



## DEVELOPMENT OF A TECHNOLOGY FOR THE ISOLATION OF LYOPHILIZED RABBIT PLASMA CITRATE

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### Abstract

This study presents a laboratory-scale methodology for the prevention of blood coagulation in rabbit blood using sodium citrate and for the isolation of blood plasma. The sequential stages of plasma separation are described in detail. In addition, particular emphasis is placed on the instruments and reagents employed during the experimental procedures, as well as strict adherence to biosafety regulations throughout the research process.

**Keywords:** rabbit blood, citrated blood, sodium citrate, blood plasma, anticoagulant, blood coagulation, centrifugation, laboratory technology, biological material, biosafety, immunological studies, biochemical analyses.

At present, the incidence of various viral diseases is increasing. Their rapid detection and accurate diagnosis remain among the most urgent and relevant challenges. In accordance with the recommendations of the World Health Organization, the number of clinical diagnostic laboratory centres equipped to European standards is increasing in our country. These centres represent laboratories operating with advanced medical technologies.

One of these analytical approaches includes the polymerase chain reaction (PCR) and immunochromatographic assay (ICA) methods, which are effective for the diagnosis of various infectious diseases, including viral hepatitis, bacterial and infectious diseases, helicobacteriosis and other conditions. These methods are widely applied in gastroenterology, urology, gynecology, neurology, oncology, hematology, neonatology, forensic medicine, clinical genetics and other fields of medicine.

The performance of these analyses requires specialized analytical instruments, as well as standardized reagent kits and auxiliary reagents.

This technology is regarded as an effective method for obtaining high-quality blood plasma in scientific research and laboratory practice. The present article provides a detailed description of a laboratory-scale technology for controlling blood coagulation and isolating blood plasma from rabbit blood through the application of sodium citrate. The scientific and diagnostic significance of enzymes, antibodies, hormones and other biologically active substances present in blood plasma is substantiated.

### Objective of the study.

The study sequentially outlines the stages of rabbit blood collection, citration, centrifugation and plasma separation. The mechanism of action of sodium citrate as an anticoagulant by binding calcium ions and thereby inhibiting blood coagulation is explained. In addition, particular attention is given to the laboratory equipment and reagents used, the maintenance of sterile conditions and strict compliance with biosafety and bioethical requirements.

This technology enables the acquisition of high-quality and purified blood plasma in laboratory practice and is of significant value due to its effective application in immunological, biochemical and clinical research.

Due to their biological and physiological characteristics, animals are widely used in scientific research, particularly in the field of pharmaceutical biotechnology. Animals such as rabbits provide essential biological material for many studies, as their physiological and biochemical processes reflect numerous characteristics of the human body. Among such investigations, the isolation and storage of blood components, especially the separation and utilization of plasma are of considerable importance in medicine and pharmaceutical sciences.

The technology for the isolation of citrated dry rabbit plasma is widely applied in scientific research and medical practice. Plasma, as the liquid component of blood, is rich in numerous biologically active substances, including proteins, immunoglobulins, electrolytes, vitamins and other essential components. The process of plasma separation is essential for many medical applications, such as blood donation procedures, vaccine development, therapeutic monitoring, and genetic research.

The use of citrate (salts of citric acid) enhances the efficiency of the plasma separation process by preventing blood coagulation. This, in turn, facilitates the isolation of coagulation factors and other components present in plasma. The production of dry plasma through lyophilization technology enables long-term storage while preserving its biological activity. Such plasma is utilized not only in scientific research but also in medical practice, for example, in the preparation of thrombocytes or plasma-derived proteins.

**Objective of the study.**

The aim of this study is to develop a technology for the isolation of citrated dry rabbit plasma and to investigate its applications in the fields of medicine and biotechnology. Particular attention is devoted to improving the efficiency of plasma separation, optimizing citrate use and refining the lyophilization process. The results of the study are expected to enhance the plasma preparation process and improve product quality, thereby facilitating its application in pharmaceutical and clinical practice. Furthermore, the study seeks to substantiate the scientific and practical significance of the technology for isolating citrated dry rabbit plasma.

**Materials and Methods.**

The study was conducted under laboratory conditions in strict compliance with established biosafety and bioethical standards. Venous blood obtained from clinically healthy experimental rabbits maintained under standard husbandry conditions was selected as the study material. Prior to the initiation of the experiments, all animals underwent veterinary examination. The principal characteristics of sodium citrate as an anticoagulant were evaluated and are presented in Table 1.

Blood samples were collected through the rabbit ear vein in strict accordance with aseptic and antiseptic procedures.

**Table 1.**

**Main characteristics of sodium citrate as an anticoagulant**

Parameter	Description
Mechanism of action	Binds Ca <sup>2+</sup> ions



Biological safety	High
Field of application	Plasma preparation, diagnostics
Advantages	Preserves proteins

A 3.8% sodium citrate solution was used as the anticoagulant. The citrate solution was pre-dispensed into sterile tubes and mixed with blood at a 9:1 (blood:citrate) ratio. To prevent blood coagulation, the tubes were gently inverted for mixing, and mechanical shaking was strictly avoided.

After the initial processing, the citrated blood samples were kept at room temperature for 10–15 minutes. In the subsequent step, centrifugation was applied to separate the blood plasma from the samples. Centrifugation was performed at 3000 rpm for 10–15 minutes using a laboratory centrifuge. As a result of this process, the formed cellular elements of the blood sedimented at the bottom of the tube, while a clear plasma layer was obtained in the supernatant.

The separated blood plasma was carefully collected under sterile conditions using single-use sterile pipettes. The obtained plasma samples were visually inspected to confirm the absence of mechanical impurities or signs of hemolysis. Prior to subsequent laboratory analyses, the plasma was stored under the specified temperature conditions.

Throughout the study, personal protective equipment was used when handling biological materials, and all instruments and equipment were disinfected and disposed of in accordance with established biosafety and waste management regulations. Humane treatment of experimental animals was ensured, and minimally invasive procedures were employed wherever possible.

**Results and Discussion.**

The conducted studies evaluated the effectiveness of the technology for isolating blood plasma from citrated rabbit blood. The application of a 3.8% sodium citrate solution to blood samples was observed to completely prevent the blood coagulation process. Mixing blood and citrate at a 9:1 ratio ensured stable and consistent results across all samples. Following centrifugation, a clear separation of the formed cellular elements was observed at the bottom of the tubes, while a transparent and clear plasma layer was formed in the upper phase.

Visual assessment of the obtained plasma samples revealed no signs of hemolysis, indicating that the selected technology is appropriate and optimal. The applied centrifugation parameters (3000 rpm for 10-15 minutes) ensured effective separation of blood components. The results demonstrated that the anticoagulant effect of sodium citrate is based on its ability to bind calcium ions, thereby blocking the blood coagulation mechanism and preserving the structural integrity of the plasma. As a result, no degradation or inactivation of biologically active substances was observed.

The isolated plasma was found to be suitable for subsequent immunological and biochemical analyses under laboratory conditions. The clarity and uniform consistency of the plasma confirmed its high quality. Comparison of the obtained results with data reported in the literature further confirmed that citrate-based plasma separation is a reliable and reproducible method.

Overall, the results of the study demonstrated that the technology for isolating blood plasma from citrated rabbit blood is an effective, safe and economically feasible method for

laboratory practice, thereby substantiating its broad applicability in scientific research and diagnostic activities.

**Conclusion.**

The results of the conducted study demonstrated that the technology for isolating blood plasma from citrated rabbit blood is an effective and reliable method under laboratory conditions. The use of sodium citrate as an anticoagulant enabled complete control of the blood coagulation process and ensured high-quality separation of blood components. Application of a 9:1 blood-to-citrate ratio and optimal centrifugation speed and duration contributed to maintaining high plasma clarity and biological integrity. The isolated plasma was characterized by the absence of hemolysis, uniform consistency, and full suitability for subsequent immunological and biochemical analyses. Overall, the findings confirm the reproducibility and practical applicability of the citrated blood processing technology.

The technology for isolating blood plasma from citrated rabbit blood is recommended for obtaining high-quality biological material in scientific research and diagnostic laboratories, and its implementation in routine laboratory practice is considered appropriate and justified

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