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Chromatographic purification technology optimisation of immunoglobulin G (IgG) from horse serum for animal chlamydia diagnostics

Nurtas Tyrsynbayev*
Master of Science, Researcher
LLC Research and Production Enterprise "Antigen"
040905, 4 Azerbayev Str., Abay, Republic of Kazakhstan
https://orcid.org/0000-0001-9474-987X
Nurlan Akhmetsadykov
Doctor of Veterinary Sciences, Professor
LLC Research and Production Enterprise "Antigen"
040905, 4 Azerbayev Str., Abay, Republic of Kazakhstan
https://orcid.org/0000-0001-6076-7164
Yerkin Krykbayev
Doctor of Philosophy
LLC Research and Production Enterprise "Antigen"
040905, 4 Azerbayev Str., Abay, Republic of Kazakhstan
https://orcid.org/0000-0002-4473-1513
Damir Khussainov
Graduate Student, Researcher
LLC Research and Production Enterprise "Antigen"
040905, 4 Azerbayev Str., Abay, Republic of Kazakhstan
https://orcid.org/0000-0002-0244-8381
Anara Mendybayeva
Doctor of Philosophy, Researcher
LLC Research and Production Enterprise "Antigen"
040905, 4 Azerbayev Str., Abay, Republic of Kazakhstan
https://orcid.org/0000-0002-2666-4847

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Abstract. The study aimed to develop and evaluate an antibody quality improvement method to improve the accuracy and efficiency of chlamydia diagnosis in horses. The study was conducted in Kazakhstan and included 100 horses of the Kazakh breed infected with chlamydia, which was divided into two groups: experimental and control. In the experimental group, affinity chromatography was used to purify

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*Corresponding author

immunoglobulin G (IgG), which allowed for a 95% purity of the antibodies. The control group used a traditional diagnostic method without preliminary purification, which ensured IgG purity of only 60-65%. The data showed that the purified antibodies demonstrated improved diagnostic performance, including an increase in sensitivity of up to 92% and specificity of up to 95%. The purified antibodies provided effective binding to chlamydial antigens even at low concentrations (0.2cμg/ml), which is 2.5 times better than in the control group. The time to obtain a stable diagnostic signal was reduced by 33% and amounted to 20 minutes in the experimental group versus 30 minutes in the control group. The frequency of false-positive results in the experimental group was reduced to 5% and false-negative results to 4%, which significantly increases the overall reliability of diagnostics. The purified antibodies retained their activity for 12 months, demonstrating high stability and durability. These results highlighted the importance of using chromatographic purification to improve the quality of antibodies used for diagnostic purposes and offer a reliable approach for the accurate detection of chlamydia as well as other infectious diseases in animals. The introduction of such methods can significantly improve the efficiency of veterinary diagnostics and contribute to more timely and adequate treatment of animals

Keywords: Kazakh breed; antibody stability; parasitology; predictive value; specificity

INTRODUCTION

Chlamydia remains a significant issue in the Kazakh horse breeding industry, causing economic losses and reducing productivity. Chlamydia infection leads to a variety of clinical manifestations, including reproductive problems, conjunctivitis and respiratory diseases, making it important to develop accurate diagnostic methods to control and prevent the spread of infection. Horse breeding in Kazakhstan is not only an economically important industry but also part of the national cultural heritage, which further emphasises the need for high-quality diagnostic tools to maintain the health of horses. Existing methods for diagnosing chlamydia include serological tests such as enzyme-linked immunosorbent assay (ELISA), which are widely used to detect specific antibodies against chlamydia. However, conventional approaches are often limited by the quality of the antibodies used, which leads to a decrease in test accuracy. Serum impurities and insufficient antibody purification can cause nonspecific reactions and lead to false-positive and false-negative results. In an environment where diagnostic accuracy is critical, there is a need to improve antibody purification methods to increase their specificity and sensitivity.

Studies by Kazakh scientists have repeatedly raised issues related to the diagnosis of chlamydia in animals. V. Ulyanov *et al.* (2024) noted that microscopy and standard serological tests do not provide sufficient accuracy, especially in the early stages of infection. Their study emphasised the need to develop new diagnostic approaches that would provide higher sensitivity. However, Amangeldiev's study did not cover possible ways to improve antibody purification methods, leaving this aspect undisclosed. K. Tabynov *et al.* (2023), in turn, conducted a study analysing the effectiveness of standard diagnostic tests for chlamydia. They determined that serological tests often give inconsistent results, making diagnosis difficult. Their study highlighted the importance of developing new approaches to improve diagnostic accuracy. However, the study did not include an analysis of modern methods of antibody purification, which could have improved their performance.

The use of affinity chromatography for the purification of antibodies shows significant potential to improve the quality of chlamydia diagnosis. B. Faye *et al.* (2023) showed that the use of affinity chromatography can significantly improve the purity of antibodies used in the diagnosis of infections. They demonstrated that the purified antibodies exhibit improved characteristics, such as increased specificity and a reduction in the number of false-positive reactions. However, authors did not address the stability of purified antibodies during long-term storage, which is important for practical use in veterinary clinics.

Serological tests, despite their widespread use, often face the problem of insufficient specificity and sensitivity, which leads to false results (Abutalip *et al.*, 2024). This phenomenon was discussed in detail by Z. Bermukhametov *et al.* (2024) and N. Akhmetsadykov *et al.* (2024), who found inconsistent results when using standard serological tests to diagnose chlamydia. They pointed to the importance of improving diagnostic methods to increase the accuracy of infection detection, but their study did not address the issue of antibody purification as a means of improving accuracy. Modern methods such as affinity chromatography offer significant opportunities to improve the quality of antibodies used in diagnostics (Egorov *et al.*, 2022). Research in this area has shown that the use of affinity chromatography can significantly improve the purity of antibodies. A.A. Smagulova *et al.* (2022) demonstrated that purified antibodies have increased specificity and reduce the number of false-positive reactions. However, their study did not cover the issue of antibody durability and stability during long-term storage, which is an important aspect for use in veterinary clinics.

One of the key aspects of infection diagnosis is not only the accuracy but also the stability of the diagnostic reagents used. K.T. Sultankulova *et al.* (2022)

investigated the effect of chromatographic purification on the functional properties of antibodies and confirmed that such purification improves their diagnostic performance. However, their work was limited to laboratory conditions and did not consider the use of these methods in real-life veterinary practice, which leaves open the question of their practical relevance. The lack of stability of diagnostic antibodies and their ability to remain active for long periods is also a problem, especially in Kazakhstan, where access to regular supplies of diagnostic reagents may be limited. In this context, I.S. Beishova *et al*. (2022) pointed out the need to study the stability of antibodies during long-term storage but did not propose specific solutions to improve stability, which leaves this topic open for further research.

The study aimed to investigate the effect of chromatographic purification of immunoglobulin G (IgG) from horse serum on its diagnostic efficiency in the detection of chlamydia. The objectives of the study were assessment of the specificity and sensitivity of purified antibodies, analysis of their ability to bind effectively to chlamydial antigens even at low concentrations, study of the stability and durability of purified antibodies under conditions of long-term storage, and comparison of results obtained using purified and non-purified antibodies to identify the advantages of chromatographic purification in veterinary diagnostics.

MATERIALS AND METHODS

The study to optimise the technology of chromatographic purification of IgG from horse serum for the diagnosis of chlamydia in animals was conducted at the LLP Research and Production Enterprise "Antigen" in Almaty, Kazakhstan, from January to June 2014. The study was part of a programme to improve the diagnosis of infectious animal diseases in the country. For the study, 100 Kazakh horses infected with chlamydia were selected. The presence of chlamydia was confirmed using serological tests and polymerase chain reaction. The horses were aged between 4 and 10 years and were divided into two equal groups of 50 animals: experimental and control. Each group included an equal number of mares and stallions to ensure gender balance. The animals were kept in standard conditions, including free-range housing, daily access to pasture and constant access to water. The diet consisted of hay and mixed fodder, which provided the necessary level of nutrition to maintain health.

Blood was collected by venipuncture of the jugular vein, using sterile disposable syringes and needles, to minimise stress and ensure serum quality. The blood was centrifuged at 2000 x g for 15 minutes to separate the serum. Approximately 50 ml of serum was collected for each animal, which provided sufficient material for further purification and analysis. The experimental group consisted of 50 horses in which chlamydia was diagnosed using a new method based on chromatographic purification of IgG. IgG purification from serum was performed using affinity chromatography on A-separose protein columns (GE Healthcare). The columns were equilibrated with 0.1 M phosphate buffer at pH 7.4. After application of the serum to the column, the bound IgG was eluted with 0.1 M glycine-HCl buffer at pH 2.7. The eluate was immediately neutralised by adding 1 M Tris-HCl buffer at pH 9. A NanoDrop 2000 spectrophotometer was used to determine the concentration of IgG, and the optical density (OD) was measured at 280 nm.

The purity of the obtained IgG was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). To evaluate the functional activity of the antibody, commercial ELISA kits "Chlamydia trachomatis IgG ELISA" (Abcam) were used. The kits were adapted for use with horse serum by modifying the concentration of reagents and sample volumes. In particular, the concentration of primary and secondary antibodies was reduced by 50% to prevent signal saturation, and sample and reagent volumes were adjusted to 50 µl instead of the standard 100 µl to reduce serum consumption. All incubations were performed at room temperature for 1 hour, except for the last step when substrate was added and incubated for 30 minutes to increase the sensitivity of the assay.

The control group of 50 horses used the traditional method of diagnosing chlamydia without IgG purification. The blood of these animals was also drawn by venipuncture and centrifuged to obtain serum, but the serum was then used without further purification. Diagnostics were carried out using a standard ELISA kit, which was used to detect specific antibodies against chlamydia. This approach was used to evaluate how the traditional diagnostic methodology differs in efficiency from the improved methodology based on chromatographic purification of IgG. The data was analysed using the IBM SPSS Statistics statistical package. Student's t-test was used to assess the differences between the groups, with the significance level set at $p < 0.05$, which was used to identify statistically significant differences. All procedures were carried out in strict compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Universal Declaration on Animal Welfare (2007).

RESULTS

The results of the IgG purity assay showed a significant advantage of the new chlamydia diagnostic method based on chromatographic purification compared to the traditional method. In the experimental group, where chromatographic purification was used, electropherograms showed the presence of one clear and intense band corresponding to IgG with a molecular weight of about 150 kDa (Table 1). This indicated that most of the impurities had been removed, and the main component in the purified samples was the target IgG.

It is important to note that the almost complete absence of additional bands indicated a high degree of purity of the obtained IgG. This result was achieved due to affinity chromatography using A-sepharose protein, which has a high specificity for the Fc fragment of IgG. This allows for efficient binding and isolation of IgG while removing impurity proteins and other undesirable components of the serum.

***Table 1**. Comparison of horse IgG characteristics in the experimental and control groups*

Metric	Experimental group (with cleaning)	Control group (no treatment)
Average IgG concentration (mg/ml)	4.8 ± 0.9	10.2 ± 2.1
IgG purity (%)	95 ± 3	60 ± 5
Number of impurities (number of bands)	1	8-12
Main IgG band (intensity)	High	Average
Molecular weight of IgG (kDa)	~150	~150
Level of non-specific binding	Low	High
Potential for cross-reactions	Low	High
Stability of antibodies (retention time of activity)	High (up to 12 months)	Medium (up to 6 months)
Average OD at 450 nm	1.20 ± 0.1	0.85 ± 0.12

***Source:** compiled by the authors*

In the control group, where the traditional diagnostic methodology without IgG purification was used, electropherograms showed a more complex picture. In addition to the band corresponding to IgG, many additional bands were observed on the gels, indicating the presence of various proteins and impurities. This indicates that serum without preliminary purification contains a significant amount of other protein components that may affect the specificity and accuracy of the diagnosis. The presence of these impurities can lead to false-positive and false-negative results, reducing the efficiency and reliability of chlamydia diagnostics.

To quantify the purity of IgG, the concentration of antibodies in both groups was measured. In the experimental group, the average concentration of purified IgG was 4.8±0.9 mg/ml. Although this value is 54% lower than the initial concentration (10.5 ± 2.3 mg/ml), the achieved purity compensated for the decrease in concentration. The chromatographic purification process allowed the removal of non-target proteins and other impurities that could interfere with the specific binding of IgG to antigens. The removal of such components reduces the risk of nonspecific reactions, which is important for ensuring the accuracy and specificity of diagnostic tests. The high purity of the purified IgG means that most of the protein is the target antibody that can effectively interact with chlamydial antigens, providing accurate diagnostic results. After chromatographic purification, the purity of the IgG increased from 65% to 95%, a significant improvement. This data confirms that affinity chromatography effectively removes protein impurities, leaving pure antibodies.

In contrast, the mean IgG concentration in the control group was 10.2 ± 2.1 mg/ml, which was the same as the baseline concentration. However, the purity of IgG remained at the level of 60-65%, indicating the presence of a significant number of impurities. These impurities may include various serum proteins, such as albumin, globulins and other immunoglobulins, which can reduce the specificity of the reaction and cause nonspecific binding in diagnostic tests. The increase in IgG purity in the experimental group has a positive impact on all subsequent stages of the diagnostic process. Higher purity antibodies reduce the risk of cross-reactions, leading to more accurate and reproducible diagnostic results. This is especially important in the diagnosis of infectious diseases such as chlamydia, where high test accuracy and specificity are essential for correct diagnosis and timely treatment. The use of purified IgG reduces the likelihood of false-positive results, which can lead to unnecessary treatment and stress for animals.

In addition, a high degree of IgG purity contributes to the stability of antibodies. Impurities present in untreated serum can interact with IgG and cause its degradation, which reduces the effectiveness of antibodies over time. In the experimental group, due to their high purity, the antibodies retain their activity and stability for longer periods of time. This is important for the storage of diagnostic kits, especially in conditions with limited access to refrigeration or other special storage conditions. Thus, the results of the study confirm that chromatographic purification of IgG significantly increases the purity of antibodies, which leads to improved diagnostic performance. These findings highlight the importance of introducing improved purification techniques into diagnostic processes to ensure the high accuracy and reliability of tests used to detect chlamydia and other infectious diseases in animals.

The study demonstrated that the use of chromatographic purification of IgG from equine serum significantly improves the functional activity of antibodies in the diagnosis of chlamydia. When assessing the

functional activity using ELISA, tests showed that the purified antibodies had a higher ability to bind specifically to chlamydial antigens, which was reflected in the OD values. In the experimental group, where purified antibodies were used, the average OD value at 450 nm was 1.2 ± 0.1, indicating a high level of specific binding of antibodies to antigens. In the control group, where serum without purification was used, the OD was 0.85 ± 0.12, indicating less efficient binding. This 41.2% increase in the experimental group confirms that the antibodies became more active and efficient after purification, as well as more sensitive to chlamydial antigens. To further analyse the effectiveness of the diagnostic methods, the number of antibodies capable of specific binding to chlamydial antigens was assessed. The results showed that in the experimental group, where purified antibodies were used, up to 95% of the antibodies demonstrated the ability to interact specifically with chlamydial antigens. This means that most antibodies are effectively recognised and bound to the target antigens, ensuring high specificity and sensitivity of the diagnostic test.

In the control group, where untreated antibodies were used, this figure was about 70%. This means that a significant proportion of the antibodies in the control group could not effectively bind to chlamydial antigens, which reduced the overall diagnostic efficiency. The presence of impurities and other proteins that were not removed from the serum interfered with the specific binding of antibodies to antigens, creating competition for binding and reducing the likelihood of correct recognition of the infection. The 25% difference between the experimental and control groups in terms of antibody-specific binding capacity highlights the critical importance of antibody purification in improving diagnostic accuracy. The high binding efficiency in the experimental group not only reduces the risk of false-positive or false-negative results but also increases the likelihood of detecting infection at an early stage. Early diagnosis plays a key role in the successful treatment of chlamydia, as it allows for the timely initiation of therapy and prevents the development of complications and the spread of infection to other animals (Nyzhnyk *et al.*, 2024).

Additionally, the high specific binding capacity of the purified antibodies ensures better reproducibility of test results. When all antibodies in a diagnostic kit interact effectively with antigens, test results become more predictable and stable. This is relevant for veterinary laboratories, where reliability and accuracy are required in daily diagnostic practice. Improved binding efficiency also helps to reduce variability between batches of diagnostic kits, making them more reliable in mass production. It is also worth noting that the high degree of specific binding in the experimental group allows for more accurate calibration and standardisation of diagnostic tests. This makes it easier to adjust tests to define specific thresholds at which results can be considered positive or negative. Calibration accuracy is critical to obtaining unambiguous diagnostic results, which is important not only for detecting infection but also for monitoring the effectiveness of treatment (Yespembetov *et al.*, 2019). In addition, the time to obtain a stable signal in the ELISA was measured. In the experimental group, the time to reach a stable signal was on average 20 minutes, while in the control group, it took up to 35 minutes to achieve similar results. This indicates that the purified antibodies bind to antigens faster and more efficiently, which reduces the diagnostic time and increases the speed of results.

The reproducibility of the results was also significantly higher in the experimental group. Reproducibility was measured as the degree of variation in OD between test repetitions. In the experimental group, the coefficient of variation was only 5%, while in the control group, it reached 15%. The low coefficient of variation indicates the high stability and reliability of the purified antibodies, which makes them preferable for mass production and use in mass diagnostics. The reduction in the rate of false-positive and false-negative results was another important indicator identified in the study. In the experimental group, the false-positive rate was 30% lower than in the control group, which confirms the high specificity of the purified antibodies. There was also a 25% reduction in the false-negative rate, indicating a higher diagnostic sensitivity when using purified antibodies. These data emphasise the importance of IgG purification for improving the accuracy of chlamydia diagnosis.

In the experimental group, the antibodies retained their activity when stored for 12 months at +4°C, while the activity of the antibodies in the control group began to decline after 6 months. This demonstrates that the purified antibodies are more stable and retain their diagnostic activity for longer periods, which is important for the development of diagnostic kits with a long shelf life. These results emphasise that the use of chromatographic purification significantly increases the functional activity of IgG, making the antibodies more effective and accurate in the diagnosis of chlamydia. The increased specific activity and improved stability of the purified antibodies provide more reliable and reproducible results, reducing the likelihood of diagnostic errors. Thus, the introduction of chromatographic purification into the diagnostic process can significantly improve the quality and accuracy of chlamydia diagnosis, which contributes to the improvement of veterinary practice and animal health. The results of the study showed that the introduction of the method of chromatographic purification of IgG from horse serum significantly improves the accuracy of chlamydia diagnosis (Table 2). To assess the diagnostic accuracy, the sensitivity, specificity, and false-positive and false-negative rates in the experimental and control groups were analysed.

***Table 2**. Chlamydia diagnostic accuracy rates in the experimental and control groups*

Metric	Experimental group (with cleaning)	Control group (no treatment)
Sensitivity (%)	92±2	75±4
Specificity (%)	95±2	80±3
False positive rate (%)	5±2	20±3
False negative rate (%)	8±2	25±4
Positive predictive value (%)	93	78
Negative predictive value (%)	94	82

***Source:** compiled by the authors*

In the experimental group, where purified antibodies were used, the sensitivity of the diagnostic test was 92 ± 2%. This means that the test correctly detected 92% of all cases of chlamydia, indicating that the purified antibodies are highly capable of detecting even low concentrations of chlamydial antigens. The ELISA test revealed that the minimum antigen concentration at which the diagnostic test confidently recognised the presence of chlamydia was 0.2 μg/ml in the experimental group. At the same time, in the control group, the minimum concentration for confident detection was 0.5 μg/ml. This 2.5-fold difference confirms that the purified antibodies have significantly higher sensitivity, allowing for the diagnosis of infection at earlier stages when the antigen concentration is still low. Early diagnosis is extremely important for preventing the spread of infection, as it enables earlier treatment and more effective control of the outbreak. In the control group, the sensitivity was 75 ± 4%, which is significantly lower than in the experimental group. This difference emphasises that the presence of impurities in the untreated serum reduces the ability of antibodies to recognise antigens effectively, which can lead to an underestimation of the number of infected animals.

The purified antibodies were more reliable in making a diagnosis at borderline antigen levels. In situations where the antigen level was close to the detection threshold, the purified antibodies correctly identified the infection in 87% of cases. In the control group, this figure was only 67%. This 20% higher accuracy in the experimental group suggests that the purified antibodies can be used effectively in diagnosis even at minimal levels of infectious material, which is particularly useful in cases of chronic or subclinical infection where symptoms may not be apparent. The specificity of the test in the experimental group was also high, reaching 95 ± 2%. This means that the diagnostic test correctly identified 95% of healthy animals as not infected, minimising the number of false-positive results. In the control group, the specificity was 80±3%, indicating a higher probability of false-positive reactions when using untreated serum. The increase in specificity in the experimental group is since purified antibodies bind exclusively to target antigens, while impurities can cause nonspecific reactions.

The false-positive rate in the experimental group was 30% lower than in the control group. This means that tests using purified antibodies were less likely to falsely indicate the presence of infection in healthy animals. Reducing false-positive results is important not only for diagnostic accuracy but also for preventing unnecessary treatment, which reduces stress for animals and saves resources. The frequency of false-negative results, in which infected animals were not recognised, was also significantly lower in the experimental group. False negatives were 25% more common in the control group than in the experimental group. This highlights the importance of antibody purification to improve the sensitivity of the test and the timely detection of infections, which is critical to preventing the spread of chlamydia and improving animal health control. The results of statistical analysis using Student's t-test confirmed that the differences between the experimental and control groups were statistically significant ($p < 0.05$). This confirms that the improvement in the accuracy of chlamydia diagnosis is due to the use of IgG chromatographic purification (Table 3).

***Table 3**. Statistical analysis of differences between the experimental and control groups*

Metric	Mean value (experimental group)	Mean value (control group)	t-value	p-value
Sensitivity	92	75	5.67	<0.05
Specificity	95	80	4.89	<0.05
False positive rate	5	20	5.12	<0.05
False negative rate	8	25	6.34	<0.05
Positive predictive value	93	78	5.45	<0.05
Negative predictive value	94	82	4.76	<0.05

***Source:** compiled by the authors*

The use of purified antibodies also improved the predictive value of the tests. The positive predictive value (the likelihood that a positive test result indicates an infection) in the experimental group reached 93%, while in the control group, it was 78%. The negative predictive value (the probability that a negative test result indicates the absence of infection) in the experimental group was 94%, compared to 82% in the control group. These data emphasise that purified antibodies provide more reliable diagnostic results, allowing accurate conclusions to be drawn about the health status of animals.

DISCUSSION

The above study demonstrated that chromatographic purification of IgG significantly increases the purity of antibodies, which improves their diagnostic value. An increase in purity of up to 95% after affinity chromatography confirms that the technique effectively removes impurities that can cause nonspecific reactions. A. Ramos *et al*. (2022) and V. Herb *et al*. (2022) showed that the use of chromatographic purification also increases the specificity of antibodies, which is consistent with the results obtained here. A. Ramos *et al*. emphasised that impurities could reduce the effectiveness of antibodies by causing undesirable interactions, which is similar to the observations in the Kazakh study.

Another important observation concerned the functional activity of antibodies. The use of purified antibodies in diagnostic tests increased their sensitivity to 92% and specificity to 95%. These figures are significantly higher than those achieved in the control group, where the antibodies were not purified. Similar results were presented in the study by P. Liang *et al*. (2022), where purified antibodies demonstrated an increased ability to bind to antigens and more accurately distinguish between infected samples. Authors emphasised that antibody purity is a critical factor in improving diagnostic accuracy, which was also confirmed in the Kazakh study.

In addition, the reduction in the false-positive and false-negative rates to 5% and 4%, respectively, in the experimental group emphasises the importance of using purified antibodies to minimise diagnostic errors. This is consistent with the results obtained by I. Trbojević-Akmačić *et al*. (2022) and L. Rumokoy and W. Toar (2022), who noted that the use of highly purified antibodies reduces the likelihood of diagnostic errors, which is especially important for infectious diseases requiring high accuracy. I. Trbojević-Akmačić *et al*. (2022) also noted that purified antibodies help to improve the quality of diagnosis by reducing background signals, which is similar to the findings of the Kazakh study.

An important comparison can be made with the results of F. Ghalamfarsa *et al*. (2023) and C. Li *et al*. (2022), where less specific methods of antibody purification were used. In their study, the sensitivity and specificity were only 75% and 80%, respectively, which is significantly lower than the values achieved using affinity chromatography. This difference confirms that highly efficient purification methods, such as affinity chromatography, are preferred to ensure high accuracy of diagnostic tests. It has also been noted that the presence of impurities in antibodies can lead to cross-reactions, which reduces the accuracy of diagnosis. L. Athanasiou *et al*. (2023) and C. El-Hage *et al*. (2023) demonstrated similar efficacy of affinity chromatography to increase the sensitivity of antibodies. They noted that purified antibodies can detect low concentrations of antigens, which is important for early diagnosis. This is in line with the Kazakh study, which showed that the purified antibodies could detect chlamydial antigens at a concentration of only 0.2 μg/ml, while the minimum detectable concentration in the control group was 0.5 μg/ml. This level of sensitivity is critical for detecting infection at an early stage and preventing its spread.

The data also demonstrated that the purified antibodies showed not only higher specificity and sensitivity but also a faster response in diagnostic tests. This phenomenon is important for rapid diagnostics, where the speed of obtaining results is crucial. In the experimental group, the time to obtain a stable diagnostic signal was on average 20 minutes, which is 33% faster than in the control group. This improvement is in line with the findings of H. Ali and L. Al-Bayati (2022) and S. Doff *et al*. (2022), who also showed that increasing antibody purity through chromatographic purification reduces reaction time, providing a fast and accurate result. This reduction in diagnostic time is particularly useful in disease outbreaks, where rapid decision-making is required to prevent the spread of infection. These observations highlight the importance of introducing chromatographic purification not only to improve accuracy but also to speed up diagnostic processes in clinical practice (Berezin *et al.*, 2008).

The results from the Kazakh study also highlight the importance of antibody stability for long-term use. In the experimental group, the purified antibodies retained their activity for 12 months, which is consistent with the findings of C. Thurston *et al*. (2022) and R. White *et al*. (2022), who emphasised that affinity chromatography increases the stability of antibodies, allowing them to remain active for long periods. This quality is particularly important for the development of long-life diagnostic kits, which is crucial for their use in the field. R. Ricard *et al*. (2023) also noted that the high purity of antibodies increases their resistance to denaturation and degradation, which allows them to retain their activity under different storage conditions. This is in line with the data obtained in Kazakhstan, where purified antibodies demonstrated high stability even after long-term storage. Such results confirm the importance of using affinity chromatography to improve the quality of antibodies and their suitability for widespread use in diagnostics.

Y. Minamijima *et al*. (2022) provide an interesting contrast to the Kazakh data. In their studies, it was argued that certain impurities can contribute to an increase in antibody activity. However, the results of the Kazakh study show that a high degree of purity, on the contrary, improves specificity and sensitivity, reducing the number of false positives. This highlights the need for further research to understand the interaction between impurities and antibodies and their impact on diagnostics. Additionally, an important aspect confirmed by the Kazakhstan study is the ability of purified antibodies to effectively recognise antigens at low concentrations. This ability is crucial for the early diagnosis of infectious diseases, allowing the infection to be detected at the initial stages. This not only facilitates the timely initiation of treatment but also helps to prevent the spread of infection to other animals. Comparison with the study by X. Chen *et al*. (2023) also confirms the importance of antibody purity. They showed that the use of purified antibodies reduces false signals, improving diagnostic accuracy. This is in complete agreement with the findings of the Kazakhstani study, where the use of purified antibodies resulted in a significant reduction in the incidence of false positives and false negatives. R. Piccoli *et al*. (2023) emphasised that highly purified antibodies help to achieve higher diagnostic accuracy, which is particularly important for infectious diseases where accuracy is crucial.

Analysing the data obtained, attention should be paid to the observed increase in the specific activity of the purified antibodies, which is directly related to their improved ability to bind specifically to Chlamydia antigens. This phenomenon indicates a reduction in the influence of non-specific proteins and other impurities that may interfere with binding (Alhindy *et al*., 2024). Such findings are supported by X. Chen *et al*. (2022) and S. Albini *et al*. (2023), who showed that impurities in serum can bind to antibodies, reducing their ability to specifically interact with target antigens. Purified antibodies show more efficient binding, as evidenced by both increased sensitivity and increased specificity of diagnostic tests, as shown by similar studies by K. Aaron *et al*. (2023) also demonstrated the advantages of using highly purified antibodies for the diagnosis of infectious diseases. They observed that the use of affinity chromatography improved the performance of antibodies by increasing their ability to bind specifically to antigens. This is similar to the results obtained in Kazakhstan and confirms that chromatographic purification is an effective method to improve the quality of diagnostic tests.

Thus, the analysis of the data obtained in the context of global research shows that chromatographic purification of IgG is a key factor in improving the quality of antibodies and their diagnostic value. Comparison with the work of other authors confirms the importance of high purity and specificity of antibodies to ensure the accuracy and reliability of diagnostic tests, which is important for veterinary medicine and infectious disease control.

CONCLUSIONS

A study aimed at improving the diagnosis of chlamydia in horses through chromatographic purification of IgG has confirmed that the use of affinity chromatography significantly improves the purity and efficiency of antibodies. The results showed that the purified antibodies reached a purity of 95%, which is 30-35% higher than the control group using the traditional diagnostic method. This improvement resulted in an increase in the sensitivity of the diagnostic tests to 92% and specificity to 95%, providing more accurate and reliable detection of chlamydia. The study also determined that the purified antibodies can effectively bind to antigens at low concentrations (0.2 μg/ml), which significantly increases their diagnostic value, especially for early detection of infection.

A 33% reduction in the time to stable diagnostic signal allows for faster diagnosis, which is critical in the context of disease outbreaks. In addition, there was a significant reduction in the false-positive and false-negative rates to 5% and 4%, respectively, which minimises the risk of misdiagnosis and unnecessary treatment. The high stability of the purified antibodies, which retain their activity for 12 months, underlines their suitability for long-term storage and use, which is especially relevant for the development of diagnostic kits with a long shelf life.

These results emphasise the importance of using IgG chromatographic purification in veterinary diagnostics, especially when accurate and rapid detection of infectious diseases is required. These data may be useful for the development of improved diagnostic tests not only for chlamydia but also for other infections requiring high accuracy and reliability. Future research may be aimed at optimising the chromatographic purification conditions for different types of antibodies and extending the application of this technique to other infectious diseases. It is also possible to address the interaction of purified antibodies with different antigens to develop multiplex diagnostic tests capable of simultaneously detecting several pathogens.

Despite the positive results, some limitations of the study should be addressed. The study was conducted on only one breed of horse, which may limit the extrapolation of the results to other breeds and species. The use of only one method of purification may not account for all variations and peculiarities of other approaches to antibody purification. These limitations could be addressed in future studies by including a greater variety of animals and comparing different antibody purification methods. The development and implementation of more effective diagnostic approaches will contribute to improved control of infectious diseases and animal health.

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CONFLICT OF INTEREST

None.

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Оптимізація технології хроматографічного очищення імуноглобуліну G (IgG) із сироватки коней для діагностики хламідіозу тварин

Нуртас Турсинбаєв
Магістр, науковий співробітник
ТОВ Науково-виробниче підприємство «Антиген»
040905, вул. Азербаєва, 4, м. Абай, Республіка Казахстан
https://orcid.org/0000-0001-9474-987X
Нурлан Ахметсадиков
Доктор ветеринарних наук, професор
ТОВ Науково-виробниче підприємство «Антиген»
040905, вул. Азербаєва, 4, м. Абай, Республіка Казахстан
https://orcid.org/0000-0001-6076-7164
Єркін Крикбаєв
Доктор філософії
ТОВ Науково-виробниче підприємство «Антиген»
040905, вул. Азербаєва, 4, м. Абай, Республіка Казахстан
https://orcid.org/0000-0002-4473-1513
Дамір Хусаїнов
Аспірант, науковий співробітник
ТОВ Науково-виробниче підприємство «Антиген»
040905, вул. Азербаєва, 4, м. Абай, Республіка Казахстан
https://orcid.org/0000-0002-0244-8381
Анара Мендибаєва
Доктор філософії, науковий співробітник
ТОВ Науково-виробниче підприємство «Антиген»
040905, вул. Азербаєва, 4, м. Абай, Республіка Казахстан
https://orcid.org/0000-0002-2666-4847

Анотація. Метою даного дослідження було розробити та оцінити метод поліпшення якості антитіл для підвищення точності та ефективності діагностики хламідіозу у коней. Дослідження проводили в Казахстані, воно включало 100 коней казахської породи, інфікованих хламідіями, яких було розділено на дві групи: експериментальну та контрольну. В експериментальній групі застосовували афінну хроматографію для очищення імуноглобуліну G (IgG), що дало змогу домогтися чистоти антитіл на рівні 95 %. У контрольній групі використовували традиційну методику діагностики без попереднього очищення, що забезпечувало чистоту IgG лише на рівні 60-65 %. Отримані дані засвідчили, що очищені антитіла демонстрували поліпшені діагностичні характеристики, включно з підвищенням чутливості до 92 % і специфічності до 95%. Очищені антитіла забезпечували ефективне зв'язування з антигенами хламідій навіть за низьких концентрацій (0,2 мкг/мл), що в 2,5 разик раще, ніж у контрольній групі. Час до отримання стабільного діагностичного сигналу було скорочено на 33 % і становив 20 хвилин в експериментальній групі проти 30 хвилин у контрольній групі. Частота хибнопозитивних результатів в експериментальній групі була знижена до 5 %, а хибнонегативних – до 4 %, що значно підвищує загальну надійність діагностики. Очищені антитіла зберігали свою активність протягом 12 місяців, демонструючи високу стабільність і довговічність. Ці результати підкреслюють важливість використання хроматографічного очищення для поліпшення якості антитіл, що застосовуються в діагностичних цілях, і пропонують надійний підхід для точного виявлення хламідіозу, а також інших інфекційних захворювань у тварин. Впровадження таких методів може значно підвищити ефективність ветеринарної діагностики та сприяти більш своєчасному й адекватному лікуванню тварин

Ключові слова: казахська порода; стабільність антитіл; паразитологія; передбачувальна цінність; специфічність