехнологические методы гель-фильтрации, ионообменной хроматографии и последующего электрофореза. Практическая значимость исследования может позволить развить технологии получения иммунобиологических препаратов на основе иммуноглобулинов и может быть применена в областях медицины, биотехнологии и научных исследований, требующих использования высокоочищенных иммуноглобулинов. Согласно представленной технологии очистки, получены иммуноглобулины массой 150 кДа, которые в восстанавливающих условиях разделяются на 2 легкие цепи (28 кДа) и на 2 тяжелые цепи (55кДа).

Ключевые слова: иммуноглобулин, технология очистки, хроматография, бешенство, сыворотка, рабический антиген, гипериммунизация, антитела.

Information about the authors

Nurmukhambetova Anara Baubekovna — PhD student, Researcher, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *anara_bn@bk.ru;*

Batanova Zhanat Mukhametkalievna — Candidate of Veterinary Sciences, Associate Professor, Head of Laboratory “Virology”, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *batanova_77@mail.ru;*

Bayandy Gulshat Askhatkyzy — PhD doctoral candidate, Researcher, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *bayandy.gulshat92@gmail.com;*

Krykbayev Yerkin Aliybekovich — PhD student, Senior Researcher, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *krykbaev_e@mail.ru;*

Bitanova Elmira Zhenyskhanovna — Candidate of Medical Sciences, Senior Researcher, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *elmira.bitanova@mail.ru;*

Akhmetsadykov Nurlan Nuroldinovich — Doctor of Veterinary Sciences, Professor, General Director, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *nurlan.akhmetsadykov@gmail.com;*

Amankeldi Diana Sabitkizy — Junior Researcher, Research and Production Enterprise “Antigen” LLP, Almaty, Kazakhstan; e-mail: *amankeldidiana@mail.ru.*