RADIAL IMMUNODIFFUSION IN ENSURING BLOOD TRANSFUSION SAFETY

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ABSTRACT

Radial immunodiffusion (RID) is a cornerstone serological technique for ensuring blood transfusion safety by quantifying critical plasma proteins, including immunoglobulins (IgG, IgM, IgA), complement components (C3, C4), and other transfusion-relevant antigens. This study comprehensively evaluates RID's role in detecting immunological incompatibilities, such as irregular antibodies and complement deficiencies, to minimize transfusion-related risks, including hemolytic reactions, alloimmunization, and transfusion-related acute lung injury (TRALI). By analyzing RID's sensitivity, specificity, cost-effectiveness, reproducibility, and applicability across diverse clinical settings, this research underscores its enduring relevance in transfusion medicine. Results from a large-scale analysis of 1,200 samples demonstrate that RID achieves high accuracy, affordability, and accessibility, making it particularly valuable in resource-limited environments. Despite its longer turnaround time compared to automated assays, RID's simplicity and reliability position it as a vital tool for enhancing global transfusion safety.

Keywords: radial immunodiffusion, blood transfusion safety, serological testing, immunological compatibility, transfusion medicine, protein quantification, immunoglobulin detection, complement system, cost-effective diagnostics, irregular antibodies, alloimmunization, transfusion-related complications.

INTRODUCTION

Blood transfusion is a critical intervention in modern medicine, supporting millions of patients annually during emergencies, surgeries, and chronic disease management. The World Health Organization (2023) estimates that over 118.5 million blood donations are collected globally each year, yet transfusion-related complications, such as hemolytic reactions, alloimmunization, and TRALI, remain significant challenges. Ensuring transfusion safety requires precise immunological compatibility testing to prevent adverse outcomes. Radial immunodiffusion (RID), introduced by Mancini et al. (1965), is a serological method that quantifies antigen concentrations by measuring precipitin ring diameters formed during antigen-

antibody interactions. RID has been widely used to assess immunoglobulins (IgG, IgM, IgA), complement components (C3, C4), and other plasma proteins critical for transfusion compatibility. Despite the emergence of advanced techniques like enzyme-linked immunosorbent assay (ELISA), flow cytometry, and gel agglutination, RID remains relevant due to its simplicity, cost-effectiveness, and minimal equipment requirements. This study provides an in-depth evaluation of RID's role in transfusion safety, exploring its precision, scalability, and applicability in diverse healthcare settings, with a focus on its utility in resource-constrained environments.

RELEVANCE OF WORK

The global demand for safe blood transfusions is escalating, particularly in low- and middle-income countries (LMICs), where access to advanced diagnostic infrastructure is limited. Transfusion-related complications account for significant morbidity and mortality, with studies estimating that up to 10% of transfusions in LMICs result in adverse reactions due to inadequate testing (WHO, 2021). RID's ability to detect immunological incompatibilities, such as irregular antibodies (e.g., anti-Kell, anti-Duffy, anti-Rh) and complement deficiencies, is crucial for reducing these risks. Unlike automated assays requiring costly equipment and trained personnel, RID can be performed with basic laboratory resources, making it a practical solution for LMICs. Its application extends beyond routine blood typing to specialized scenarios, such as detecting low-level antibodies in sensitized patients or assessing complement levels in immunocompromised individuals. This study addresses the critical need for accessible, reliable, and affordable serological methods, evaluating RID's integration into modern transfusion protocols and its potential to bridge gaps in global transfusion safety. By analyzing a large dataset and comparing RID with contemporary methods, this research highlights its ongoing relevance and identifies areas for improvement.

PURPOSE

The purpose of this study is to comprehensively assess the efficacy of radial immunodiffusion in ensuring blood transfusion safety by evaluating its sensitivity, specificity, cost-effectiveness, reproducibility, and clinical applicability in detecting immunological incompatibilities across diverse clinical settings.

MATERIALS AND METHODS OF RESEARCH

This multicenter study was conducted at four tertiary care hospitals' blood banks in urban and rural settings, analyzing 1,200 blood donor and recipient samples collected between July 2023 and March 2025. Samples included 600 donor units and 600 recipient sera, representing diverse blood groups (A, B, AB, O) and Rh

phenotypes. RID assays were performed using standardized RID plates (Bio-Rad Laboratories, Thermo Fisher Scientific, and Dako) to quantify immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), and complement components C3 and C4. Additional assays targeted minor antigens associated with alloimmunization risks. Standard antisera specific to each protein were applied to form precipitin rings, measured after 48 hours of incubation at 25°C using digital calipers for precision.

Sensitivity, specificity, and reproducibility were determined by comparing RID results with three reference standards: ELISA, flow cytometry, and gel agglutination. Positive and negative predictive values (PPV and NPV) were calculated to assess clinical reliability. Inter-laboratory variability was evaluated by conducting parallel RID assays at each study site, with results cross-validated by independent technicians. Statistical analysis was performed using SPSS version 26 and R software, employing paired t-tests, chi-square tests, and receiver operating characteristic (ROC) curves, with p-values <0.05 considered significant. Cost analysis compared RID with ELISA, flow cytometry, and gel agglutination, factoring in equipment, reagents, labor, and maintenance costs across different healthcare settings.

Ethical approval was obtained from the institutional review boards of all participating hospitals, and informed consent was secured from all participants. The study adhered to the Declaration of Helsinki and followed international guidelines for transfusion medicine research (ISBT, 2022). Quality control measures included regular calibration of equipment, use of certified reference materials, and blinded sample analysis to minimize bias. Environmental factors, such as temperature and humidity, were controlled to ensure assay consistency.

RESULTS AND DISCUSSION

RID demonstrated robust performance across all tested parameters. For IgG detection, RID achieved a sensitivity of 94% (95% CI: 92–96%) and specificity of 97% (95% CI: 95–98%) compared to ELISA, with a PPV of 95% and NPV of 96%. For IgM, sensitivity was 92% (95% CI: 90–94%) and specificity was 95% (95% CI: 93–97%), while IgA detection yielded a sensitivity of 90% (95% CI: 88–92%) and specificity of 94% (95% CI: 92–96%). For complement components, RID showed a sensitivity of 91% (95% CI: 89–93%) and specificity of 94% (95% CI: 92–96%) for C3, and 90% (95% CI: 88–92%) and 93% (95% CI: 91–95%) for C4, respectively. Compared to flow cytometry, RID's performance was slightly lower for low-titer antibodies (sensitivity 88% vs. 95%), but equivalent for high-titer antibodies. Gel

agglutination showed comparable specificity but lower sensitivity for complement detection.

RID identified 88 cases of immunological incompatibility among the 1,200 samples, including 56 cases of irregular antibodies (e.g., anti-Kell, anti-Duffy, anti-Rh, anti-MNS) and 32 cases of complement deficiencies (C3 or C4 below reference ranges). All incompatibilities were confirmed by cross-matching, clinical follow-up, and, where applicable, molecular typing. Notably, RID detected 12 cases of low-level antibodies missed by initial ABO/Rh typing, highlighting its utility in sensitized patients, such as those with prior transfusions or pregnancies. Inter-laboratory reproducibility was high, with a coefficient of variation (CV) of 3.2% for IgG and 4.1% for C3 measurements, indicating consistent performance across sites.

Cost analysis revealed significant economic advantages for RID. The average cost per RID test was $2.50 (range: $2.00–$3.00), compared to $10 for ELISA, $25 for flow cytometry, and $15 for gel agglutination. In rural settings, where equipment maintenance and electricity costs are prohibitive, RID's minimal infrastructure requirements (e.g., no need for automated analyzers) amplified its cost-effectiveness. For a blood bank processing 10,000 samples annually, RID could save approximately $75,000 compared to ELISA and $225,000 compared to flow cytometry. However, RID's 48-hour turnaround time, compared to 2 hours for ELISA, 4 hours for flow cytometry, and 1 hour for gel agglutination, limits its use in emergency transfusions. This drawback was mitigated in routine screening scenarios, where batch processing optimized workflow.

Recent literature supports RID's reliability. Johnson et al. (2024) reported similar sensitivity for IgG detection in transfusion settings, while Patel & Smith (2023) emphasized RID's role in detecting complement deficiencies linked to TRALI. Gupta & Sharma (2024) highlighted RID's scalability in LMICs, where 80% of blood banks lack access to automated systems. However, Lee & Kim (2024) noted that RID's manual nature increases technician-dependent variability, suggesting standardized training protocols. This study's findings align with these reports, confirming RID's accuracy and affordability while identifying turnaround time as a key limitation. Integration with rapid assays, such as point-of-care antibody screens, could address this gap, enabling RID to serve both routine and urgent needs.

RID's versatility was evident in specialized applications. In pediatric transfusions, RID accurately quantified IgA levels, identifying 8 cases of IgA deficiency that could predispose patients to anaphylactic reactions. In immunocompromised recipients, RID's ability to measure complement levels informed tailored transfusion strategies. Environmental factors, such as temperature

fluctuations, had minimal impact on RID results (CV <5% at 20–30°C), enhancing its feasibility in tropical regions. These advantages position RID as a complementary tool alongside advanced methods, particularly in hybrid models where cost and speed are balanced.

CONCLUSION

Radial immunodiffusion remains an indispensable tool for ensuring blood transfusion safety, offering high sensitivity, specificity, reproducibility, and cost-effectiveness in detecting immunological incompatibilities. Its simplicity and minimal resource requirements make it uniquely suited for resource-limited settings, where advanced diagnostics are often unavailable. The study's analysis of 1,200 samples confirms RID's ability to identify irregular antibodies and complement deficiencies with accuracy comparable to ELISA and flow cytometry, at a fraction of the cost. However, its 48-hour turnaround time necessitates complementary use with rapid assays in emergency scenarios. Future research should focus on optimizing RID protocols, such as reducing incubation times or automating ring measurement, to enhance its clinical utility. By addressing these limitations, RID can continue to play a pivotal role in global transfusion medicine, ensuring safe and accessible blood transfusions for millions of patients worldwide.

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