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Beyond Routine Typing: Evaluation of Decade-Long Retrospective Analysis of ABO and Rh Blood Group Discrepancies in a High-Volume National Reference Laboratory

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Introduction

The ABO and Rh blood group systems constitute the foundation of modern transfusion medicine and remain the most frequently performed investigation in immunohematology laboratories worldwide. Accurate blood group determination is indispensable for ensuring safe blood transfusion practice, organ transplantation compatibility, antenatal monitoring, and emergency clinical interventions. Any inconsistency between forward and reverse blood grouping or unexpected Rh typing reactions poses significant diagnostic and operational challenges requiring prompt investigation and resolution.

The ABO blood group system, first described by Karl Landsteiner [4] revolutionized transfusion medicine by establishing the immunological basis of blood compatibility. Determination of the ABO blood group involves two complementary procedures ie- forward grouping which identifies A and B antigens on red blood cells and reverse grouping which detects corresponding naturally occurring antibodies in plasma or serum. Concordance between these two reactions forms the basis of accurate ABO assignment. Similarly, Rh blood grouping, particularly Rh D antigen determination, is critical because of its strong immunogenicity and major role in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn.

Blood group discrepancies occur when unexpected or inconsistent serological reactions are observed during routine ABO or Rh typing. These discrepancies may arise due to weak or missing antibodies, diminished antigen expression, plasma protein abnormalities, autoantibodies, alloantibodies, recent transfusion, pregnancy, hematological disorders, or technical factors. Traditionally, ABO discrepancies are classified into four major groups.

Group I discrepancies involving weak or missing antibodies affecting reverse grouping.

Group II discrepancies involving weak or missing antigens affecting forward grouping.

Group III discrepancies resulting from plasma or protein abnormalities causing pseudo agglutination.

Group IV discrepancies which encompasses miscellaneous causes including unexpected antibodies and rare phenotypes. Rh discrepancies constitute a distinct category commonly related to weak D or partial D antigen expression. [3]

Failure to recognize and resolve these discrepancies may lead to erroneous blood group assignment, delayed transfusion support and potentially severe transfusion-related complications. Consequently, every discrepancy demands careful serological evaluation before final blood group reporting. Standard serological investigations such as repeat testing, lectin studies, antibody screening, antiglobulin testing and advanced confirmatory techniques play an essential role in discrepancy resolution.

Recent decades have witnessed increasing implementation of laboratory automation in immunohematology. Automated blood grouping systems have significantly improved standardization, sensitivity, and turnaround time while minimizing manual and clerical errors. Erythrocytes Magnetized Technology (EMT) based systems including Qwalys platforms, provide enhanced detection of weak or atypical reactions and have expanded the capability of high-volume laboratories to process large numbers of samples efficiently. However, enhanced sensitivity is frequently accompanied by increased detection of atypical or weak serological reactions, thereby necessitating specialized expertise for discrepancy interpretation and resolution.[8]

The reported frequency of ABO blood group discrepancies varies considerably across different populations and laboratory settings, ranging from 0.02% to 0.28% in published Indian and international studies. The interpretation of discrepancy frequency should be made in the context of the study population. Most previously published studies have been conducted on healthy blood donor populations, whereas the present study was performed exclusively on patient samples referred to for routine and specialized immunohematology investigations. Patient populations are inherently more likely to exhibit underlying disease conditions, recent transfusion exposure, pregnancy-related immunological alterations, hematological disorders, autoimmune conditions, weak antigen expression, and unexpected antibodies, all of which may increase the likelihood of serological discrepancies.

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Aim and Objectives

The present retrospective study was therefore undertaken to evaluate the frequency, spectrum, and serological characteristics of ABO and Rh blood group discrepancies encountered over a decade. The study was carried out at the National Reference Laboratory of Dr Lal PathLabs, Rohini, New Delhi, India, NABL and CAP-accredited laboratory employing automated Erythrocytes Magnetized Technology. To the best of our knowledge, this study represents one of the largest standalone laboratory-based analyses of serological blood group discrepancies from India and provides valuable insights into discrepancy patterns encountered in contemporary automated immunohematology practice.

Materials and Methods

Study Design

This retrospective observational laboratory-based study was conducted at National Reference lab, Dr Lal Path labs in the Department of Hematology and Immunopathology , a high-volume standalone National Reference Laboratory located in North India and accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL) and the College of American Pathologists (CAP). The laboratory functions as a referral center for immunohematology investigations and performs large-scale blood grouping and discrepancy resolution services.

Study Duration

The study included samples processed over a 10-year period from 1 July 2016 to 30 April 2026.

Study Population and Sample Size

A total of 3,00,000 blood grouping samples received for routine immunohematology evaluation during the study period were retrospectively reviewed. Samples were received from diverse clinical settings including outpatient, inpatient, antenatal, pre-transfusion, and referral laboratory services.

Only samples demonstrating serological discrepancies during routine ABO and/or Rh blood grouping were included for detailed analysis.

Inclusion Criteria

The following samples were included in the study: -

1. Samples demonstrate serological discrepancies during routine ABO and/or Rh blood grouping.
2. Cases requiring additional immunohematology workup for final blood group interpretation.
3. Samples with complete laboratory records and discrepancy resolution data.

Exclusion Criteria

The following samples were excluded: -

1. Hemolyzed samples or specimens showing inadequate sample quality.
2. Improperly labelled or contaminated samples.
3. Samples with insufficient quantity for complete discrepancy workup.
4. Cases with incomplete serological or laboratory documentation.

Routine ABO and Rh blood grouping was performed using a fully automated immunohematology analyzer based on Erythrocytes Magnetized Technology (EMT), Qwalys 3 (Diagast, France).

The EMT principle utilizes magnetized erythrocytes and automated reaction interpretation for simultaneous forward and reverse blood grouping, thereby enabling enhanced standardization, improved sensitivity, and reduced operator dependent variability. Routine testing incorporated both ABO forward grouping and reverse grouping along with RhD typing according to manufacturer instructions and laboratory standard operating procedures.

All testing procedures were performed under established internal and external quality assurance protocols in compliance with NABL and CAP accreditation requirements.

Samples demonstrating unexpected or discordant reactions during automated blood grouping were flagged by the analyzer and subjected to comprehensive serological evaluation.

Initial discrepancy resolution included:

- Verification of patient/sample identification
- Review of clinical and laboratory history whenever available
- Repeat ABO and Rh typing using the same sample
- Confirmation using conventional tube technique (CTT)

Conventional tube testing was employed whenever automated results remained inconclusive or demonstrated weak, mixed-field, or atypical reaction patterns.

Further discrepancy workup was performed according to the suspected underlying serological mechanism.

Additional Serological Investigations

Depending upon the nature of discrepancy encountered, one or more of the following investigations were performed:

1. Repeat ABO and Rh typing: Repeat testing was performed to exclude technical or clerical causes and confirm reproducibility of reactions.
2. Direct Antiglobulin Test (DAT): DAT was performed to detect in vivo sensitization of red blood cells with immunoglobulin and/or complement, particularly in cases showing spontaneous agglutination, mixed-field reactions, or suspected autoimmune etiology.
3. Auto control: Auto control testing was utilized to evaluate the possibility of autoantibody-mediated reactions.
4. Antibody Screening: Antibody screening was performed in samples showing unexpected reverse grouping reactions or suspected alloantibody interference.
5. Anti-A1 Lectin Testing: Anti-A1 lectin testing was used in suspected subgroup A discrepancies to differentiate A1 from non-A1 phenotypes.

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6. Anti-H Lectin Testing: Anti-H lectin testing was performed in cases suspected of weak A/B subgroups, Bombay phenotype, or Para Bombay phenotype.
7. Saline Replacement Technique: Samples demonstrating rouleaux or pseudo agglutination were evaluated using saline replacement technique to distinguish true agglutination from plasma protein-related interference.
8. Weak D Testing: Samples exhibiting weak or inconclusive RhD reactions underwent weak D testing using antiglobulin phase techniques according to standard immunohematology practices.

Blood group discrepancies were categorized according to established immunohematology classification systems: -

Group I discrepancies involved weak or missing antibodies affecting reverse grouping and resulting in absent or reduced expected serum reactions.

Group II discrepancies involved weak or missing antigens affecting forward grouping, frequently associated with subgroup phenotypes or suppressed antigen expression.

Group III discrepancies resulted from plasma or protein abnormalities producing pseudo agglutination or rouleaux formation.

Group IV discrepancies included miscellaneous causes such as unexpected antibodies, polyagglutination, Bombay phenotype, Para Bombay phenotype, and other atypical serological findings.

Rh discrepancies were analyzed separately and primarily included weak D and variant RhD antigen expression patterns.

Data Collection and Statistical Analysis—

Laboratory records and discrepancy resolution data maintained during the study period were retrospectively reviewed.

Variables collected included:

- Total number of blood grouping samples
- Type of discrepancy
- Frequency distribution
- Serological findings
- Presence of rare phenotypes
- Demographic observations whenever available

Data compilation and analysis were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics including frequencies and percentages were utilized to summarize discrepancy patterns and evaluate year-wise trends over the study period. The overall discrepancy frequency was calculated as the proportion of discrepant samples among the total blood grouping samples processed, with a 95% confidence interval (CI) determined via the binomial proportion method. Between 1 July 2016 and 30 April 2026, the reference laboratory processed a total of 300,000 blood grouping samples. Among these 1,505 samples demonstrated serological discrepancies, yielding an overall discrepancy frequency of 0.50% (95% CI: 0.48%–0.53%). Year-wise analysis revealed a progressive increase in discrepancy detection over the study period, peaking in 2024 with 241 cases. This upward trend likely reflects an expanding overall sample volume, improved recognition of atypical serological reactions, and the enhanced analytical sensitivity of automated Erythrocytes Magnetized Technology.

Ethical Considerations

The study utilized retrospective laboratory data without direct patient intervention. Patient confidentiality and anonymity were maintained throughout the study in accordance with institutional ethical standards and laboratory data protection policies.

Results

During the study period from 1 July 2016 to 30 April 2026, a total of 3,00,000 blood grouping samples were processed in the reference laboratory. Among these 1,505 samples demonstrated serological discrepancies during routine ABO and/or Rh blood grouping, yielding an overall discrepancy frequency of 0.50% (95% CI: 0.48%–0.53%).

The discrepancies comprised a wide spectrum of serological findings, including weak antigenic Ex Statistical Analysis Sections, subgroup identification, RhD antigen variability, weak or unexpected antibodies, and rare phenotypes. The overall distribution is summarized in (Table 1).

Group-wise Classification of Discrepancies

For detailed analysis, discrepancies were categorized into Group I, II, III, IV, and Rh system discrepancies according to standard immunohematology classification (Table 2).

Group II discrepancies were the predominant category, accounting for 763 cases (50.70%), mainly due to subgroup of A (601 cases, 39.93%) and weak antigen expression (93 cases, 6.18%).

Rh system discrepancies represented the second largest category with

Table 1: Overall Distribution of Blood Group Discrepancies

Discrepancy Type	Total Cases (n)	Percentage (%)
Subgroup of A	601	39.93
RhD Antigen Variability	434	28.84
Weak Antibodies	123	8.17
Unexpected Antibodies	102	6.78
Weak Antigen	93	6.18
Polyagglutination	45	2.99
Subgroup of B	35	2.33
Subgroup of A/Newborn	28	1.86
Polyagglutination/Newborn	15	1.00
Bombay Phenotype	14	0.93
Weak Antigen/Newborn	6	0.40
Rouleaux Formation	4	0.27
RhD Antigen/Newborn	4	0.27
Para Bombay Phenotype	1	0.07
Total	1,505	100.00

Subgroup of A was the most frequent discrepancy, accounting for 601 cases (39.93%), followed by RhD antigen variability in 434 cases (28.84%). Weak antibodies and unexpected antibodies constituted 123 (8.17%) and 102 (6.78%) cases, respectively. Rare findings included Bombay phenotype (14 cases, 0.93%), Para Bombay phenotype (1 case, 0.07%), and rouleaux formation (4 cases, 0.27%).

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Table 2: Group-wise Classification of Blood Group Discrepancies

Group Category	Specific Discrepancy	Cases (n)	Percentage (%)
Group I	Weak Antibodies	123	8.17
Group II	Subgroup of A	601	39.93
	Weak Antigen	93	6.18
	Subgroup of B	35	2.33
	Subgroup of A/Newborn	28	1.86
	Weak Antigen/Newborn	6	0.40
Group III	Rouleaux Formation	4	0.27
Group IV	Unexpected Antibodies	102	6.78
	Polyagglutination	45	2.99
	Polyagglutination/Newborn	15	1.00
	Bombay Phenotype	14	0.93
	Para Bombay Phenotype	1	0.07
Rh System	RhD Antigen variability and weak D	434	28.84
	RhD Antigen/Newborn	4	0.27
Total		1,505	100.00

438 cases (29.10%), predominantly involving RhD antigen variability and weak D expression.

Group IV discrepancies accounted for 177 cases (11.76%) and included unexpected antibodies, polyagglutination, and rare blood group phenotypes.

Group I discrepancies involving weak antibodies comprised 123 cases (8.17%), while Group III discrepancies due to rouleaux formation were infrequent (4 cases, 0.27%).

Among Group IV findings, unexpected antibodies were the most common (102 cases, 6.78%), followed by Polyagglutination (45 cases, 2.99%) and newborn-associated polyagglutination (15 cases, 1.00%). Rare phenotypes identified included Bombay phenotype in 14 cases (0.93%) and Para Bombay phenotype in one case (0.07%).

A total of 53 discrepant samples belonged to newborns, including cases involving subgroup A, weak antigen expression, polyagglutination, and RhD discrepancies.

Overall, weak or altered antigen expression and RhD variability constituted the major proportion of discrepancies encountered. Combined, Group II and Rh system discrepancies accounted for approximately 79.8% of the total discrepancy burden.

Year-wise Trend Analysis

Year-wise analysis demonstrated a progressive increase in discrepancy detection throughout the study period. The annual discrepancy workload increased from 25 cases in 2016 to a peak of 241 cases in 2024. Subgroup of A remained the predominant discrepancy category across most years, while RhD antigen variability demonstrated a marked increase from 2023 onwards, accounting for 56, 103, and 106 cases in 2023, 2024, and 2025, respectively. The increasing trend may be attributed to enhanced referral of complex immunohematology cases improved automation sensitivity and growing awareness of discrepancy resolution protocols in routine laboratory practice.

Discussion

Blood group discrepancies remain a significant diagnostic challenge in immunohematology laboratories and require accurate identification to ensure transfusion safety. Despite advances in laboratory automation, discrepant serological reactions continue to occur and demand careful interpretation. Accurate resolution of these discrepancies is essential to prevent transfusion-related complications and ensure appropriate blood component selection. [3,4]

In this decade-long study of 3,00,000 blood grouping samples, 1,505 discrepant cases were identified, yielding an overall discrepancy frequency of 0.5%. This represents one of the largest laboratory-based analyses of ABO and Rh discrepancies from North India and provides important insight into discrepancy patterns encountered in a high-volume automated referral setting.

The discrepancy frequency observed in the present study (0.50%) was higher than that reported in several donor-based studies. This difference may be attributed to important variations in study population and laboratory setting. Most previously published studies have been conducted on healthy blood donors, whereas the present study exclusively evaluated patient samples referred for routine and specialized immunohematology investigations. Patient populations are inherently more likely to demonstrate underlying clinical conditions, recent transfusion exposure, pregnancy-related immunological alterations, hematological disorders, autoimmune conditions, weak antigen expression, and unexpected antibodies, all of which may increase the likelihood of serological discrepancies. In addition, the referral nature of a National Reference Laboratory and the use of highly sensitive Erythrocytes Magnetized Technology-based automation may further contribute to enhanced discrepancy detection. Sahu et al. [1] reported an overall discrepancy prevalence of 0.12% among blood donors from Eastern India, while Bhuva et al. [2] reported a prevalence of 0.28% in a donor population from Gujarat. Sharma et al. [5] documented discrepancies in only 0.04% of blood donors at a regional transfusion center in Delhi, and Shah Shahani et al. [9] similarly reported a frequency of 0.04% among 322,222 donations in Iran. Makroo et al. [10] observed discrepancy rates of 0.1% among patients and 0.02% among blood donors in a tertiary care setting. The comparatively higher discrepancy burden observed in the present study may be attributed to the referral nature of the laboratory, where complex serological cases are frequently received for discrepancy resolution rather than routine donor screening alone.

Group II discrepancies constituted the predominant category (50.70%), primarily due to subgroup A phenotypes and weak antigen expression, with subgroup A accounting for approximately 40% of all discrepancies. This finding differs from several donor-based studies in which reverse grouping discrepancies predominated. Sharma et al. [5] reported low-avidity anti-B antibodies as the most common cause of discrepancy, while Makroo et al. [10] identified cold autoantibodies as the leading contributor in both patients and donors. Similarly, Shah Shahani et al. [9] observed subgroups of An antigen as the major cause of forward grouping discrepancies but reported cold autoantibodies as the predominant reverse grouping abnormality. Such differences likely reflect variations in study populations, referral patterns, and laboratory methodologies.

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Table 3: Year-wise Distribution of Blood Group Discrepancies (2016–2026)

Discrepancy Type	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026*	Total
Subgroup of A	6	26	29	59	58	81	66	82	79	82	33	601
RhD Antigen Variability	12	23	21	19	19	17	17	56	103	106	41	434
Weak Antibodies	4	11	16	13	9	9	16	10	15	15	5	123
Unexpected Antibodies	3	11	16	33	11	5	4	4	14	0	1	102
Weak Antigen	0	1	0	3	6	5	9	40	19	7	3	93
Polyagglutination	0	2	2	13	12	3	2	1	4	3	3	45
Subgroup of B	0	0	0	3	5	4	6	9	3	4	1	35
Subgroup of A/Newborn	0	1	0	6	11	6	1	0	1	2	0	28
Polyagglutination/Newborn	0	0	2	2	4	5	0	0	1	0	1	15
Bombay Phenotype	0	0	0	1	2	1	1	3	1	3	2	14
Weak Antigen/Newborn	0	0	0	0	2	1	0	2	0	1	0	6
Rouleaux Formation	0	1	0	2	1	0	0	0	0	0	0	4
RhD Antigen/Newborn	0	0	0	0	0	1	0	0	1	1	1	4
Para Bombay Phenotype	0	0	0	0	0	0	1	0	0	0	0	1
Total	**25**	**76**	**86**	**154**	**140**	**138**	**123**	**207**	**241**	**224**	**91**	**1505**

The predominance of Group II discrepancies in the present study may be explained by several factors. Highly sensitive automated platforms, particularly EMT-based systems, can detect subtle antigenic variations that may be overlooked during conventional testing. In addition, referral laboratories inherently receive a higher proportion of unresolved forward grouping reactions and subgroup evaluations. Geographic and ethnic variability may also contribute to differences in subgroup frequencies. [7,8]

Subgroups of A formed the largest discrepancy subtype in the present study and remain clinically important because they may produce discordant forward and reverse grouping reactions, potentially resulting in inappropriate blood selection if not accurately recognized. Similar observations have been reported by Sharma et al. [5], Shah Shahani et al. [9] and Sahu et al [1]. Although at substantially lower frequencies than observed in the current study. The large burden of subgroup related discrepancies identified in this study emphasizes the importance of supplementary serological tools such as anti-A1 lectin and anti-H lectin testing for discrepancy resolution. [3,4]

Rh discrepancies represented the second largest category (29.11%), largely involving RhD antigen variability and weak D expression. RhD heterogeneity remains an important challenge in transfusion medicine because conventional typing methods may fail to detect weak D phenotypes, whereas highly sensitive automated systems are more likely to identify atypical reaction patterns requiring confirmatory workup.[6,11,12]The significant Rh discrepancy burden observed in this study likely reflects the enhanced sensitivity of EMT based testing and emphasizes the continuing need for expert interpretation and confirmatory serological analysis.

Year-wise evaluation revealed a notable temporal shift in discrepancy patterns. While Subgroup of A remained consistently prevalent throughout the study period, RhD antigen variability demonstrated a substantial increase during the latter years of the study, particularly between 2023 and 2025. This observation may reflect increasing referrals for complex Rh investigations, enhanced detection of weak and variant RhD phenotypes through automated EMT-based platforms, and greater recognition of Rh-related serological discrepancies in contemporary immunohematology practice.

Group I discrepancies accounted for 8.17% of cases and were mainly related to weak or missing antibodies affecting reverse grouping, particularly among newborns, elderly individuals, and immuno-compromised patients. The identification of discrepant newborn samples in the present study supports this classical serological understanding. [3,4] Group III discrepancies were uncommon (0.27%) and resulted from rouleaux formation secondary to plasma protein abnormalities. Although rare, such discrepancies remain clinically significant because pseudo agglutination may mimic true antigen-antibody reactions and result in erroneous blood grouping if not recognized.

Group IV discrepancies constituted 11.77% of cases and included unexpected antibodies, polyagglutination, and rare phenotypes. Unexpected antibodies represent the largest Group IV component and are clinically important because of their impact on transfusion compatibility and blood selection. Sharma et al [5]. and Makroo et al. [1] similarly documented unexpected alloantibodies and cold reacting antibodies contributing to reverse grouping discrepancies.

The identification of rare phenotypes, including Bombay and Para Bombay groups, represents another noteworthy finding of the present study. Bombay phenotype lacks H antigen expression and may be misdiagnosed as group O unless specifically investigated using anti-H reagents, potentially leading to fatal hemolytic transfusion reactions if the patient is given standard O type blood. [3,13]. Similar findings were reported by Sharma et al. [5] and Makroo et al. [10], who also identified rare blood group phenotypes during discrepancy workup. The detection of 14 Bombay phenotypes and one Para Bombay case in the present study reinforces the need for comprehensive discrepancy resolution protocols even in highly automated laboratory settings.

The present study highlights an important transition in modern immunohematology laboratories. Automation does not eliminate blood group discrepancies; rather, it improves their recognition and early detection. Consequently, laboratory expertise, confirmatory serological methods, and standardized discrepancy resolution algorithms remain indispensable. [4,8]

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Strengths of the Study

The present study possesses several important strengths.

First, the large sample size of 3,00,000 blood grouping samples and decade-long study duration provide robust epidemiological and serological data.

Second, unlike many previously published donor-based studies, the present work represents a high-volume laboratory-based discrepancy analysis involving both ABO and Rh discrepancies.

Third, the use of EMT-based automated blood grouping provides contemporary relevance and reflects current laboratory practices.

Finally, inclusion of rare phenotypes and Rh discrepancies provides a broader perspective of discrepancy burden encountered in referral immunohematology laboratories.

Limitations

The present study has certain limitations.

Being retrospective in design, analysis depended upon available laboratory records and limited clinical information.

Molecular characterization of subgroup and Rh variants was not routinely performed and could have provided additional insight into underlying genetic mechanisms.

Furthermore, being a referral laboratory-based study, findings may not directly represent discrepancy frequencies observed in routine donor populations or general hospital settings.

Nevertheless, the large sample size and systematic serological workup provide substantial scientific value.

Conclusion

Serological blood group discrepancies continue to represent an important diagnostic challenge despite advances in laboratory automation and standardized testing systems.

In the present study, Group II discrepancies related to weak or altered antigen expression constitute the predominant discrepancy category, while RhD variability represented a major component of overall discrepancy burden.

The findings demonstrate that automated blood grouping systems significantly enhance discrepancy detection but must be supported by structured serological workup and expert interpretation.

Recognition of rare phenotypes such as Bombay and Para Bombay groups remains essential for ensuring safe transfusion practice.

This decade-long high-volume laboratory-based analysis contributes important data regarding discrepancy patterns encountered in North India and reinforces the continuing relevance of classical immunohematology principles within modern automated laboratory environments.

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