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The Paradox of Negative Antibody Screening and Positive Crossmatching: Implications for Transfusion Safety in a Developing Country

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A B S T R A C T

Modern antibody screening is a pillar of transfusion safety, intended to prevent hemolytic reactions. However, its ultimate effectiveness, particularly in diverse populations within developing nations where abbreviated crossmatch protocols are not feasible, remains a critical question. This study investigates the "safety gap" between negative screening results and final patient-donor compatibility, aiming to quantify the incidence of serological incompatibility detected by mandatory crossmatching in a tertiary hospital in Indonesia. A descriptive, cross-sectional study was conducted from March to June 2024 at the Blood Bank of PKU Muhammadiyah Yogyakarta Hospital. A total of 299 donor-recipient pairs were analyzed via total sampling. Donor units were screened for irregular antibodies using an automated Column Agglutination Technology (Gel Test) with comprehensive three-cell panels. Subsequently, major and minor crossmatches for all pairs were performed using a Gel Test-based Indirect Antiglobulin Test (IAT) incubated for 15 minutes at 37°C. All 299 donor samples (100%) yielded negative results for irregular antibodies during screening. However, the final crossmatch revealed serological incompatibilities. Major crossmatching (patient serum vs. donor cells) identified incompatibility in 4 cases (1.34%). Minor crossmatching (donor serum vs. patient cells) showed a significantly higher rate of incompatibility, found in 21 cases (7.03%). The predominant blood component transfused was Packed Red Cells (91.97%). In conclusion, the findings demonstrate a significant paradox where a substantial rate of serological incompatibility is only detected by the final crossmatch. This study quantitatively confirms that antibody screening alone is insufficient to guarantee blood compatibility. These results challenge the safety of adopting abbreviated crossmatch protocols in this setting and affirm that the physical crossmatch remains an indispensable, non-negotiable safeguard. This provides critical, region-specific evidence for strengthening hemovigilance systems and reinforcing transfusion policies in Indonesia and other resource-limited nations.

1. Introduction

Blood transfusion is an indispensable, life-saving therapeutic intervention in modern medicine, critical in the management of a vast spectrum of clinical conditions ranging from acute massive hemorrhage and surgical support to the chronic management of severe anemia and hematological malignancies.[1] The World Health Organization (WHO) estimates that millions of lives are saved annually through this procedure, yet it underscores that the process is not without inherent risks.[2] The ultimate success and safety of a blood transfusion hinge upon the precise immunological compatibility between the donor's blood and the recipient's circulatory system. An incompatible transfusion can trigger a cascade of devastating immunological sequelae, most notably acute and delayed hemolytic transfusion reactions (HTRs), which can lead to severe morbidity, multi-organ failure, and mortality.[3] The scientific foundation of transfusion safety began with Karl Landsteiner's discovery of the ABO blood group system in 1901, a landmark that transformed transfusion from a high-risk gamble into a viable medical procedure. Since then, our understanding has expanded to encompass over 43 different blood group systems, comprising more than 360 red blood cell (RBC) antigens. While the ABO and Rhesus (Rh) systems are the most immunogenic, antibodies directed against antigens in other "minor" blood group systems—such as Kell (K), Duffy (Fy), and Kidd (Jk)—are also of immense clinical importance. These antibodies, typically IgG, are capable of causing severe HTRs and hemolytic disease of the fetus and newborn (HDFN).[4] The formation of these "irregular" antibodies, or alloantibodies, is typically a consequence of sensitization through prior transfusion, pregnancy, or transplantation.[5] In an ideal, high-resource healthcare setting, the paradigm of transfusion safety has evolved towards elegant, technology-driven solutions. Comprehensive donor screening programs, validated laboratory information systems, and the use of sensitive antibody detection methods have enabled many institutions to adopt computer or electronic crossmatching.[6] This practice relies on the statistical certainty that for a patient with a negative antibody screen and no history of alloantibodies, the probability of a major crossmatch being incompatible is vanishingly small. This represents the pinnacle of a systems-based approach to safety.[7]

However, this ideal stands in stark contrast to the reality faced in many developing countries, including Indonesia. Here, the "patient journey" through the healthcare system is subject to a different set of variables. Consider the case of a multi-transfused thalassemia patient in a regional Indonesian hospital or a woman suffering from postpartum hemorrhage. For them, the safety of each unit of blood is not an abstract concept but a matter of immediate survival. The reliability of pre-transfusion testing is the bedrock of their clinical management. In this context, economic constraints often preclude the adoption of advanced technologies like routine molecular genotyping; supply chains for high-quality reagents can be inconsistent; and the operational interface between the central blood collection center (Unit Transfusi Darah, UTD) and the hospital blood bank (Bank Darah Rumah Sakit, BDRS) introduces a critical control point that requires meticulous oversight.[8] These hurdles mean the conditions necessary for safely implementing electronic crossmatching are not met. Consequently, reliance on the final physical crossmatch remains the paramount, non-negotiable safeguard. This study is framed as an evaluation of this crucial two-stage safety process. The Indonesian Ministry of Health, through Regulation No. 83 of 2014, has mandated standards for blood services, yet national data on transfusion incompatibility rates remain sparse. This gap in local, empirical data creates a challenge for evidence-based policymaking. Is the antibody screening performed at the centralized UTD sufficient to ensure safety? What is the tangible, quantifiable value added by the second, definitive check—the crossmatch—performed at the hospital BDRS just before blood is issued? Answering these questions is vital for understanding and strengthening the national transfusion system.[9]

While the imperative for crossmatching is a foundational principle of transfusion medicine, a significant knowledge gap persists regarding the precise frequency at which modern, sensitive screening technologies fail to predict serological incompatibilities, particularly within the unique population genetics and healthcare infrastructures of Southeast Asia. There is a scarcity of recent, systematic data from Indonesia that quantifies this residual risk.[10] Therefore, the primary aim of this study was to meticulously document and analyze the incidence of serological incompatibility detected by major and minor crossmatching in a cohort of donor-recipient pairs where all donors had previously tested negative for irregular antibodies using a contemporary gel-based screening platform. The novelty of this research lies in its direct quantification of this "serological paradox" or "hidden risk." By providing a precise, empirical measure of incompatibilities that bypass initial screening, this study generates critical, region-specific evidence. This evidence is intended to inform and reinforce national transfusion safety policies, underscore the non-negotiable role of the final crossmatch, and serve as a foundational data point for the advocacy and development of a more robust national hemovigilance system in Indonesia and other comparable resource-limited nations.

2. Methods

This study was conducted using a descriptive, observational design with a cross-sectional approach. This design was deemed appropriate to capture a snapshot of the prevalence and characteristics of serological incompatibility within a defined timeframe without any experimental intervention. The study was carried out from March 2024 to June 2024. The research involved a collaboration between two key institutions integral to the transfusion service workflow in Yogyakarta, Indonesia: the Blood Transfusion Unit (Unit Transfusi Darah, UTD) of the Indonesian Red Cross (Palang Merah Indonesia, PMI), Yogyakarta City, and the Hospital Blood Bank (Bank Darah Rumah Sakit, BDRS) of PKU Muhammadiyah Yogyakarta Hospital. The BDRS at PKU Muhammadiyah Yogyakarta Hospital serves as a major tertiary care and teaching hospital with high-volume services in internal medicine, surgery, obstetrics, and pediatrics, driving a consistent and varied demand for blood transfusions. The UTD PMI is the central blood establishment responsible for donor recruitment, blood collection, infectious disease screening, component preparation, and initial serological testing. The BDRS is responsible for receiving, storing, and performing the final pre-transfusion compatibility testing for hospital patients. This dual-site approach accurately reflects the standard operational procedure for transfusion services in the region. No unusual operational changes or public health events that might have skewed the patient population occurred during the study period.

The study population consisted of all donor blood units processed at the UTD PMI Yogyakarta and subsequently requested for transfusion and subjected to crossmatching at the BDRS of PKU Muhammadiyah Yogyakarta Hospital during the four-month study period. A total sampling methodology was employed, whereby every donor-recipient pair that met the defined inclusion criteria during this period was included in the analysis. This method was chosen to ensure a comprehensive dataset, minimize selection bias, and accurately reflect the real-world prevalence of the phenomena under investigation. The inclusion criterion was any donor blood unit with a complete, documented antibody screening result from the UTD PMI that was subsequently crossmatched against a recipient sample at the hospital's BDRS. Exclusion criteria included any donor units or recipient samples with incomplete or ambiguous laboratory records, samples with quality issues (such as hemolysis or lipemia) that could interfere with serological interpretation, or records where the final disposition of the crossmatched unit could not be confirmed. Based on these criteria, a total of 299 unique donor-recipient pairs were deemed eligible and were included in the final analysis.

The data for this study were secondary in nature, retrospectively collected from the laboratory information systems and official paper-based records maintained at the UTD PMI Yogyakarta and the BDRS of PKU Muhammadiyah Yogyakarta Hospital. A standardized data collection form was designed to extract the relevant variables while ensuring anonymity. The collected variables included Recipient Data (Age, gender, ABO/Rh blood group), Donor Data (ABO/Rh blood group, antibody screen result), Transfusion Data (type of blood component), and Compatibility Testing Results (major and minor crossmatch interpretation). All data were carefully transcribed and cross-verified to ensure accuracy. All serological testing followed the standard operating procedures of the respective institutions, which are aligned with national and international guidelines. Daily quality control for all serological tests was performed using commercial control reagents with known antigen and antibody profiles, and all results were confirmed to be within established limits. Antibody Screening: The screening of donor plasma for irregular RBC antibodies was performed at the UTD PMI using automated Column Agglutination Technology (Gel Test; ID-System, Bio-Rad, Cressier, Switzerland). The principle of this technology involves the use of microtubes containing a dextran-acrylamide gel matrix. Donor plasma was incubated with commercial three-cell screening panels (ID-DiaCell I-II-III, Bio-Rad), which are selected to collectively express the most clinically significant RBC antigens, including D, C, c, E, e, K, k, Fya, Fyb, Jka, Jkb, M, N, S, and s. In a negative reaction, RBCs pass through the gel to form a pellet at the bottom. In a positive reaction, antibody-mediated agglutinates are trapped within the gel matrix. For this study, any degree of agglutination (graded 1+ to 4+) was considered a positive result. Crossmatching: Final compatibility testing was performed at the BDRS using the Gel Test-based Indirect Antiglobulin Test (IAT): Major Crossmatch: This test involved incubating the recipient's serum/plasma with a 0.8% suspension of the donor's RBCs at 37°C for 15 minutes. This step is designed to detect any recipient antibodies that could destroy the transfused donor cells; Minor Crossmatch: This test involved incubating the donor's serum/plasma with a 0.8% suspension of the recipient's RBCs under the same conditions. This step is designed to detect any donor antibodies that could react with the recipient's own cells. Following incubation, microtubes containing the cell-serum mixture within a gel matrix impregnated with anti-human globulin (AHG) were centrifuged. A compatible result was defined by the absence of agglutination, while an incompatible result was defined by the presence of agglutination trapped in the gel.

All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA). The analysis was purely descriptive. Frequencies and percentages were calculated for all categorical variables. The results were tabulated to describe the sample characteristics and the prevalence of serological incompatibility. No inferential statistical tests were performed, consistent with the study's descriptive objectives. This study was conducted with strict adherence to ethical principles outlined in the Declaration of Helsinki. As the research utilized anonymized secondary data from pre-existing laboratory records, there was no direct contact with human subjects. All personal identifiers were removed to ensure confidentiality. Formal approval for accessing and utilizing the anonymized laboratory data was obtained from the institutional authorities at PKU Muhammadiyah Yogyakarta Hospital.

3. Results and Discussion

During the four-month study period, a total of 299 donor-recipient pairs met the inclusion criteria and were analyzed. The demographic and blood group characteristics of this cohort are presented in detail in Figure 1. The recipient population showed a balanced gender distribution, comprising 152 females (50.84%) and 147 males (49.16%). The ABO blood group distribution among recipients was consistent with known frequencies in the Southeast Asian population, with blood group O being the most common (142 patients, 47.49%). This was followed by blood groups B (73 patients, 24.41%) and A (72 patients, 24.08%), with group AB being the least frequent (12 patients, 4.02%). An analogous distribution was observed among the 299 blood donors, where group O was also predominant (145 donors, 48.49%). This parity between donor and recipient blood group frequencies suggests a well-calibrated blood supply system meeting the local clinical demand.

The types of blood components requested for transfusion were analyzed to understand the clinical context of the compatibility testing. As shown in Figure 2, there was an overwhelming use of red cell-based products. Packed Red Cells (PRC) constituted the vast majority of transfusions, with 275 units, accounting for 91.97% of the total. This highlights that the primary indication for transfusion in this cohort was the restoration of oxygen-carrying capacity. Other components were utilized with much lower frequency: Thrombocyte Concentrate (TC) was used in 19 cases (6.35%), Fresh Frozen Plasma (FFP) in 3 cases (1.00%), and Whole Blood (WB) in 2 cases (0.67%). This distribution is typical for a tertiary hospital setting managing both medical and surgical patients with anemia.

The central finding of this investigation is the discrepancy between the initial donor antibody screen and the final donor-recipient crossmatch. The results of this comparative analysis are detailed in Figure 3. The initial screening phase, conducted at the central blood unit, yielded a uniform result: all 299 donor units (100%) were determined to be negative for irregular red blood cell antibodies using the automated Column Agglutination Technology. Despite this universal negative screening outcome, the subsequent crossmatching phase revealed a notable number of serological incompatibilities. In the major crossmatch, 4 of the 299 pairs (1.34%) were found to be incompatible. Conversely, 295 pairs (98.66%) were compatible. The minor crossmatch demonstrated a markedly higher frequency of incompatibility. In this test, 21 of the 299 pairs (7.03%) were identified as incompatible, while 278 pairs (92.97%) were compatible. This quantitative data clearly establishes a serological paradox where a final, direct compatibility test uncovers risks not apparent from the preliminary screening step.

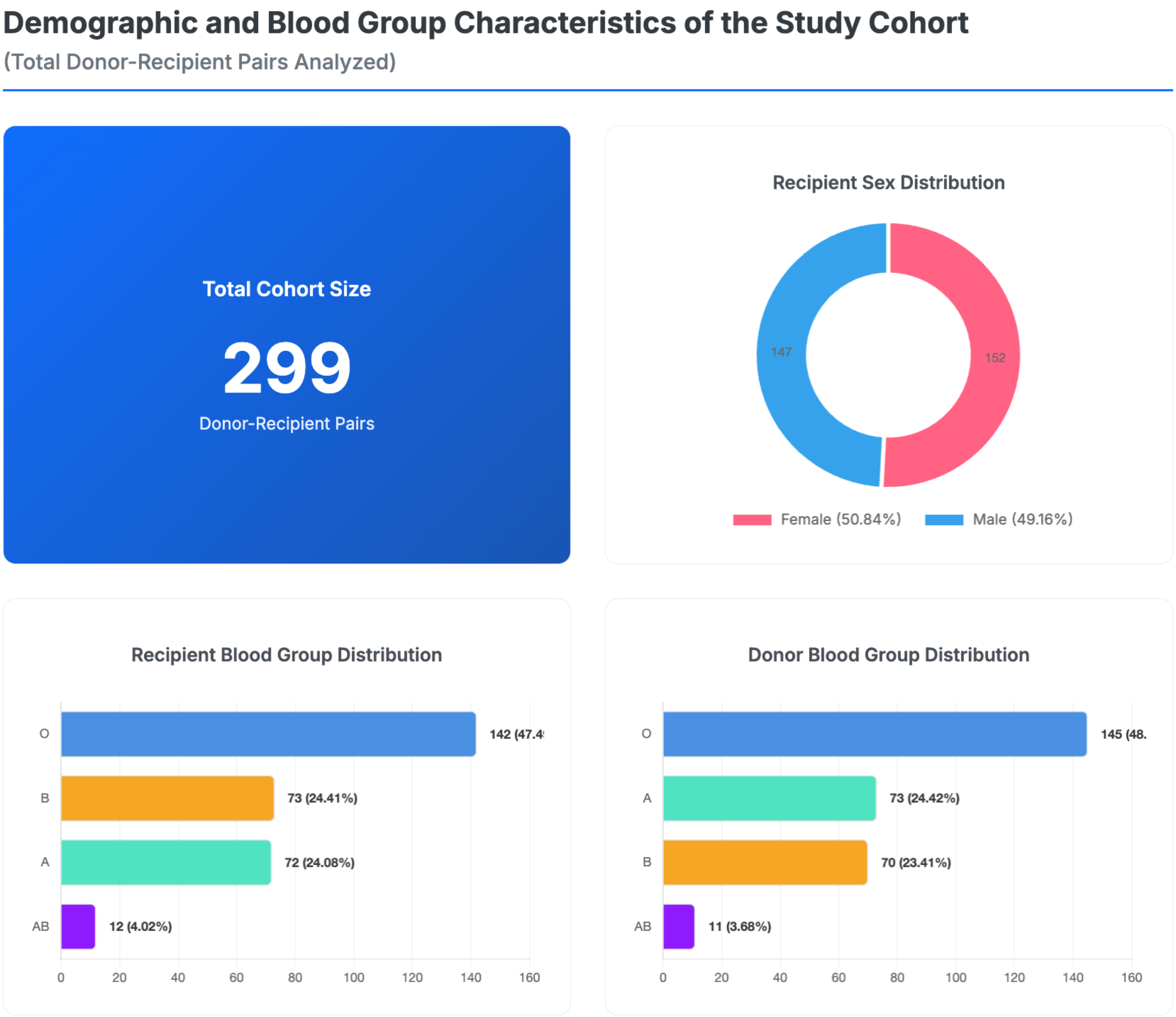

Figure 1. Demographic and blood group characteristics of the study cohort (n=299).

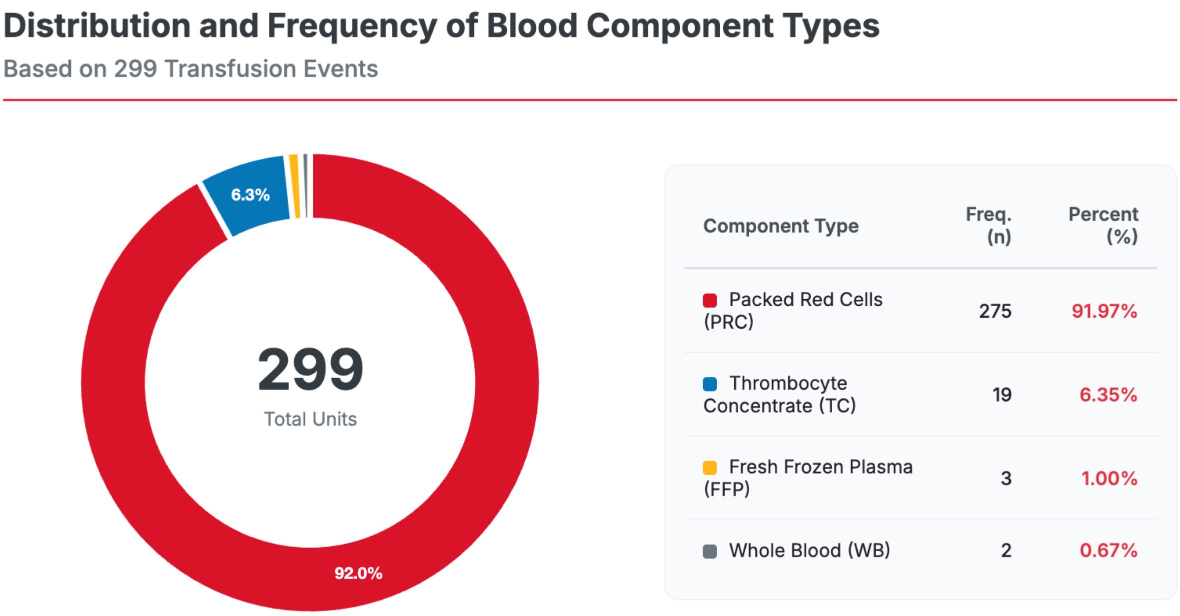

Figure 2. Distribution and frequency of blood component types (n=299).

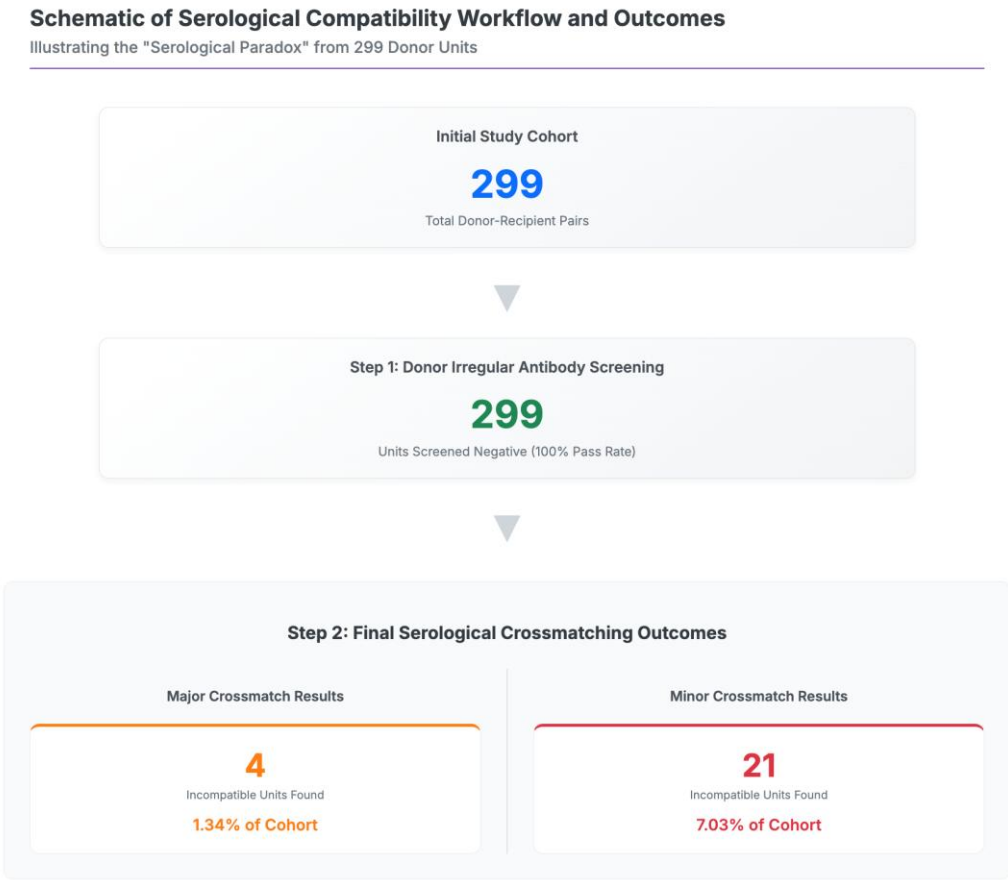

Figure 3. Comparative results of antibody screening and crossmatching (n=299).

This study provides a critical and quantitative evaluation of pre-transfusion testing efficacy in a representative Indonesian tertiary hospital. The central finding is a compelling paradox: while a modern, sensitive antibody screening method cleared 100% of donor units, the definitive crossmatch still identified a significant number of serological incompatibilities—1.34% in the major crossmatch and 7.03% in the minor crossmatch. This discrepancy is not merely a statistical curiosity; it represents a successful "near-miss analysis" where potentially harmful transfusion events were intercepted. It provides a profound insight into the theoretical and pathophysiological limitations of relying on screening alone, especially within specific health systems contexts.[11] Figure 4 provides a comprehensive and detailed conceptual framework that elucidates the core findings of this study, bridging the observed serological results with their underlying pathophysiological mechanisms and immunohematological principles. The figure is meticulously structured into three distinct, yet interconnected, columns, each serving to dissect a crucial aspect of the "screening paradox." This narrative description will unpack each component of the schematic, offering a scholarly interpretation of the visual information in the context of advanced laboratory medicine and clinical transfusion safety. The first column of the figure addresses the most critical finding from a patient safety perspective: the detection of a 1.34% major incompatibility rate. This section visually and conceptually frames this result not as a mere statistic, but as the successful interception of potentially catastrophic clinical events. The schematic begins by illustrating the fundamental immunological conflict of a major incompatibility. The diagram depicts antibodies from the recipient's plasma directly targeting a donor red blood cell. This visual metaphor represents the pre-sensitized state of the recipient, who has developed irregular alloantibodies from a prior exposure event, such as a previous transfusion, pregnancy, or organ transplant. The "ATTACK" label underscores the aggressive and destructive nature of this interaction. Upon transfusion of antigen-positive donor cells into such a recipient, these pre-formed antibodies, primarily of the IgG or IgM class, would immediately bind to the corresponding antigens on the surface of the transfused RBCs. This binding event is the initiating trigger for a cascade of deleterious downstream effects, which the figure categorizes into two primary clinical outcomes. The schematic correctly identifies acute hemolytic transfusion reaction (AHTR) as a rapid and severe event, typically mediated by potent, complement-binding antibodies, most classically IgM but also certain high-titer IgG subclasses (IgG1 and IgG3).[12] The binding of these antibodies to the donor RBCs initiates the classical complement cascade, a powerful component of the innate immune system. This cascade culminates in the formation of the C5b-9 complex, known as the Membrane Attack Complex (MAC). The MAC effectively punctures the RBC membrane, leading to massive and immediate intravascular hemolysis—the explosive destruction of red cells within the circulation. The figure's listed consequences—hemoglobin release, renal failure, shock, and Disseminated Intravascular Coagulation (DIC)—are the direct pathophysiological sequelae of this event. The sudden release of vast quantities of free hemoglobin into the plasma overwhelms its natural scavenger protein, haptoglobin. This unbound hemoglobin is filtered by the kidneys, where it is directly toxic to the renal tubules, causing acute tubular necrosis and subsequent renal failure.[13] Furthermore, free hemoglobin scavenges nitric oxide, a critical endogenous vasodilator, leading to systemic vasoconstriction, severe hypertension, and ischemic organ damage. Simultaneously, the exposed phospholipid membranes of the lysed RBCs are highly prothrombotic, activating the coagulation cascade on a massive scale. This leads to DIC, a paradoxical and life-threatening condition characterized by widespread microvascular thrombosis (leading to further ischemic organ damage) and a consumptive coagulopathy that results in uncontrolled bleeding. The 1.34% major incompatibility rate identified in this study represents the successful prevention of four such potential AHTR events, highlighting the indispensable role of the final crossmatch as a critical safety checkpoint. A delayed hemolytic transfusion reaction (DHTR) is typically mediated by IgG antibodies that are less potent at activating the complement system.[14] In this scenario, the transfused RBCs become opsonized (coated) by these antibodies. Instead of being destroyed within the blood vessels, these antibody-coated cells are recognized as abnormal by the reticuloendothelial system. Macrophages, primarily within the spleen and liver, possess Fc receptors that bind to the IgG-coated RBCs, leading to their phagocytosis and gradual destruction. This process is termed extravascular hemolysis. Clinically, a DHTR manifests insidiously, typically 3 to 14 days following the transfusion. The patient may present with an unexplained fever, falling hemoglobin levels, and jaundice (from the breakdown of heme into bilirubin). While less acutely dramatic than an AHTR, a DHTR completely negates the therapeutic benefit of the transfusion, can cause significant morbidity in already compromised patients, and can further alloimmunize the patient, making future transfusions even more difficult and hazardous. The interception of the four major incompatibilities also prevented these potential DHTR outcomes, preserving the efficacy of the intended therapy. The third column of the figure is dedicated to the study's most provocative finding—the high 7.03% rate of minor incompatibility. This section serves to educate the reader on why this often-dismissed finding carries significant, tangible clinical weight. The schematic correctly reverses the immunological conflict. Here, it is the antibodies present within the residual plasma of the donor blood component that recognize and bind to antigens on the recipient's own RBCs. While modern component therapy, particularly the use of Packed Red Cells, minimizes the volume of transfused plasma, the argument that the donor antibodies are simply diluted to insignificance is an oversimplification that this study's data challenge.[15] This panel details the nuanced but important clinical risks associated with transfusing a minor-incompatible unit. Reduced Transfusion Efficacy, this is perhaps the most overlooked consequence. The binding of donor antibodies to recipient RBCs, even at low levels, can opsonize them for premature clearance. This can lead to a mild, compensated hemolysis that results in a blunted or negligible rise in the patient's hemoglobin level, thereby defeating the primary purpose of the transfusion. Positive Direct Antiglobulin Test (DAT), the coating of the recipient's RBCs with donor antibodies will result in a positive DAT post-transfusion. This can create significant diagnostic confusion for the clinical and laboratory teams, potentially mimicking an autoimmune hemolytic anemia or complicating the investigation of a suspected delayed transfusion reaction. It also makes future pre-transfusion testing for that patient more complex and time-consuming. Risk in Massive Transfusion, in trauma or major surgery, a patient may receive numerous units of blood. The cumulative dose of an irregular antibody from multiple "minor-incompatible" units can reach a clinically significant concentration, sufficient to cause overt hemolysis of the recipient's cells. High Risk in Pediatrics, the dilution argument is weakest and most dangerous in neonatal and pediatric patients. Due to their very small total blood volume, the plasma from a single PRC unit is not insignificant. A minor incompatibility in this population can lead to severe hemolysis, dangerous hyperbilirubinemia, and the need for exchange transfusion.[16] The 7.03% finding underscores a significant and often underestimated risk to the most vulnerable patient populations. The central column of the figure is the conceptual core, dedicated to answering the study's primary question: Why does the screening test fail where the crossmatch succeeds? This section visually deconstructs the complex immunohematological principles that create the "serological paradox." This panel provides an elegant visual explanation of one of the most important concepts in immunohematology. Many blood group antigens are products of codominant alleles, meaning an individual can be homozygous (possessing two copies of the same allele) or heterozygous (possessing two different alleles) for a given gene. RBCs from a homozygous individual express a "double dose" of the antigen on their surface. The schematic perfectly illustrates this, showing a "Screening Cell" with a single blue antigen marker (representing a heterozygous state) and a "Crossmatch Cell" with two blue markers (representing a homozygous state). Many alloantibodies are known to exhibit dosage; they react strongly with homozygous cells but may react very weakly, or not at all, with heterozygous cells. Commercial screening cells are often sourced from heterozygous individuals to maximize the number of antigens on a single cell. The diagram shows a "Weak Antibody" failing to react with the heterozygous screening cell, resulting in a false-negative screen. However, when this same weak antibody encounters the homozygous cell in the direct crossmatch, the higher antigen density allows for a stable antigen-antibody lattice to form, producing a detectable positive reaction. This is a classic and common cause of screen-negative, crossmatch-positive events. Low-Prevalence Antigens (LPA) are rare antigens present in less than 1% of the population. It is impractical and economically unfeasible for commercial screening panels to include cells positive for the hundreds of known LPAs. The diagram shows a "Screening Panel" of three RBCs, none of which possess the rare triangular antigen. A recipient may have formed an antibody against this LPA from a prior transfusion. Their antibody screen will always be negative because the screening cells lack the target antigen. However, the schematic then shows a "Donor Cell" which, by chance, possesses this rare triangular LPA. When the recipient's anti-LPA antibody is mixed with this donor cell in the crossmatch, a strong positive reaction occurs. This elegantly demonstrates how a major incompatibility can suddenly appear in a patient with a long history of negative antibody screens, a scenario that only the final, direct biological crossmatch is capable of detecting. An antibody's concentration in plasma is referred to as its titer.[17] It is entirely possible for a patient to have a clinically significant antibody at a very low titer, below the lower limit of detection of the standardized screening assay. The optimized conditions of the crossmatch—which involves a direct mixture of patient serum and donor cells, often with ideal incubation times and enhancement media—can provide a more sensitive environment for this weak antibody to bind and produce a detectable agglutination reaction. This cause underscores that even with the most advanced screening technologies, a residual risk from very weak but potentially harmful antibodies will always remain, necessitating the final safety check of the crossmatch. Figure 4 is not merely a depiction of results but a sophisticated educational tool. It masterfully translates complex scientific data and theory into an accessible visual narrative, making a powerful and unequivocal case for the continued, indispensable role of the serological crossmatch in ensuring the safety of every blood transfusion.[18]

The 1.34% major incompatibility rate represents the prevention of four potentially catastrophic transfusion events. A major incompatibility signifies the presence of recipient alloantibodies targeting antigens on the donor's red blood cells. Had these units been transfused, the clinical outcome would depend on the class and thermal range of the responsible antibody. If the antibody were a potent, complement-binding IgM or a high-titer IgG, the patient would be at high risk for an Acute Hemolytic Transfusion Reaction (AHTR). The pathophysiology of AHTR is a rapid and destructive cascade. Upon infusion, recipient antibodies bind to donor RBCs, activating the classical complement pathway and forming the C5b-9 Membrane Attack Complex (MAC).[19] The MAC causes massive, immediate intravascular hemolysis, releasing free hemoglobin that overwhelms haptoglobin. This free hemoglobin is nephrotoxic, scavenges nitric oxide (causing vasoconstriction), and the exposed RBC stroma triggers Disseminated Intravascular Coagulation (DIC). Clinically, the patient would present within minutes with fever, back pain, hemoglobinuria, and hypotension, potentially progressing to shock and death.

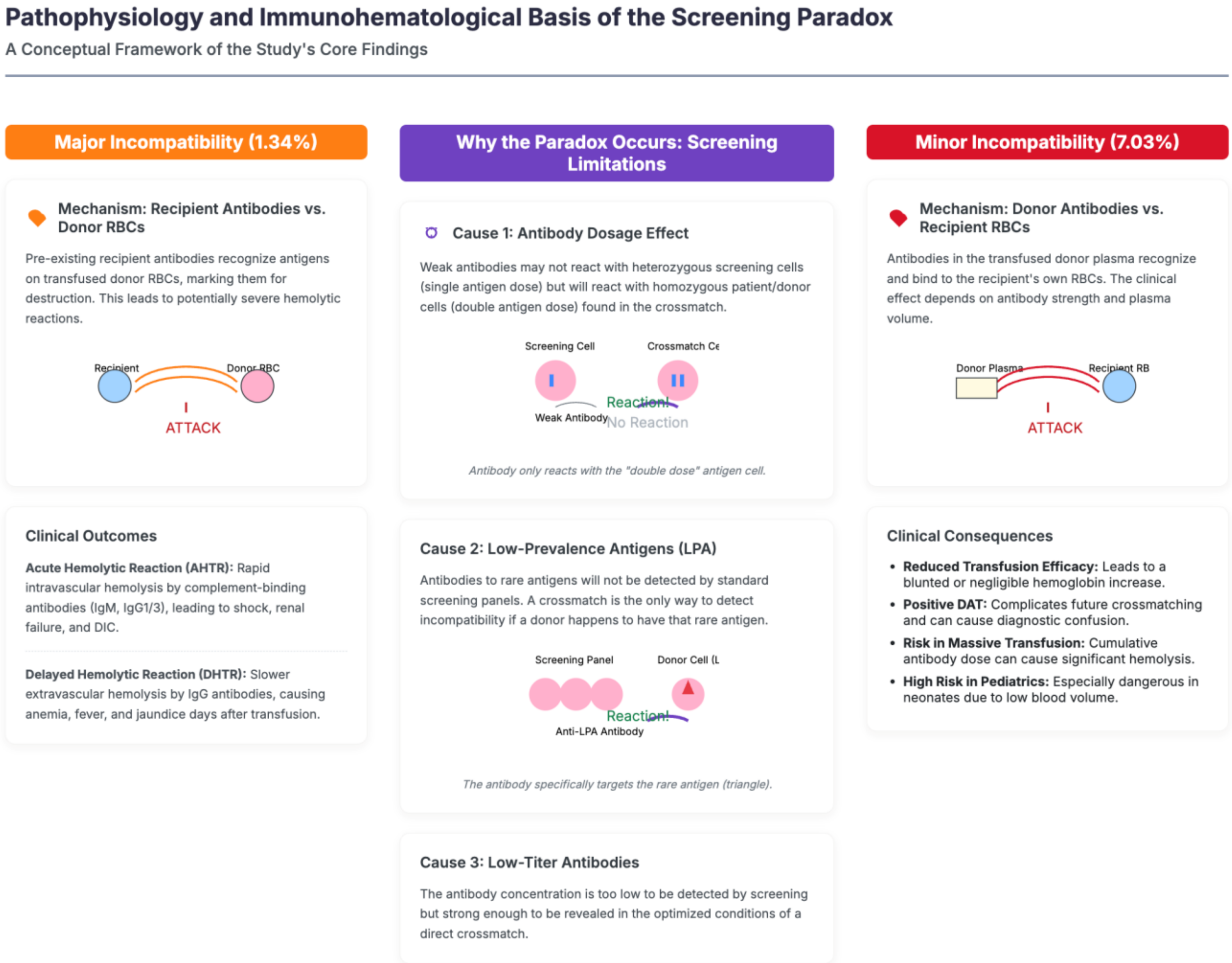

Figure 4. Pathophysiology and immunohematological basis of the screening paradox.

By identifying these four cases, the crossmatch served as the final, critical firewall preventing this devastating outcome. Alternatively, if the antibody were a non-complement-binding IgG, the likely result would be a Delayed Hemolytic Transfusion Reaction (DHTR). In this scenario, opsonized RBCs are cleared by macrophages in the spleen and liver via extravascular hemolysis. This typically occurs 3 to 14 days post-transfusion, manifesting as an unexplained drop in hemoglobin, fever, and jaundice. A DHTR negates the benefit of the transfusion and can cause significant morbidity. The interception of these four cases, therefore prevented either immediate, life-threatening hemolysis or a delayed destruction of the transfused cells. The observed major incompatibility rate of 1.34% is comparable to studies in other resource-limited regions, which report rates that underscore the persistent risk of alloimmunization that bypasses initial screening. The finding of a 7.03% minor incompatibility rate is the most provocative result of this study. This finding, suggesting alloantibodies in the donor plasma, directly challenges the adequacy of donor screening and the widespread assumption that minor incompatibilities are clinically benign due to dilution. While the plasma volume in PRC units is small, our data compel a more cautious interpretation. From a clinical perspective, these 21 intercepted events represent the prevention of diagnostic confusion and suboptimal therapeutic outcomes. A patient receiving a minor-incompatible unit could develop a low-grade fever, mild jaundice, or a blunted hemoglobin response. A clinician at the bedside might easily attribute these subtle signs to the patient's underlying illness. This study demonstrates that in a significant number of cases, an iatrogenic, immunological cause is a real possibility, a crucial consideration for the clinician's differential diagnosis. The pathophysiological risk, while often subtle, is tangible. Even diluted donor antibodies can lead to a positive DAT and low-grade hemolysis. In scenarios of massive transfusion, the cumulative antibody dose from multiple units can become significant. The risk is magnified exponentially with high-plasma-volume components like FFP or platelets. Most critically, in vulnerable pediatric patients, the dilution argument is weakest, and a minor incompatibility can lead to severe hemolysis. Therefore, the 7.03% rate is not a trivial finding; it is a key patient safety indicator. The routine performance of the minor crossmatch in this setting successfully prevented these 21 units from being transfused, protecting patients from potential transfusion inefficacy and other adverse outcomes.[20]

The discrepancy at the heart of this study can be explained by several established immunohematological principles. All laboratory tests have a lower limit of detection. A low-titer alloantibody may be insufficient to produce a visible reaction with standardized screening cells but may be detected in the direct crossmatch procedure. The Phenomenon of Dosage is a critical concept in immunohematology and a likely major contributor to our findings. Many blood group antigens (in the Rh, Duffy, Kidd, MNS systems) are encoded by codominant alleles. Red cells from a homozygous individual express a "double dose" of the antigen compared to a heterozygous individual. Many alloantibodies exhibit dosage, reacting weakly or not at all with heterozygous cells. As commercial screening cells are often heterozygous, it is highly plausible that a weak antibody was non-reactive with screening cells but produced a clear positive reaction when it encountered homozygous cells in the crossmatch. This is a classic "hidden" incompatibility that only a direct crossmatch can reveal. Antibodies to Low-Prevalence Antigens (LPAs), present in <1% of the population, will not be detected by screening panels that lack these antigens. An incompatibility will only appear if a sensitized patient is crossmatched against a donor who happens to carry the same LPA. Conversely, a donor with a rare null phenotype may have an antibody to a High-Prevalence Antigen (HPA). This antibody will cause a minor incompatibility when crossmatched against a recipient who, like most of the population, is positive for the HPA. The prevalence of these specific antigens and their null phenotypes may be unique to the Indonesian and broader Southeast Asian populations, highlighting the importance of local data.[17,18]

This study's findings extend beyond the laboratory bench to crucial health systems and economic considerations. The observed 8.37% total incompatibility rate (major + minor) has significant operational implications for the hospital blood bank. It means that for nearly one in twelve blood requests, the transfusion process is delayed. This requires additional technologist time to find an alternative compatible unit, consumes more reagents, and can impact clinical urgency. Furthermore, these results provide a powerful health economics argument for the "cost of safety." While performing 299 crossmatches to prevent 25 incompatible transfusions seems resource-intensive, the cost of managing a single severe AHTR—including intensive care, dialysis, and extended hospitalization—is exponentially higher. This study provides the quantitative evidence to justify the continued allocation of resources for comprehensive crossmatching as a highly cost-effective, life-saving intervention. Based on these findings, it is recommended that Indonesian national transfusion guidelines explicitly state that electronic or abbreviated crossmatching is not appropriate. A pilot program for extended antigen matching (at a minimum for Rh and K antigens) for all chronically transfused patients, such as those with thalassemia, should also be considered to proactively reduce alloimmunization rates.

4. Conclusion

This study successfully illuminates a critical paradox in modern pre-transfusion testing within a developing country context. Despite the use of sensitive, automated Gel Test technology that resulted in 100% negative irregular antibody screens in blood donors, a final crossmatch still detected clinically significant incompatibilities in 1.34% of major tests and 7.03% of minor tests. In conclusion, this study quantitatively demonstrates the immense value of the final serological crossmatch as a critical safety procedure. It serves not as a redundant step, but as an active and essential process of vigilance that successfully intercepts tangible risks to patients. These findings unequivocally reaffirm that the serological crossmatch is an indispensable and life-saving final step in the transfusion workflow that cannot be safely omitted or replaced by antibody screening alone. The results provide compelling, local evidence to support the mandatory continuation of both major and minor crossmatching in Indonesia and highlight the urgent need to strengthen the national hemovigilance system to continually enhance the safety and quality of blood transfusion services for all patients.

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