

## Review

# Evaluation of Centrifugation Technique and the Effect of Centrifugation Condition on the Laboratory Samples

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### Abstract

Centrifugation is a critical laboratory technique used to separate components within a mixture based on density differences. The efficiency and reliability of this process depend on the precise control of variables such as speed, time, temperature, and rotor design. Improper centrifugation settings can compromise sample integrity, leading to analytical inaccuracies. High-speed centrifugation, while effective for rapid separation, often imposes mechanical stress on samples, resulting in hemolysis or cellular damage. Time duration is equally significant, as insufficient centrifugation can cause incomplete separation, while prolonged exposure may induce thermal stress and biochemical degradation. Temperature control plays a pivotal role, particularly for temperature-sensitive biomolecules like enzymes, nucleic acids, and proteins. Unregulated temperatures during high-speed centrifugation can denature proteins and degrade nucleic acids, affecting their utility in downstream applications such as diagnostics and molecular biology. Automated centrifuges equipped with programmable cooling systems have proven effective in mitigating these risks. Centrifugation protocols require customization based on sample type. Blood, urine, and cellular samples exhibit distinct sensitivities to centrifugal force and duration, necessitating tailored approaches. Specialized techniques, such as density-gradient centrifugation, enhance separation precision for applications like exosome isolation. Recent innovations in centrifuge technology, including programmable speed ramps and advanced rotor designs, have significantly improved the reproducibility and reliability of centrifugation processes. Standardization of centrifugation protocols is essential to ensure consistency in laboratory results. Advances in centrifuge design and optimization strategies have enabled better preservation of sample integrity across diverse applications. These developments highlight the need for continuous refinement of protocols to accommodate emerging diagnostic and research requirements, ensuring high-quality outcomes in both clinical and experimental settings.

**Keywords:** *centrifugation, sample integrity, temperature control, protocol optimization, laboratory diagnostics*

**Introduction**

Centrifugation is a cornerstone of laboratory processing and sample preparation, widely employed across various fields such as clinical diagnostics, biochemistry, molecular biology, and forensic science. Its primary function is to separate components of a mixture based on density by applying centrifugal force, which accelerates sedimentation. The effectiveness of centrifugation depends on several critical parameters, including speed, time, temperature, and rotor type, all of which significantly influence the integrity and usability of laboratory samples (1). A well-executed centrifugation process ensures the preservation of sample quality while enabling efficient separation, paving the way for accurate downstream analyses.

The application of centrifugation spans diverse biological materials, such as blood, urine, and cell cultures, highlighting its importance in clinical and research laboratories. In blood sample processing, centrifugation separates plasma, serum, and cellular components, providing a critical step for biochemical, immunological, and hematological assessments. However, improper centrifugation conditions, such as excessive speed or inadequate time, can lead to hemolysis, protein denaturation, or suboptimal separation, thereby compromising sample quality and analytical accuracy (2). Similarly, temperature control during centrifugation plays an essential role in preserving thermolabile biomolecules, such as enzymes and nucleic acids, emphasizing the need for meticulous protocol optimization. The choice of centrifugation speed, typically measured in revolutions per minute or relative centrifugal force (RCF), is dictated by the density and physical properties of the sample. High-speed centrifugation is often required for ultra clear separations, such as isolating subcellular organelles or nucleic acids, while lower speeds are suitable for general applications, including serum or plasma preparation (3, 4). The relationship between centrifugal force and sedimentation efficiency underscores the importance of selecting appropriate settings tailored to the specific application.

Advances in centrifugation technology have further refined the precision and versatility of this technique. Modern centrifuges offer enhanced control features, such as programmable speed ramps, temperature settings, and imbalance detection systems, to safeguard sample quality and operator safety. Additionally, the availability of various rotor designs, including fixed-angle, swinging-bucket, and vertical rotors, expands the applicability of centrifugation to accommodate diverse sample types and volumes (5). Despite these advancements, the risk of sample degradation persists if optimal centrifugation conditions are not adhered to. The critical influence of centrifugation on sample integrity necessitates standardized protocols, particularly in high-stakes applications such as diagnostic testing and pharmaceutical development. Variations in centrifugation practices across laboratories can lead to discrepancies in analytical outcomes, highlighting the need for rigorous quality control measures. Understanding the effects of centrifugation parameters on sample characteristics is vital for ensuring reproducibility and reliability in laboratory investigations (6).

**Review**

Centrifugation is a highly sensitive process that directly impacts the quality and usability of laboratory samples. Factors such as speed, temperature, and duration require careful optimization to prevent sample degradation. High-speed centrifugation, while effective in achieving rapid separation, poses risks such as protein denaturation, mechanical stress on cellular components, and hemolysis in blood samples. Studies highlight that improper speed settings can lead to suboptimal separation, interfering with diagnostic accuracy and research outcomes (7, 8).

Temperature control is another critical aspect, particularly for temperature-sensitive biomolecules. Fluctuations in temperature during centrifugation can compromise enzyme activity, nucleic acid stability, and protein conformation. For instance, chilled centrifugation has proven effective in preserving thermolabile compounds, especially in the preparation of plasma and serum for clinical

diagnostics. Research indicates that maintaining a stable temperature of 4°C is optimal for minimizing protein aggregation and other degradation processes (9, 10). Additionally, rotor types play a significant role in influencing separation outcomes. Swinging-bucket rotors offer superior resolution for density-gradient separations, while fixed-angle rotors are preferred for rapid sedimentation of particulates. This diversity underscores the importance of selecting appropriate centrifugation protocols to align with specific analytical objectives, ensuring consistency and reliability in results.

### ***Impact of Centrifugation Speed and Time on Sample Integrity***

Centrifugation speed and time are pivotal variables that determine the success of sample processing. Variations in these parameters can lead to either incomplete separation or physical damage to the sample. High-speed centrifugation is commonly used for tasks such as pelleting subcellular organelles or isolating nucleic acids, but it often imposes mechanical stress on the samples. Excessive centrifugal force has been shown to cause hemolysis in blood samples, releasing intracellular contents and distorting downstream analytical results. Hemolysis is particularly problematic in diagnostic settings, as it may falsely elevate levels of certain biomarkers such as potassium or lactate dehydrogenase (11).

Time spent under centrifugal force also plays a key role. Insufficient time can result in incomplete sedimentation, leading to a poor separation of sample components. For instance, shorter durations might leave suspended particulates in serum, which could interfere with optical density measurements in spectrophotometric assays. On the other hand, prolonged centrifugation exacerbates thermal and mechanical stress, especially when the samples are exposed to higher speeds. This is particularly critical in plasma preparation for coagulation studies, where platelet activation during extended centrifugation could lead to altered clotting profiles (12). Different sample types exhibit unique sensitivities to speed and time variations. For example, cellular components, such as white blood cells, tend to fragment when exposed to high speeds for

prolonged periods. This can be problematic in flow cytometry or cell culture applications where intact cell populations are required. By contrast, sedimentation of denser particles like calcium oxalate crystals in urine samples often demands higher speeds but shorter durations to minimize shearing forces while achieving complete separation (13-15). Consequently, standardizing protocols based on the nature of the sample is essential for consistent and reproducible results.

The interaction between speed and time becomes particularly significant in advanced techniques such as density-gradient centrifugation. This approach relies on precise adjustments of centrifugal force to fractionate components with minimal overlap in density. Excessive speed in such cases can disrupt the gradient, reducing resolution and complicating the retrieval of target fractions. For sensitive applications like isolating exosomes or purifying viruses, these disruptions can significantly affect the yield and purity of the isolated particles. Optimized speed-time protocols have shown remarkable success in mitigating these risks and enhancing the quality of separated fractions (15). Emerging research suggests that incremental increases in speed over a defined period, rather than abrupt accelerations, reduce the risk of sample damage. This controlled approach allows denser particles to sediment without creating excessive shear forces on more fragile components. Automated centrifuges equipped with programmable ramps provide the flexibility to fine-tune these parameters, addressing the variability observed with manual operation. These advancements have contributed significantly to achieving greater precision in modern laboratory practices, particularly for proteomics and genomics applications where sample integrity is paramount.

### ***Role of Temperature Control During Centrifugation***

Temperature regulation during centrifugation significantly influences the stability and functionality of biological samples. Many biomolecules, including enzymes, nucleic acids, and proteins, are sensitive to temperature fluctuations, making precise control essential. Elevated temperatures generated by high-speed

centrifugation can lead to protein denaturation and enzymatic degradation, especially in samples such as serum or plasma intended for diagnostic purposes. Studies demonstrate that maintaining a constant low temperature, typically around 4°C, helps preserve the biological activity and structural integrity of these molecules during centrifugation processes (9, 10).

Thermal stability is particularly important in the preparation of samples for molecular biology applications, such as RNA or DNA extraction. Nucleic acids are prone to degradation at elevated temperatures, which compromises their usability for downstream applications like polymerase chain reaction (PCR) or sequencing. Research has shown that even minor deviations from optimal cooling conditions can result in detectable loss of sample quality, with significant implications for experimental reproducibility. Temperature-controlled centrifuges equipped with advanced cooling systems have been developed to mitigate these risks, ensuring the preservation of nucleic acid integrity throughout the centrifugation process (16).

Clinical specimens often require stringent temperature management to ensure the accuracy of diagnostic tests. In hematology, for instance, platelet-rich plasma preparation is highly sensitive to temperature variations. Cooling during centrifugation prevents platelet activation, a process that alters the sample's coagulation profile and affects its utility in therapeutic or analytical applications. Furthermore, biochemical assays for hormones or metabolites often rely on temperature-sensitive reactions. Failure to maintain the required cooling conditions can alter the concentration of analytes, leading to false readings and diagnostic errors (17).

Temperature control becomes even more critical when handling thermolabile proteins or compounds. For instance, certain cytokines and growth factors are extremely unstable at temperatures exceeding their threshold limits. The heat generated during prolonged or high-speed centrifugation can lead to their degradation, reducing the reliability of immunoassays and other protein-based analyses.

Experimental data indicate that immediate cooling post-centrifugation is equally vital for minimizing thermal damage, particularly in samples stored for extended periods before analysis (18). Recent advancements in centrifuge technology have introduced features such as real-time temperature monitoring and programmable cooling profiles. These innovations allow precise regulation of thermal conditions throughout the centrifugation cycle, even under varying rotor speeds and load distributions. Additionally, the integration of insulation and heat-dissipating designs has enhanced the efficiency of temperature management in modern centrifuges, further reducing the risk of thermal fluctuations affecting sample quality. These developments underline the importance of optimizing centrifugation parameters to align with the specific thermal sensitivities of various sample types, ensuring consistency and reliability across applications.

### ***Optimization of Centrifugation Protocols for Different Sample Types***

Centrifugation protocols must be tailored to the unique characteristics of each sample type to achieve optimal separation and maintain sample integrity. Blood, urine, and cellular samples all demand distinct approaches in terms of speed, time, and rotor configuration. In blood processing, the separation of plasma, serum, and cellular components requires careful consideration of centrifugal force and duration. Studies have highlighted that variations in these parameters can result in incomplete separation or hemolysis, directly impacting the reliability of downstream tests. Protocols designed for serum preparation typically employ RCF between 1,500 and 2,000  $\times$  g for 10 to 15 minutes to ensure clear separation without damaging red blood cells (19, 20).

Urine samples, often analyzed for sedimentation of particulates like crystals, casts, and cells, present a different challenge. The density and fragility of these components necessitate lower speeds and shorter durations to avoid fragmentation or loss of diagnostic features. Research indicates that using an RCF of approximately 400 to 600  $\times$  g for 5 to 10 minutes achieves sufficient sedimentation while



preserving the morphology of urinary elements critical for microscopy-based diagnostics. Adjusting centrifugation protocols based on sample volume and specific diagnostic objectives further enhances accuracy (20, 21).

Cell culture applications, particularly in the context of harvesting cells or separating subpopulations, demand precision in protocol customization. Centrifugation settings must strike a balance between sedimentation efficiency and cell viability. Excessive force can lead to cell lysis or disruption of fragile cell membranes, compromising viability and functionality. Protocols optimized for live cell collection often utilize swinging-bucket rotors at lower speeds, such as 300 to 500 × g, for durations that align with cell density and medium viscosity. For subcellular fractionation, stepwise increases in centrifugal force are applied, with each step designed to isolate specific organelles or molecular components (21, 22).

Protocols for specialized applications, such as isolating extracellular vesicles (EVs) or exosomes, underscore the importance of fine-tuning centrifugation parameters. These nanoparticles require ultracentrifugation at forces exceeding 100,000 × g for extended durations. However, the success of such protocols depends on intermediate steps that remove larger contaminants without losing target particles. Gradient centrifugation methods, employing density gradients to enhance separation precision, have been widely adopted for isolating EVs. This approach ensures high yield and purity, critical for applications in diagnostics and therapeutics (23).

Advancements in automated centrifugation technology have contributed significantly to protocol optimization across diverse sample types. Programmable features now allow precise control over speed ramps, deceleration profiles, and temperature settings, enabling greater reproducibility. Incorporating these tools into standard laboratory workflows has minimized variability and enhanced the reliability of centrifugation-dependent processes. These innovations reflect the growing recognition of the

need for tailored protocols that accommodate the specific properties of each sample type, ensuring the accuracy and reproducibility of analytical outcomes.

## **Conclusion**

Optimizing centrifugation protocols is essential to maintain the integrity and functionality of laboratory samples across diverse applications. Tailored settings for speed, time, and temperature ensure reliable separation while preserving sample quality. Advances in centrifuge technology have further enhanced precision and reproducibility. These improvements underscore the importance of standardization and protocol refinement to achieve consistent analytical outcomes.

## **Disclosure**

### ***Conflict of interest***

There is no conflict of interest.

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### ***Ethical consideration***

Non applicable.

### ***Data availability***

Data that support the findings of this study are embedded within the manuscript.

### ***Author contribution***

All authors contributed to conceptualizing, data drafting, collection and final writing of the manuscript.

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