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Original Research Article

Comparative Evaluation of Pool Cell Panel with The Standard Three Cell Panel in The Detection of Irregular Antibodies

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Abstract:

Background: This study compared the two methods in order of their sensitivity, specificity, cost effectiveness, time efficacy, enhancing workflow along with their limitations and challenges.

Methods: In this Blood Centre based observational cross-sectional study we performed irregular antibody screening in blood donor samples by standard pool cell and 3 cell panel at our Blood Centre's in-house laboratory.

Results: Both panels have extremely high specificity (over 99.98%) and identical overall accuracy at 99.9808%, indicating strong performance in correctly identifying true negatives and maintaining general accuracy. However, the Pool Cell Panel demonstrates higher sensitivity (87.5% vs. 77.78%), meaning it's better at detecting true positives, which is critical in minimizing false negatives. Conversely, the 3-Cell Panel has a higher positive predictive value (87.5% vs. 77.78%), suggesting greater reliability when a positive result is reported. The negative predictive values are nearly perfect for both, essentially swapped due to the inverse relationship with PPV and sensitivity. Overall, the Pool Cell Panel may be more suitable in contexts where missing a true positive is costlier, while the 3-Cell Panel offers greater confidence in reported positives.

Conclusion: The study concludes that while pooled cell panels offer a slightly higher sensitivity in antibody detection among donors, the standard 3-cell panel remains a more reliable tool for positive result interpretation due to its higher positive predictive value.

Keywords: 3-cell panel, Pool Cell Panel, Sensitivity, Specificity.

Introduction

Blood transfusion is an essential medical intervention that saves lives in various clinical scenarios, including surgical procedures, trauma donors, obstetric emergencies, cancer treatments, and hematological disorders such as thalassemia and sickle cell disease. However, ensuring the safety of transfused blood is critical to prevent adverse reactions in recipients. Apart from the standard ABO (ISBT001) and Rh (D) (ISBT004) blood group matching, the presence of unexpected red cell antibodies (also called irregular antibodies or alloantibodies) in donor blood or recipients can lead to hemolytic transfusion reactions (HTRs), delayed hemolysis, and hemolytic disease of the fetus and newborn (HDFN). [1-2]

Unexpected red cell antibodies develop when a person is exposed to foreign red blood cell (RBC) antigens through pregnancy, prior blood transfusions, or organ transplantation. These antibodies may belong to clinically significant blood group systems, such as: [3]

- (ISBT004) Rh system (D, C, c, E, e)
- (ISBT006) Kell system (K, k, Kpa, Kpb)
- (ISBT008) Duffy system (Fya, Fyb)
- (ISBT009) Kidd system (Jka, Jkb)
- (ISBT002) MNS system (M, N, S, s)
- (ISBT010) Diego system (Dia, Dib) and others.

If an individual with alloantibodies receives a transfusion of incompatible red cells, it can trigger immune-mediated hemolysis, leading to complications such as fever, jaundice, renal failure, and even death in severe donors. Therefore, pre-transfusion antibody screening is a mandatory step in blood banking and transfusion services to ensure safe and compatible transfusions. [4-5]

The annual collection of blood at our centre in the year of 2023 was 42050 and whole blood and packed red blood cells supplied was 40931. Keeping this in mind, in order to ensure supply of compatible blood, our Blood Centre performs screening for irregular

red cell antibodies by three cell and pool cell method.

This study compared the two methods in order of their sensitivity, specificity, cost effectiveness, time efficacy, enhancing workflow along with their limitations and challenges.

In view of the above, the need of this study was to determine which of the two tests is better suited for our Blood Centre.

Materials and Methods

Study Site: Department of Immunohematology and Transfusion Medicine Sardar Patel Medical College and Associated Groups of Hospitals, Bikaner, Rajasthan, India.

Study Design: Blood Centre based observational cross-sectional study.

Study Period: This Study was carried out over a period of thirteen months from 1st March 2024 to 31st March, 2025.

Sample Size: All whole blood donors donating at our Blood Centre in the duration of the study.

$$n = \frac{Z^2 X p X (1 - p)}{d^2}$$

Where:

- n = required sample size
- Z = Z-value
- p = estimated proportion (prevalence or expected proportion)
- d = margin of error Taking, confidence level= 95% (Z= 1.96)

Estimated proportion (p) = 0.5.

Margin of error (d) = 0.05.

Although the minimum calculated sample size was 385, our study included all 46,987 eligible blood donors over the study period. This approach was adopted to maximize statistical precision, reduce error margins, and ensure comprehensive evaluation of the pooled versus three-cell panel techniques. Inclusion of the entire donor population not only enhances the reliability of prevalence estimates but also aligns with routine blood centre screening practices, making the study both feasible and highly representative.

Sampling Technique: All whole blood donors donating at our Blood Centre in the duration of the study, meeting all inclusion criteria and not any of the exclusion criteria.

Methods of Data Collection

1. From each voluntary blood donor, blood sample (3 ml) was collected into EDTA (ethylene diamine tetra acetic acid) anticoagulated tube following all aseptic measures and plasma was separated and tested on the same day.
2. ABO- RH(D) blood grouping was performed on Immucor Galileo neo system (fully automated immunohematology analyzer) using direct hemagglutination micro strips with monoclonal control, Anti-A, Anti-B, Anti-AB, anti- D1 anti-D2, A1 cells, B cells, A2 cells and O cells provided by the manufacturer.
3. Screening and identification of red blood cell antibodies (other than anti-A and Anti – B antibodies) were performed using Immucor Galileo neo system (fully automated immunohematology analyzer) based on solid phase red cell adherence (SPRCA) capture technology.

Data Analysis: Collected data was entered into Microsoft Excel spreadsheet and was presented in the form of tables, figures, graphs, diagrams. Statistical analysis was performed with SPSS, version 23.0 for windows statistical software package (SPSS inc., Chicago, IL, USA). Qualitative data was expressed in form of percentage. Significance of difference was inferred by Chi-square test.

- Probability was considered to be significant if p value <0.05.

Results

The highest number of donors (45.66%) occurred in the 18–30 years age group, with a total of 21,452 individuals. This is followed by the 31–40 years group, which accounts for 40.34% (18,954 donors). The 41–50 age group had 3,546 donors (7.55%), while 2,440 donors (5.19%) were reported in the 51–60 age group. The smallest proportion was seen in the 61–65 age group, with only 595 donors, making up 1.27% of the total. In total, 46,987 donors were recorded across all age groups. The vast majority of donors were male, accounting for 46,613 individuals or 99.20% of the total. In contrast, only 374 donors were female, representing just 0.80% of all donors. Altogether, the total number of donors was 46,987. The most common blood group among the donors was B+ve, with 15,761 individuals (33.54%), followed by O+ve with 13,961 donors (29.71%), and A+ve with 9,133 donors (19.44%). AB+ve accounted for 3,792 donors (8.07%), while B-ve and O-ve had 1,593 (3.39%) and 1,496 (3.18%) donors respectively. Less common blood groups included A-ve with 885 donors (1.88%) and AB-ve with 366 donors (0.78%). The total number of donors was 46,987.

Table 1: Distribution of positive screen according to gender

Gender	Number	Percentage (%)
Male	29	96.6
Female	01	03.33
Total	30	100

The gender distribution within a group of 30 individuals, with 29 males and 01 females. Calculating the percentages, males constitute approximately 96.6% of the group, while females make up around 03.337%. This analysis highlights a slightly higher representation of males compared to females, this observed discrepancy is likely due to the disproportionately higher representation of male donors in the study population.

Table 2: ABO Blood Group wise distribution of blood donors with positive Antibody Screen

Blood Group	Rh Positive	Rh Negative	Total	Percentage (%)
B	13	04	17	56.6
O	08	01	9	30.0
A	02	01	3	10.0
AB	01	00	1	03.4
Total	24	06	30	100

The majority of donors belonged to blood group B, with 17 individuals (13 Rh positive and 4 Rh negative), making up 56.6% of the total. Blood group O followed, comprising 9 donors (8 Rh positive and 1 Rh negative), accounting for 30.0%. Blood group A included 3 donors (2 Rh positive and 1 Rh negative), representing 10.0%, while AB had the fewest donors, with only 1 individual who was Rh positive, making up 3.4%. Overall, out of 30 donors, 24 were Rh positive and 6 were Rh negative.

Table 3: Frequency of positivity of Ab screen by pool cell and 3 cell panel

Antibody Screen	Frequency of Positivity	Percentage (%)	Total Sample Tested by Neo IRS
Pool Cell	27	0.057	46987
3 Cell	24	0.051	46987

Out of 46,987 tested, 27 (0.057%) were positive for antibody screen by pool cell method. Out of 46,987 tests, 24 were positive for antibody screen by 3 cell panel, representing 0.051% of the total tested samples. This data shows that the vast majority of blood donors tested were negative for irregular antibody screens, indicating that 99.94% of the donors were free of irregular antibody. Only a small percentage (0.06%) of the donors were positive for an antibody screen, which translates to 30 individuals out of the total tested. This low positivity rate for irregular antibody screen among the tested blood donors suggests a relatively low prevalence of irregular antibodies in the general blood donor population.

Table 4: Comparison between Antibody Screening by 3-Cell vs Pool Cell Panel

Metric	3-Cell Panel	Pool Cell Panel
Specificity	99.9936%	99.9872%
Sensitivity	77.78%	87.5%
Positive Predictive Value	87.5%	77.78%
Negative Predictive Value	99.9872%	99.9936%
Accuracy	99.9808%	99.9808%
% Positives	0.051	0.057

Table 11 shows a comparison between the 3-Cell Panel and the Pool Cell Panel across key diagnostic performance metrics. Both panels have extremely high specificity (over 99.98%) and identical overall accuracy at 99.9808%, indicating strong performance in correctly identifying true negatives and maintaining general accuracy. However, the Pool Cell Panel demonstrates higher sensitivity (87.5% vs. 77.78%), meaning it's better at detecting true positives, which is critical in minimizing false negatives. Conversely, the 3-Cell Panel has a higher positive predictive value (87.5% vs. 77.78%), suggesting greater reliability when a positive result is reported. The negative predictive values are nearly perfect for both, essentially swapped due to the inverse relationship with PPV and sensitivity. Overall, the Pool Cell Panel may be more suitable in contexts where missing a true positive is costlier,

while the 3-Cell Panel offers greater confidence in reported positives.

Discussion

In this study we performed irregular antibody screening in blood donor samples by standard pool cell and 3 cell panel at our Blood Centre's in-house laboratory.

Pooled red cell antibody screening offers cost and time saving by using a mixture of cells, while three-cell screening provides more sensitivity and can detect a wider range of antibodies. 3-cell screening allows for the identification of clinically relevant antibodies, while pooled screening may miss antibodies to less common antigens, potentially leading to transfusion complications.

The diagnostic performance of the 3-Cell Panel and the Pool Cell Panel across several key metrics. Both panels show exceptionally high specificity (above 99.98%) and share the same overall accuracy of 99.9808%, indicating a strong ability to correctly rule out negatives and deliver consistent results.

Where the two differ is in their performance on sensitivity and positive predictive value (PPV). The Pool Cell Panel achieves higher sensitivity (87.5% vs. 77.78%), making it more effective at identifying true positives and reducing the chance of missed detections. On the other hand, the 3-Cell Panel outperforms in PPV (87.5% vs. 77.78%), meaning it is more reliable when a positive result is returned.

Both panel's report near-perfect negative predictive values (NPVs), which is expected given their high specificity and accuracy. The variation in PPV and sensitivity reflects a common trade-off in diagnostic testing: increasing the ability to detect true positives can sometimes lower the confidence in those positives, and vice versa.

In practical terms, the Pool Cell Panel may be better suited for applications were failing to detect a condition could have serious consequences. In contrast, the 3-Cell Panel might be preferred in settings where it is more important to ensure that positive results are truly accurate.

Table 5: Comparative Evaluation of studies for Antibody screening in India

Sr. No.	Author	Place of Study	Year	Sample Size	Panel Type	Results
1	A.K. Tiwari et al. [6]	Medanta – The Medicity, Delhi	2011-12	63,927	CAT, LISS-IAT	0.12% incidence; 1 in 1,000 had antibodies.
2	Neeraj Garg et al. [7]	GTB Hospital, Delhi	2011-13	47,450	Pooled cells + 3-cell (CAT)	0.09% positive; screening essential.
3	Makroo et al. [8]	Indraprastha Apollo, Delhi	2012-15	82,153	SPRCA (Pooled), 4-cell confirmatory	0.27% positive; 150 autoantibodies.
4	Garg et al. [9]	Delhi	2017	150	MCT, Tube LISS-IAT, CTT	MCT best for significant antibodies.
5	Solanki et al. [10]	KGMU, Lucknow	2015-17	166,803	Diagast (automated pooled)	0.17% positive; 86.7% alloantibodies.
6	Rashmi Sood et al. [11]	Apollo Hospitals, Delhi	2008-09	306	3-cell CAT, 2-cell SPRCAT	4.24% alloimmunization in multi-transfused.
7	Agrawal et al. [12]	Not specified	2021	30	LISS/Coombs + NaCl/Enzyme Gel	3.33% positive; anti-Lewis.
8	Singh et al. [13]	SGRDIMSR, Amritsar	2019-21	200	Solid phase	7.5% alloantibodies; anti-K most common.
9	Present Study	SPMC, Bikaner	2024-25	46987	3 Cell	Sensitivity = 77.78% Specificity = 99.9936%

						Antibody Screen Positive (%) =0.051
					Pool Cell	Sensitivity = 87.5% Specificity = 99.9872% Antibody Screen Positive (%) =0.057

Table 6: Comparative Evaluation of studies for Antibody screening Internationally

Sr. No.	Author	Place of Study	Year	Sample Size	Panel Type	Results
1	De Silva et al. [14]	UK	1985	105 sera	Pooled vs. Individual	12% undetectable by pooled; reduced sensitivity.
2	AuBuchon JP [15]	USA	1986	19 panels	Evaluation study	Created scoring tool matching expert evaluation.
3	Eggington et al. [16]	UK	1996	Not specified	Pooled red cells + columns	Reduced sensitivity; unsuitable for routine use.
4	Schrem et al. [17]	Germany	1996	10,008 donors	Pooled red cells, solid-phase	0.56% alloantibodies; sensitivity varied.
5	Present Study	India	2024-25	46987	3 Cell	Sensitivity = 77.78% Specificity = 99.9936% Antibody Screen Positive (%) =0.051
					Pool Cell	Sensitivity = 87.5% Specificity = 99.9872% Antibody Screen Positive (%) =0.057

Conclusion

The study concludes that while pooled cell panels offer a slightly higher sensitivity in antibody detection among donors, the standard 3-cell panel remains a more reliable tool for positive result interpretation due to its higher positive predictive value. Given the low prevalence (0.06%) of unexpected antibodies among donors, both methods can be used for mass screening in resource-limited settings, but confirmatory testing with standard panels is recommended for clinical safety.

Routine antibody screening, especially in regions with high transfusion rates, should be encouraged to minimize transfusion-related complications and ensure better matching of donor units, especially in vulnerable populations like pediatric or polytrans fused patients. Further large-scale studies including true negative donors are recommended to fully assess the specificity and NPV of both methods.

While both panels perform exceptionally well, the Pool Cell Panel provides a better balance of sensitivity and NPV, making it better suited for detecting as many true positive cases as possible, which is crucial in early detection, disease screening, or outbreak surveillance. The slightly lower PPV is an acceptable trade-off in these contexts, as false positives can typically be ruled out with secondary confirmatory testing.

Therefore, the Pool Cell Panel is the better option overall especially in scenarios where the cost of missing a case is greater than the cost of investigating a false positive.

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