

Comparative Evaluation of In-House and Commercial Low Ionic Strength Solutions For Immunohematological Testing

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Abstract

BACKGROUND: Low ionic strength solution (LISS) is widely used in immunohematological testing to enhance antibody detection and reduce incubation time. Although commercially available LISS reagents are routinely used in blood banks, their cost may present a challenge, particularly in resource-limited settings.

OBJECTIVE: To compare the quality and performance of an in-house prepared LISS with a commercially available LISS for immunohematological testing.

METHODOLOGY: The in-house LISS was prepared according to standard procedures and evaluated for relevant quality-control parameters. Comparative testing was performed using samples subjected to antibody screening and compatibility testing with both in-house and commercial LISS. Reaction strength, antibody detection, crossmatching performance, and selected quality-control parameters were compared.

RESULTS: The in-house LISS demonstrated quality-control characteristics comparable to those of the commercial preparation, including appropriate physicochemical parameters. Comparable serological reaction strengths and performance in antibody screening and compatibility testing were observed between the two preparations. Clinically significant antibody specificities were detected, and the in-house LISS showed satisfactory performance during crossmatching.

CONCLUSION: A properly prepared and quality-controlled in-house LISS may provide a practical alternative to commercial LISS for routine immunohematological testing. Further evaluation using larger sample sizes and a broader range of clinically significant antibodies may help establish its wider applicability in transfusion services.

Keywords: Antibody screening, Blood bank, Crossmatching, Immunohematology, Low ionic strength solution, Quality control.

Introduction

Low ionic strength solution (LISS) is an established enhancement medium used in immunohematology to facilitate antigen–antibody interactions during indirect antiglobulin testing (IAT). It is commonly incorporated into antibody screening, antibody identification, and compatibility testing because reducing the ionic strength of the reaction medium enhances antibody uptake by red blood cells and permits shorter incubation periods while maintaining appropriate test performance.^[1] The reliable detection of clinically significant red cell antibodies is a central component of pretransfusion testing, as these antibodies may result in incompatible transfusion, shortened survival of transfused red cells, or hemolytic transfusion reactions.^[2,3] Recent studies from different transfusion settings continue to emphasize the importance of sensitive and reproducible antibody screening and compatibility testing for the selection of appropriate blood components.^[4-6]

The choice and performance of reagents used during immunohematological testing can influence the reliability and reproducibility of laboratory results. Commercially prepared LISS reagents are widely used in transfusion laboratories because they provide standardized formulations and established performance characteristics. However, routine dependence on commercially available reagents may present practical challenges related to procurement, uninterrupted availability, and laboratory expenditure, particularly in settings with limited resources. These challenges have encouraged interest in locally prepared or in-house alternatives for selected transfusion laboratory applications. A previous study from Uganda demonstrated that appropriately prepared and quality-assured home-made reagent red blood cells could be successfully used for pretransfusion antibody screening, with results comparable to confirmatory testing performed at an external reference laboratory.^[7] Similarly, recent studies have shown the continued value of locally developed or in-house approaches when commercially available reagents or conventional testing methods are associated with practical limitations, provided that appropriate validation and quality assurance procedures are followed.^[8,9]

Nevertheless, the introduction of an in-house reagent into routine laboratory practice requires more than demonstrating its potential economic or logistical advantages. The reagent must be prepared using a standardized procedure and evaluated for relevant physicochemical and quality-control characteristics. In addition, its intended clinical application should be assessed against an established method or commercially available reagent to determine whether it provides consistent and satisfactory analytical performance. In

immunohematology, such evaluation is particularly important because variation in reagent performance may affect antibody detection or compatibility testing and consequently influence transfusion safety.^[3,5,6] Comparative studies of pretransfusion testing methods have shown high levels of concordance among established immunohematological platforms when testing is appropriately standardized, highlighting the importance of systematic performance evaluation before changes are introduced into routine practice.^[10]

LISS therefore remains relevant not only as an enhancement medium but also as a reagent whose preparation and performance require careful standardization. Although commercially prepared products offer convenience and established specifications, an appropriately formulated in-house preparation may represent a practical alternative for transfusion laboratories if it demonstrates acceptable quality-control characteristics and comparable serological performance. However, evidence supporting the routine use of an in-house LISS should be based on direct comparison with an established commercial preparation under conditions relevant to its intended use.

Therefore, the present prospective comparative study was conducted at Saveetha Medical College and Hospital to evaluate an in-house prepared LISS in comparison with a commercially available LISS reagent (DiaMed GmbH, Switzerland). A total of 90 blood samples were evaluated over a 3-month period. The two preparations were compared with respect to selected quality-control parameters, antibody detection, serological reaction strength, and compatibility testing. The study aimed to assess the practical suitability of a properly prepared and quality-controlled in-house LISS for routine immunohematological testing in a transfusion service.

Methodology

Study design and population

This prospective comparative study was carried out at Saveetha Medical College and Hospital from February 1 to April 30, 2025. A total of 90 patient blood samples were included in the study. Each sample was evaluated using both the in-house prepared low ionic strength solution (LISS) and a commercially available LISS reagent (DiaMed GmbH, Switzerland) for comparative assessment.

Preparation and quality control of in-house LISS

The in-house LISS was prepared according to standard procedures described for LISS preparation in transfusion medicine practice.^[11] The use of standardized and validated procedures for pretransfusion testing and reagent systems is essential to ensure consistent analytical performance.^[12]

Non-serological quality control parameters included conductivity and osmolarity, which were measured using calibrated instruments. The predefined acceptable ranges were 3.6–3.7 mmho/cm for conductivity and 270–285 mmol/L for osmolarity. Serological quality control included determination of the anti-D (IgG) titre and assessment for hemolysis. The target anti-D (IgG) titre was $\geq 1:32$, and a titre of 1:64 was achieved in all batches. Hemolysis testing was performed using 10 samples, and no visible hemolysis was observed. All assessed quality control parameters met the predefined acceptance criteria. Quality assurance and validation of reagents and testing procedures are important components of pretransfusion laboratory practice.^[12,13]

Testing method

Antibody screening and compatibility testing were performed using the conventional tube technique (CTT) in accordance with established pretransfusion testing procedures.^[11,12] Pooled group O red blood cells were used as indicator cells in both test setups. Each sample was tested using both the in-house and commercial LISS preparations.

The LISS indirect antiglobulin test is an established method for the detection of clinically significant red cell antibodies and is widely used in pretransfusion testing because of its sensitivity and suitability for compatibility procedures.^[12,13] Reaction strengths were graded from 0 to 4+ according to standard agglutination grading criteria used for tube-based immunohematological testing.^[11] Any discrepancies observed between the in-house and commercial LISS preparations were recorded and analyzed.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 21. Descriptive statistics were used to summarize the study findings. Categorical variables, including antibody detection rates and reaction strengths, were evaluated using the chi-square test. A p value of <0.05 was considered statistically significant.

Results

A total of 90 blood samples were included in the comparative evaluation, with 45 samples tested using in-house LISS and 45 samples tested using commercial LISS. The two reagents were compared with respect to serological reaction strength, antibody detection performance, crossmatch compatibility, and selected quality-control parameters.

Serological reaction strength

The distribution of serological reaction grades was comparable between the in-house and commercial LISS preparations. Strong reactions were predominantly observed in both groups, with 4+ reactions recorded in 16 (35.6%) samples using in-house LISS and 17 (37.8%) samples using commercial LISS. Similarly, 3+ reactions were observed in 14 (31.1%) and 15 (33.3%) samples, respectively. The proportions of 2+ reactions were 22.2% and 20.0%, while weak 1+ reactions were observed in 6.7% and 4.4% of samples tested with in-house and commercial LISS, respectively. Negative reactions were identical in both groups, with two samples (4.4%) in each preparation. Overall, only minor differences of one reaction grade were observed between the two reagents, indicating comparable serological reaction strength (Table 1; Figure 1).

Table 1. Distribution of serological reaction strength obtained with in-house and commercial LISS.

Reaction Grade	In-house LISS (n = 45)	Commercial LISS (n = 45)	Difference (Δ)
4+	16 (35.6%)	17 (37.8%)	-1
3+	14 (31.1%)	15 (33.3%)	-1
2+	10 (22.2%)	9 (20.0%)	+1
1+	3 (6.7%)	2 (4.4%)	+1
0	2 (4.4%)	2 (4.4%)	0

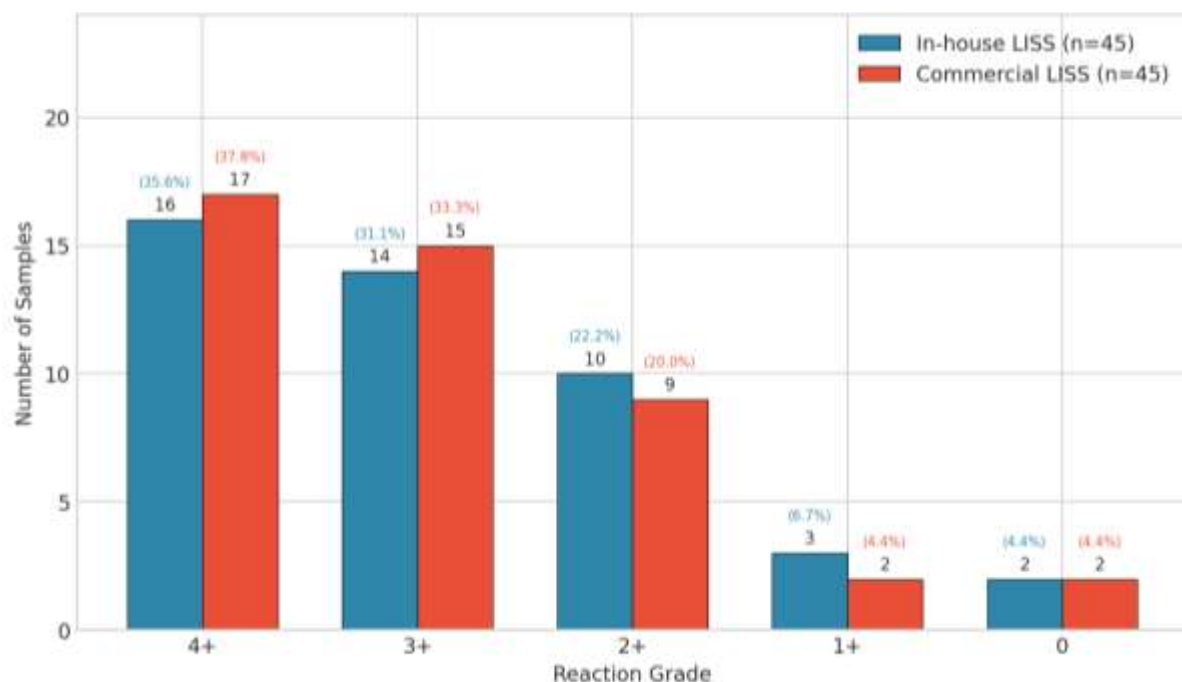


Figure 1. Serological reaction strength distribution between in-house and commercial LISS.

Antibody detection performance

Both in-house and commercial LISS demonstrated complete detection of the evaluated clinically significant antibodies, including Anti-D, Anti-E, Anti-K, Anti-M, and Anti-c. All cases of the respective antibody specificities were detected by both preparations. Minor variation was observed only among weak reactions, with three weak reactions detected using in-house LISS and four using commercial LISS. The overall sensitivity was 93.3% for in-house LISS and 95.6% for commercial LISS, with no statistically significant difference between the two preparations ($p = 0.64$). These findings indicate comparable antibody detection performance under the study conditions (Table 2; Figure 2).

Table 2. Comparison of antibody detection performance and sensitivity between in-house and commercial LISS

Antibody Type	In-house LISS	Commercial LISS	p-value
Anti-D	12/12	12/12	1.000
Anti-E	8/8	8/8	1.000
Anti-K	6/6	6/6	1.000
Anti-M	5/5	5/5	1.000
Anti-c	4/4	4/4	1.000
Weak reactions (1+)	3	4	0.667
Overall sensitivity	93.3%	95.6%	0.64 (NS)

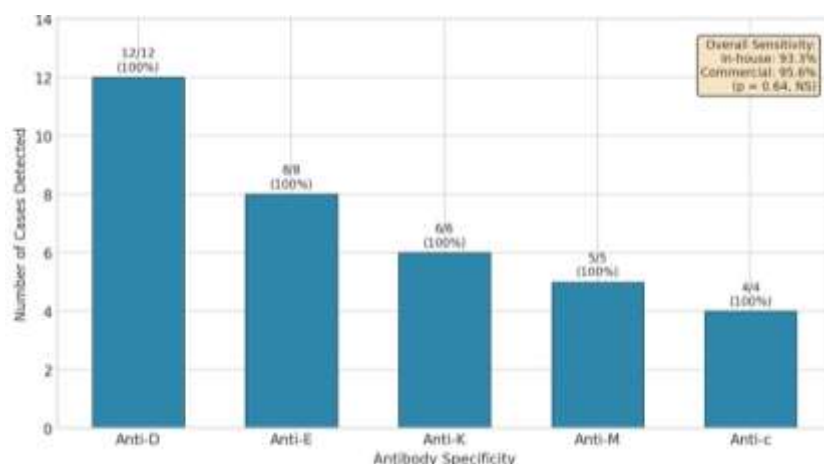


Figure 2. Antibody detection performance of in-house and commercial LISS.

Crossmatch compatibility

Crossmatch testing showed comparable compatibility results between the two LISS preparations. Using in-house LISS, 39 samples (86.7%) were classified as compatible and six samples (13.3%) as incompatible. With commercial LISS, 41 samples (91.1%) were compatible and four samples (8.9%) were incompatible. Two discrepant cases were observed between the testing approaches. These discrepancies did not result in the transfusion of incompatible blood units and were considered to have no clinical impact within the study period (**Table 3; Figure 3**).

Table 3. Comparison of crossmatch compatibility results between in-house and commercial LISS

Crossmatch Result	In-house LISS (n = 45)	Commercial LISS (n = 45)	Discrepant Cases
Compatible (0-1+)	39 (86.7%)	41 (91.1%)	2
Incompatible ($\geq 2+$)	6 (13.3%)	4 (8.9%)	2

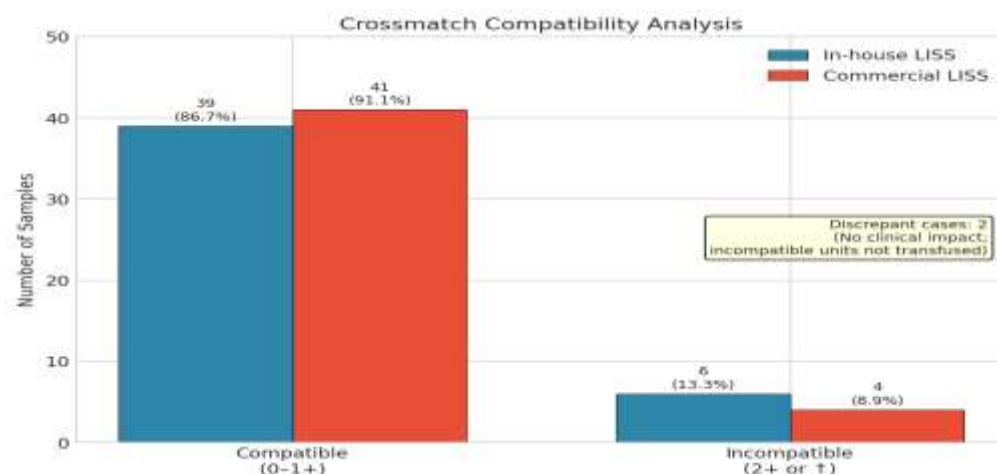


Figure 3. Crossmatch compatibility analysis using in-house and commercial LISS.

Quality-control evaluation

The quality-control assessment demonstrated that both the in-house and commercial LISS preparations met the predefined acceptable criteria for all evaluated parameters. The mean conductivity of the in-house and commercial preparations was 3.67 ± 0.03 mmho/cm and 3.68 ± 0.02 mmho/cm, respectively, which were within the acceptable range of 3.6–3.7 mmho/cm. Similarly, the osmolarity values were 275.5 ± 2.5 mmol/L for in-house LISS and 277 ± 2.0 mmol/L for commercial LISS, both within the predefined acceptable range of 270–285 mmol/L. The Anti-D (IgG) titer was 1:64 in all batches of both preparations, exceeding the minimum target value of $\geq 1:32$. In addition, no visible hemolysis was observed in any of the 10 samples evaluated for each preparation. Overall, both reagents demonstrated 100% compliance with the selected physicochemical and serological quality-control parameters (Table 4).

Overall, the findings demonstrated that the in-house LISS preparation showed comparable performance to the commercial LISS reagent in terms of reaction strength, antibody detection, compatibility testing, and the evaluated quality-control characteristics.

Table 4. Quality-control evaluation of physicochemical and serological parameters of in-house and commercial LISS

Parameter	Acceptable Range	In-house LISS	% Compliance	Commercial LISS	% Compliance
Conductivity	3.6–3.7 mmho/cm	3.67 ± 0.03	100%	3.68 ± 0.02	100%
Osmolarity	270–285 mmol/L	275.5 ± 2.5	100%	277.0 ± 2.0	100%
Anti-D titer (IgG)	$\geq 1:32$	1:64 (all batches)	100%	1:64 (all batches)	100%
Hemolysis test	No visible hemolysis	0/10 failed	100%	0/10 failed	100%

Discussion

The present prospective comparative study demonstrated that the in-house prepared low ionic strength solution (LISS) showed overall comparable performance to the commercially available LISS in terms of serological reaction strength, antibody detection, crossmatch compatibility, and quality-control characteristics. The distribution of agglutination reaction grades was similar between the two preparations, with only minor differences across the reaction categories. No statistically significant difference was observed between the two groups, indicating that an appropriately prepared LISS can provide consistent enhancement of antigen–antibody reactions when used under standardized testing conditions.^[1,14]

Antibody detection performance was comparable between the in-house and commercial LISS preparations. Both reagents detected all evaluated cases involving Anti-D, Anti-E, Anti-K, Anti-M, and Anti-c antibodies. Although minor variation was observed in weak reactions, the difference was not statistically significant and did not result in any false-negative antibody detection. This finding is particularly important because the reliable identification of clinically significant red cell antibodies remains a fundamental component of pretransfusion testing and transfusion safety.^[2,3,14]

Crossmatch compatibility was observed in 86.7% and 91.1% of samples tested using the in-house and commercial LISS preparations, respectively. Although the commercial reagent showed a slightly higher proportion of compatible results, the observed difference was not associated with a clinically significant adverse outcome. The discrepant cases were limited to minor differences in reaction grading or incompatible crossmatch results, and incompatible units were appropriately excluded from transfusion. These findings suggest that minor analytical variation between reagent preparations does not necessarily translate into clinically relevant differences when appropriate interpretation, quality assurance, and transfusion laboratory procedures are maintained.^[5,6,15]

An important finding of the present study was the satisfactory quality-control performance of the in-house LISS. The measured conductivity and osmolarity values remained within the predefined acceptable ranges, while the Anti-D IgG titre achieved the required performance level in all evaluated batches. In addition, no visible hemolysis was observed during testing. These findings indicate that the in-house preparation demonstrated acceptable physicochemical and serological consistency under the conditions of the present study. Standardization of reagent preparation and appropriate quality-control procedures are essential when introducing locally prepared reagents into routine immunohematological testing, particularly because variations in reagent composition or performance may influence the reliability of antibody detection and compatibility testing.^[11-13,16]

The findings of this study have practical relevance for transfusion services, particularly in laboratories where the procurement, cost, or uninterrupted availability of commercial reagents may represent operational challenges. An in-house LISS that is prepared according to a standardized protocol and subjected to appropriate physicochemical and serological quality-control assessment may provide a practical alternative to commercially available preparations. However, the results should be interpreted within the scope of the present study, which involved a relatively limited sample size and a single-center evaluation. Larger multicenter studies involving a broader range of clinically significant antibodies and different testing platforms would be useful to further establish the reproducibility and routine applicability of in-house LISS preparations. Overall, the present study demonstrated that the in-house prepared LISS showed comparable serological performance to the commercial LISS under the evaluated testing conditions. The in-house preparation fulfilled the predefined quality-control criteria and demonstrated satisfactory antibody detection and crossmatch performance. These findings support the potential use of a properly standardized and quality-controlled in-house LISS as a practical alternative for routine immunohematological testing, while emphasizing the need for continued validation and quality assurance before wider implementation.^[14-16]

Limitations of the Study

This study has certain limitations that should be considered when interpreting the findings. First, the study was conducted at a single center with a relatively small sample size of 90 blood samples, which may limit the generalizability of the results. Second, the evaluation was performed over a limited study period of three months, and therefore long-term batch-to-batch consistency and stability of the in-house LISS were not assessed. Third, although several clinically relevant antibody specificities were evaluated, the study did not include a wider range of rare or complex antibodies.

In addition, the comparison was limited to the conventional tube technique (CTT) and a single commercially available LISS preparation; therefore, the performance of the in-house LISS with other immunohematological platforms or commercial reagents was not evaluated. Future multicenter studies involving larger and more diverse sample populations, a broader range of antibody specificities, and extended stability assessments are required to further establish the reproducibility and wider applicability of the in-house LISS.

Conclusion

The present study demonstrated that the in-house prepared low ionic strength solution (LISS) showed comparable performance to the commercially available LISS in terms of antibody detection, serological reaction strength, and crossmatch compatibility. Minor differences observed between the two preparations, including slight variations in reaction strength and the number of incompatible crossmatches, did not result in clinically significant discrepancies affecting transfusion safety.

The in-house LISS met all predefined quality-control requirements, including conductivity, osmolarity, Anti-D IgG titre, and hemolysis testing, demonstrating satisfactory formulation consistency and performance. These findings suggest that, when prepared using a standardized protocol and subjected to appropriate quality-control and validation procedures, in-house LISS may serve as a cost-effective and reliable alternative to commercially available preparations.

However, further studies involving larger sample sizes and multicenter evaluations are required to confirm its reproducibility and broader applicability. Nevertheless, the present findings support the potential use of properly standardized and quality-controlled in-house LISS for routine immunohematological testing, particularly in resource-limited settings where cost and dependency on commercially available reagents may present practical challenges.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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