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Literature Review

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Hemolysis in Rodent Blood Sampling and Biomarker Validity - A Narrative Review

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ABSTRACT

Background: Blood sampling is essential in rodent biomedical research, yet hemolysis, remains a major source of bias. Hemolysis is common, often underreported, and can significantly distort serum and plasma biomarker results, compromising reproducibility, translational validity, and adherence to 3Rs principles.

Aims: This narrative review synthesizes current evidence on mechanisms, technique-related risk patterns, biomarker interference, and prevention strategies for hemolysis in laboratory rodent blood sampling.

Methods: The literature was searched using PubMed, Scopus, and Google Scholar to identify publications published from 2000 onward that addressed hemolysis in rodent blood sampling: search terms combined hemolysis, blood collection techniques, and laboratory rodents. Experimental studies, methodological reports, guidelines, and relevant reviews were included when they examined pre-analytical factors affecting serum or plasma biomarkers. Findings were integrated qualitatively due to heterogeneity in study design, analytical endpoints, and hemolysis assessment approaches.

Results: Hemolysis risk varies by sampling technique and procedural handling, with peripheral serial methods generally more susceptible to mechanical stress than terminal approaches. Analytical impact is biomarker dependent, particularly for intracellular analytes. Objective hemolysis indices improve assessment consistency. Standardized collection, processing, and reporting practices are essential to enhance reproducibility and 3Rs alignment.

Conclusion: Hemolysis is a modifiable source of pre-analytical variability in rodent studies. Standardizing sampling procedures, implementing objective quality assessments, and ensuring transparent reporting can enhance biomarker validity while promoting methodological rigor and ethical compliance.

INTRODUCTION

Blood sampling is fundamental in rodent-based biomedical research, supporting hematology, clinical chemistry, toxicology, and translational biomarker studies (Parasuraman et al., 2010; Port Louis et al., 2023). However, analytical validity is highly dependent on pre-analytical quality. The pre-analytical phase, including restraint, collection technique, sampled volume, and processing, accounts for 60–70% of laboratory errors (Lippi et al., 2019; Plebani, 2012). In rodents, this vulnerability is amplified by limited circulating blood volume and strain-specific physiological variability, increasing susceptibility to stress-induced and mechanical artifacts (Patel et al., 2024; Teilmann et al., 2014). Poor technique may therefore compromise data

integrity and increase animal use, conflicting with 3Rs principles (Stuart & Robinson, 2015).

Hemolysis is defined as the rupture of erythrocyte with release of hemoglobin, is among the most frequent and consequential pre-analytical errors (Lippi & Plebani, 2019). In vitro hemolysis commonly results from excessive aspiration force, small-gauge needles, improper anticoagulant ratios, delayed processing, or inadequate mixing (Lippi et al., 2019; Oliveira & Fernandes, 2016). Hemolyzed samples exhibit pink-to-red discoloration and introduce analytical bias through spectrophotometric interference and release of intracellular analytes such as potassium, lactate dehydrogenase, and transaminases (Lippi et al., 2013). Reported hemolysis rates in rodents range from 15–50%, exceeding those in human laboratories (3–6%), likely reflecting greater procedural variability and less standardized protocols (Çeken et al., 2025; Simundic, 2014). Notably, hemolysis status is frequently unreported, limiting reproducibility (Plebani, 2006).

Multiple sampling techniques are used in mice and rats. Tail and saphenous vein sampling permit serial collection without anesthesia but require technical proficiency (Port Louis et al., 2023). Retro-orbital sampling allows rapid volume acquisition but requires anesthesia and carries ocular risk (Sharma et al., 2014). Jugular catheterization enables repeated sampling with reduced handling stress post-recovery, whereas terminal cardiac puncture yields maximal volume but precludes longitudinal studies (Beeton et al., 2007; Park et al., 2018). Across methods, suboptimal handling remains a major determinant of hemolysis.

Key gaps persist, including limited quantitative comparisons across techniques, incomplete data on biomarker-specific susceptibility (e.g., molecular analytes), reliance on subjective visual hemolysis assessment, and inconsistent reporting. Strengthening objective hemolysis evaluation and standardized reporting is essential to improve data quality and reproducibility in rodent research.

MATERIALS AND METHODS

A narrative literature review was undertaken to synthesize current evidence on blood sampling techniques in laboratory rodents and their association with hemolysis occurrence and the validity of serum and plasma biomarkers. Given the heterogeneity of study designs, analytical endpoints, and hemolysis assessment methods in this field, a narrative rather than systematic approach was considered most appropriate to enable interpretive integration of the available literature.

Literature searches were conducted using PubMed, Scopus, and Google Scholar to identify relevant publications from January 2000 to December 2025. Earlier seminal studies were included when they were frequently cited and provided foundational methodological or conceptual insights. Search strategies combined three core concepts: hemolysis ("hemolysis" OR "haemolysis"), blood sampling ("blood sampling," "blood collection," "phlebotomy," OR "venipuncture"), and laboratory rodents ("mouse," "mice," "rat," OR "rodent"), with additional terms related to analytical matrices and outcomes (e.g., "serum," "plasma," "biomarker"). Reference lists of selected articles were manually screened to identify additional pertinent studies, including technical guidelines and consensus documents not readily captured through database searches.

The literature selection process aimed to enhance transparency in the narrative synthesis. Initially, 446 records were identified through database and manual searches. After removing duplicates, 297 records were screened based on titles and abstracts, excluding irrelevant articles on rodent blood sampling and related topics. Ultimately, 148 full-text articles were assessed for eligibility, resulting in 33 publications included in the narrative synthesis, encompassing experimental studies, methodological reports, reviews, and guidelines.

Studies were considered for inclusion if they involved laboratory rodents (mice or rats), addressed hemolysis occurrence or assessment in the context of blood sampling, and reported serum or plasma biomarker data. Eligible sources included experimental studies, methodological or technical reports, official guidelines, and relevant review articles published in English with full-text availability. Studies exclusively involving non-rodent species, focusing solely on in vivo hemolysis due to pathological conditions without a pre-analytical component, or lacking sufficient methodological detail relevant to hemolysis assessment were excluded. Abstracts, case reports, and editorials were also excluded.

The relevance and methodological informativeness of the selected literature were qualitatively assessed, focusing on peer-reviewed experimental studies, methodological reports, official guidelines, consensus documents, and review articles that addressed hemolysis, blood sampling procedures, pre-analytical variability, or serum/plasma biomarker validity in laboratory rodents. Higher informativeness was attributed to studies that detailed animal species or strain, sampling sites, needle gauge, sample handling, hemolysis assessment methods, and biomarker outcomes. Sources with limited methodological detail

were excluded from primary evidence for rodent-specific conclusions.

Due to the broader study of hemolysis assessment and biomarker interference in clinical laboratory medicine compared to rodent models, non-rodent references were included for contextual support, while rodent-specific conclusions were primarily drawn from studies on laboratory mice or rats. The review synthesized findings narratively due to substantial methodological heterogeneity, organizing results thematically and utilizing comparative tables for cross-study interpretation. It aimed not for exhaustive systematic synthesis or formal risk-of-bias assessment, but for a critical integration of methodologically informative studies relevant to hemolysis and biomarker validity in rodent blood sampling.

RESULTS AND DISCUSSION

Mechanisms and Causes of Hemolysis

Hemolysis in laboratory rodent blood samples arises from a complex interplay of mechanical, chemical, thermal, and biological factors occurring during collection and subsequent handling. Mechanical contributors remain the most prominent and modifiable, particularly needle gauge selection, aspiration force, and handling technique (Lindstrom et al., 2015; Lippi et al., 2013). The use of excessively small-gauge needles, typically smaller than 25–27G in mice or 23–25G in rats, markedly increases shear stress, predisposing erythrocytes to membrane rupture (Bowen & Dasgupta, 2025). Similarly, rapid aspiration, mechanical agitation during transport or mixing, and forcing blood through needles into collection tubes exacerbate cellular trauma (Ialongo & Bernardini, 2016). Temperature extremes further destabilize erythrocyte membranes, amplifying hemolysis risk.

Chemical and biological factors also play a significant role. Incorrect blood-to-anticoagulant ratios can induce osmotic lysis, while delayed processing beyond 30–60 minutes increases erythrocyte fragility. Species-related differences are particularly relevant in rodent models: mouse erythrocytes are inherently more fragile than those of rats, largely due to their smaller size and higher surface area-to-volume ratio. Additional modifiers include strain, age, and disease state, all of which influence membrane stability and baseline susceptibility to hemolysis (Igbokwe, 2019; Layssol-Lamour et al., 2019; Washington & Van Hoosier, 2012). Collectively, these observations indicate that hemolysis in rodent blood sampling is rarely attributable to a single factor but rather reflects the cumulative impact of multiple procedural and biological variables (Igbokwe, 2019; Marques-Garcia, 2020).

Importantly, unlike larger animals or humans, where controlling one dominant variable may be sufficient, rodent blood sampling demands meticulous attention to multiple parameters. Protocols optimized for rats cannot be directly transferred to mice without modification, particularly with respect to needle size, aspiration speed, and allowable handling time. Failure to account for these interspecies differences contributes substantially to preventable pre-analytical error.

Blood Sampling Techniques and Relative Hemolysis Risk

Hemolysis rates vary markedly across commonly used rodent blood sampling techniques, reflecting differences in vessel size, invasiveness, and procedural complexity (Table 1). Among non-terminal approaches, tail vein sampling consistently demonstrates the highest hemolysis rates, ranging from approximately 10–25%. This elevated risk is attributable to small vessel diameter, the need for tail warming, repeated punctures, and frequent manipulation (David & Chen, 2018; Lee & Goosens, 2015; Liu et al., 2019; Toft et al., 2006). While tail vein sampling permits serial collection without anesthesia, the associated compromise in sample quality may necessitate increased sample sizes to offset analytical variability.

Saphenous vein sampling exhibits intermediate hemolysis rates (approximately 8–18%), benefiting from improved vessel visualization and reduced compression-related trauma (Tsai et al., 2015; Van Herck et al., 2001; Vangala, 2015). This technique represents a practical compromise for longitudinal studies, balancing acceptable sample quality with animal welfare considerations. However, its performance is highly operator-dependent, and inter-operator variability remains a major determinant of hemolysis risk.

Serial peripheral sampling is intrinsically more prone to hemolysis because blood is withdrawn from small, low-flow vessels that are more susceptible to collapse and shear stress during aspiration (Lippi et al., 2013, 2019). Repeated puncture, warming, compression, and repositioning during serial collection further increase local vascular trauma and sample instability. Restraint-associated stress induces sympathetic-mediated peripheral vasoconstriction, reducing peripheral blood flow and prolong collection time, indirectly increasing

the risk of clotting and erythrocyte disruption (Meyer et al., 2020; Parasuraman et al., 2010; Tsai et al., 2015). In contrast, terminal cardiac puncture or central access permits collection from larger, higher-flow vessels with fewer repeated manipulations, thereby reducing mechanical hemolysis risk when performed appropriately (Beeton et al., 2007; Park et al., 2018).

Retro-orbital sinus sampling yields lower hemolysis rates (approximately 5–15%) compared with peripheral techniques, largely due to rapid blood flow and reduced need for repeated puncture. Nevertheless, its reliance on anesthesia and potential for ocular injury limit its suitability for repeated sampling (Sharma et al., 2014; Vangala, 2015). Terminal techniques, particularly cardiac puncture, consistently demonstrate the lowest hemolysis rates (approximately 3–10%) due to minimal manipulation and collection of fresh central blood. While optimal for sample quality, the terminal nature of this approach restricts its use to end-point analyses (Beeton et al., 2007; Williams et al., 2020).

The inverse relationship between hemolysis risk and procedural invasiveness presents a fundamental trade-off in experimental design. Techniques that maximize sample quality often limit longitudinal data collection, whereas methods enabling serial sampling introduce greater pre-analytical variability. From a 3Rs perspective, this trade-off has direct implications: high hemolysis rates may paradoxically increase animal use through repeat sampling or inflated group sizes, undermining the principle of Reduction while also affecting Refinement.

Biomarker-Specific Interference from Hemolysis

The analytical impact of hemolysis is highly biomarker dependent and must be interpreted within the context of erythrocyte composition and assay methodology (Table 2).

In rodent blood samples, the analytical impact of hemolysis is biomarker dependent, with potassium, LDH, AST, iron, and cardiac troponin exhibiting high susceptibility even at subvisual hemolysis levels (<0.5–1%). Moderate effects are observed for ALT, CK, phosphate, and albumin, whereas creatinine, urea, and major electrolytes are generally robust. For emerging molecular biomarkers, hemolysis status must be explicitly reported due to erythrocyte-derived contamination.

Biomarkers with high intracellular concentrations, such as potassium, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), are particularly

Table 1. Blood sampling techniques in laboratory rodents and relative hemolysis risk

Technique	Typical Application	Relative Hemolysis Risk*	Principal Contributors	Advantages	Limitations / Ethical Considerations	Selected References
Tail vein	Serial sampling	Higher (particularly in mice)	Small vessel diameter, repeated puncture, mechanical compression, tail warming	No anesthesia required; suitable for longitudinal studies	Operator-dependent; stress-related variability; potential thrombosis or tissue injury	David & Chen, 2018; Lee & Goosens, 2015; Liu et al., 2019; Toft et al., 2006
Saphenous vein	Serial sampling	Moderate	Needle angle, vessel stabilization, operator technique	Repeatable; no anesthesia; moderate volume	Training-intensive; bruising or hematoma risk	Tsai et al., 2015; Van Herck et al., 2001; Vangala, 2015
Retro-orbital sinus	Limited or controlled sampling	Lower relative to peripheral veins when properly performed	Orbital pressure, anesthesia effects	Rapid collection; relatively large volume	Risk of ocular injury; limited suitability for repeated sampling	Sharma et al., 2014; Mahl et al., 2000; Teilmann et al., 2014
Jugular catheter	Intensive serial sampling	Low (after recovery period)	Surgical manipulation; catheter handling	Enables repeated sampling with reduced handling stress	Requires surgery; infection and thrombosis risk	Bardelmeijer et al., 2003; Park et al., 2018
Cardiac puncture (terminal)	Terminal studies	Low	Minimal mechanical manipulation	Large volume; typically high analytical quality	Terminal procedure; requires deep anesthesia	Beeton et al., 2007; de Oliveira et al., 2021

susceptible to even minimal hemolysis. For example, hemolysis as low as 0.5–1% can produce clinically meaningful pseudohyperkalemia or artifactual enzyme elevation, distorting experimental outcomes (Nigro et al., 2023; Ünl et al., 2018; Wu & Peacock, 2024).

Moderately affected biomarkers, including alanine aminotransferase (ALT), creatine kinase, phosphate, and albumin, exhibit more gradual interference patterns and may tolerate limited hemolysis with cautious interpretation. In contrast, biomarkers such as creatinine, urea, sodium, chloride, and calcium, characterized by low erythrocyte-to-plasma concentration gradients, are relatively resistant to hemolysis-related bias and may remain analytically valid even in moderately hemolyzed samples (Mahl et al., 2000; Vangala, 2015).

Emerging molecular biomarkers, including microRNAs, circulating cell-free DNA, and extracellular vesicles, present a distinct challenge. Erythrocyte contamination can profoundly alter measured concentrations, yet standardized hemolysis thresholds for these analytes are lacking. In rodent models, where blood volumes are limited and hemolysis risk is intrinsically higher, the absence of validated acceptance criteria represents a critical methodological gap. Until such standards are established, rigorous hemolysis assessment and transparent reporting should be considered mandatory for molecular biomarker studies (Enders et al., 2025; Guerrero-Alba et al., 2024).

Hemolysis-related interference also has direct implications for translational reproducibility. Even minor or visually undetectable hemolysis may introduce systematic bias in biomarker interpretation, particularly in toxicology, inflammation, cardiovascular injury, and molecular biomarker studies, where small differences between experimental groups may be considered biologically meaningful (Ho et al., 2021; Lippi et al., 2006; Monneret et al., 2015). In rodent models, this risk is amplified by limited circulating blood volume, which restricts repeat sampling and lowers tolerance for pre-analytical error compared with human clinical studies (Parasuraman et al., 2010; Port Louis et al., 2023).

Consequently, unrecognized hemolysis may distort interpretation of disease mechanisms, treatment effects, and translational validity. Transparent reporting of hemolysis status, detection methods, and biomarker-specific acceptance thresholds is therefore essential to improve reproducibility and comparability across studies (Enders et al., 2025; Guerrero-Alba et al., 2024; Lippi et al., 2013; Lippi & Plebani, 2019).

Stratifying biomarkers according to hemolysis susceptibility enables risk-based quality control. High-interference biomarkers require stringent hemolysis thresholds, whereas more robust analytes may tolerate mild hemolysis without compromising interpretability. This approach facilitates efficient allocation of resources while preserving analytical validity.

Table 2. Biomarker susceptibility to hemolysis in rodent serum/plasma

Interference Category	Representative Biomarkers	Mechanism of Interference	Practical Interpretation	Selected References
High susceptibility	Potassium, LDH, AST, Iron, Cardiac troponin	Intracellular release; spectrophotometric interference	Even minimal hemoglobin contamination may alter results; consider strict acceptance criteria	Monneret et al., 2015; Ünl et al., 2018; Nigro et al., 2023
Moderate susceptibility	ALT, CK, Phosphate, Albumin	Partial intracellular contribution; optical interference	Mild hemolysis may require cautious interpretation	Mahl et al., 2000; Vangala, 2015
Lower susceptibility	Creatinine, Urea, Sodium, Chloride, Calcium	Low erythrocyte-to-plasma concentration gradient	Often analytically stable under mild hemolysis	Washington & Van Hoosier, 2012
Emerging molecular biomarkers	miRNA, cfDNA, Extracellular vesicles	Erythrocyte-derived contamination	Hemolysis status should be explicitly documented due to lack of standardized thresholds	Enders et al., 2025; Guerrero-Alba et al., 2024

Note: Where rodent-specific interference validation was unavailable, mechanistic insight from clinical laboratory studies was considered for contextual interpretation.

Detection, Prevention, and Management of Hemolysis

Reliable hemolysis assessment is central to effective quality control. Visual inspection, although rapid and inexpensive, is limited by subjectivity, inter-observer variability, and low sensitivity, particularly at low hemoglobin concentrations. Objective spectrophotometric measurement using hemolysis indices (H-index) offers superior sensitivity and reproducibility, enabling detection of hemolysis levels well below the visual threshold and providing a quantitative basis for sample acceptance or rejection (Simundic et al., 2012; Dolci et al., 2017). Practical thresholds and interpretive guidance are summarized in Table 3.

Preventive strategies span all phases of sample handling. Pre-collection measures include appropriate needle selection, matching syringe size to target volume, and minimizing stress through acclimatization. During collection, gentle aspiration, avoidance of excessive negative pressure, removal of the needle prior to dispensing blood into collection tubes, and gentle mixing are critical. Post-collection, rapid processing, ideally within 30–60 minutes, remains one of the most influential determinants of hemolysis risk. Notably, many effective preventive measures require minimal financial investment and rely primarily on operator training and protocol standardization rather than specialized equipment.

When hemolysis cannot be avoided, structured sample management is essential. A stratified approach, accepting mildly hemolyzed samples with documentation, applying biomarker-specific rejection criteria, and avoiding unnecessary re-sampling, balances scientific rigor with practical and ethical constraints (Table 3). This approach is particularly relevant for terminal procedures or studies in which sample repetition is not feasible.

Implications for Research Quality and the 3Rs

Collectively, the evidence demonstrates that hemolysis represents a substantial yet underappreciated threat to data quality and reproducibility in rodent biomedical research. Reported hemolysis rates of up to 25% for commonly used techniques imply that a significant proportion of samples may yield biased results, particularly for hemolysis-sensitive biomarkers. These biases can propagate through study design, leading to inflated sample size requirements, repeat procedures, and potentially erroneous scientific conclusions.

The frequent omission of hemolysis status in published studies further compounds this problem,

Table 3. Integrated framework for hemolysis detection, prevention, and sample management

Phase	Key Considerations	Practical Guidance	Impact on Analytical Validity	Selected References
Detection	Objective hemolysis assessment preferred over visual inspection	Use automated hemolysis indices when available; interpret according to analyzer-specific documentation	Improves reproducibility and reduces subjective variability	Dolci & Panteghini, 2014; Lippi & Plebani, 2019
Pre-collection	Appropriate needle selection and restraint technique	Select gauge appropriate to species and vessel size; minimize stress	Reduces mechanical erythrocyte injury	Lippi et al., 2013
Collection	Gentle aspiration; avoid excessive negative pressure	Remove needle before dispensing; gentle mixing	Minimizes shear-induced hemolysis	Sharma et al., 2014
Post-collection	Timely processing and standardized centrifugation	Separate plasma/serum promptly according to laboratory SOP	Limits progressive in vitro hemolysis	Layssol-Lamour et al., 2019
Sample Management	Biomarker-specific acceptance criteria	Define rejection or documentation policy prior to analysis	Preserves interpretive integrity	Lippi et al., 2006

Note: Hemolysis index thresholds are analyzer-dependent and should follow manufacturer validation and institutional quality control policies.

creating a hidden source of irreproducibility. Routine reporting of collection technique, hemolysis assessment method, and acceptance thresholds should therefore be considered a minimum standard, particularly for studies relying on intracellular or spectrophotometrically sensitive biomarkers. Implementation of evidence-based sampling practices has the potential to reduce hemolysis rates substantially, improving data reliability while simultaneously supporting the principles of Reduction and Refinement.

CONCLUSION

Hemolysis is a significant yet often overlooked bias in rodent biomedical research, affecting serum and plasma biomarker validity. It varies by sampling technique and is influenced by procedural factors. Preventative measures and objective reporting standards are essential to improve data quality and reduce animal use in research.

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AUTHORS CONTRIBUTION

A.N.P.R. and H.V.M. conducted the literature search, screening, data extraction, and initial drafting of the manuscript. D.A.W.S. conceived and designed the study, supervised the methodological framework, and performed critical revisions for important intellectual content. N.H. critically reviewed and refined the manuscript. All authors approved the final version of the manuscript.

"The authors declare that there is no conflict of interest with the parties involved in this research".

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