



OPEN Clinical evaluation and comparative study of a fully automated erythrocyte osmotic fragility analysis system based on scatter turbidimetry

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Accurate assessment of erythrocyte osmotic fragility (EOF) is essential for diagnosing hemolytic disorders, yet traditional methods are labor-intensive and time-consuming, limiting their clinical efficiency. This study aimed to evaluate the performance of a fully automated EOF analysis system developed by Shenzhen Pumen Technology Co., Ltd., which utilizes scatter turbidimetry, and to determine its accuracy and consistency compared with the conventional spectrophotometric method. A total of 114 whole blood samples were analyzed using the automated system with reagents from Changchun Huili Biotechnology Co., Ltd., and results were compared with those obtained from the Shimadzu UV-2450 spectrophotometer as the reference method. Consistency between the two systems was assessed using linear regression, Kappa statistics, Bland-Altman analysis, and medical decision level bias evaluation. The automated EOF system demonstrated a strong linear correlation with the reference method, achieving a Kappa value of 1.00, indicating complete agreement in classification results. Moreover, 99.11% of data points fell within acceptable ranges, and bias confidence intervals at the medical decision level were below threshold values. These findings indicate that the fully automated EOF system provides stable and reliable results that align with clinical needs and offer high consistency with traditional methods, supporting its potential for broader clinical application and promotion.

Keywords Erythrocyte osmotic fragility, Scattering nephelometry, Consistency analysis, Automation systems, Clinical performance

Erythrocyte osmotic fragility (EOF) is a critical laboratory parameter used in the diagnosis and management of a variety of hematological disorders, including hemolytic anemia, hereditary spherocytosis, and immune-mediated hemolytic conditions¹. The EOF test measures the susceptibility of red blood cells (RBCs) to hemolysis when exposed to hypotonic solutions, providing insight into the integrity and stability of the erythrocyte membrane¹. Under physiological conditions, RBCs maintain membrane stability and resist lysis in mild hypotonic environments; however, pathological membrane composition or structure alterations increase fragility, leading to premature hemolysis and clinical manifestations of anemia.

Traditional EOF testing methods, such as multi-tube and single-tube assays, have been widely employed in clinical laboratories for decades². Despite their clinical utility, these techniques are labor-intensive, time-consuming, and prone to variability due to manual handling and subjective interpretation, which can affect diagnostic accuracy and throughput³. In the era of precision medicine and high-throughput diagnostics, there

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is a growing demand for automated, rapid, and reliable assays that reduce operator dependence and improve workflow efficiency without compromising analytical performance⁴.

Scatter turbidimetry is based on the principle of light scattering, which infers the osmotic fragility of red blood cells by measuring the intensity of light scattering in the blood in a hypotonic environment⁵. Unlike traditional colorimetric methods, scatter turbidimetry has high sensitivity and specificity and can obtain results for detecting erythrocyte osmotic fragility in a short time⁴. The automated system developed by Shenzhen Pumen integrates nephelometric scatter turbidimetry with automatic sample injection, enhancing detection accuracy and operational efficiency while minimizing manual intervention⁶. A stable laser beam is focused through a lens into the blood sample, where erythrocytes scatter the light. The scattered light is detected by a photosensitive sensor and converted into electrical signals, which are analyzed to determine erythrocyte osmotic fragility. By analyzing the intensity and pattern of these scattered signals, the system can accurately assess the osmotic fragility of erythrocytes, enabling rapid detection⁷.

Despite adopting automated erythrocyte osmotic fragility analyzers in some laboratories, their performance across different clinical settings has not been comprehensively validated^{4,8}. This study aims to evaluate the clinical consistency and measurement performance of Shenzhen Pumen Technology's nephelometry-based system compared to the traditional reference system from Changchun Huili Biotechnology Co., Ltd., using whole blood samples. The study will assess the substitutability and clinical applicability of the automated system through rigorous statistical analyses. The findings of this study could provide valuable insights into the feasibility and reliability of using the automated system in clinical practice, offering an alternative to manual EOF testing that could lead to more efficient, accurate, and standardized diagnostic workflows in hematology.

Materials and methods

Study design

This study employed a single-center, comparative trial design to evaluate the clinical consistency and accuracy of the fully automated erythrocyte osmotic fragility analysis system developed by Shenzhen Pumen Technology Co., Ltd., compared to the traditional reference method. The study was conducted from February to May 2021 at Chenjiaqiao Hospital in accordance with the Declaration of Helsinki and relevant ethical guidelines (No. 2021-35). Informed consent was obtained from all participants prior to enrollment, ensuring transparency and adherence to ethical standards. All eligible samples were randomly assigned for analysis to minimize potential selection bias, and sample handling was standardized to ensure consistency in timing and conditions of testing. Samples were obtained from healthy individuals undergoing routine physical examinations. Exclusion criteria included history of cardiovascular, pulmonary, renal, hepatic, or pancreatic diseases, recent surgery or blood transfusion, medication or dietary interventions, and women who were pregnant, lactating, or menstruating. Samples showing hemolysis, jaundice, or improper storage were also excluded. A total of 114 whole blood samples were collected, of which 112 met the inclusion criteria and were subsequently used for data analysis to ensure both sample representativeness and the reliability of the results.

Sample selection and processing

All research samples were collected following approval from the Ethics Committee and in strict adherence to ethical guidelines. Whole-blood samples were collected in EDTA, heparin, or sodium citrate anticoagulant tubes according to routine clinical practice and the device's technical requirements. Because anticoagulants may influence erythrocyte fragility, a preliminary stability comparison was performed across the three anticoagulants, and no significant differences were observed. To prevent hemolysis or clotting, samples were maintained at 2–8 °C during storage and equilibrated to room temperature (20–25 °C) prior to testing. All samples were processed and analyzed within 24 h of collection. To ensure data quality and accuracy, all samples underwent quality control (QC) prior to the experiment to confirm compliance with analytical requirements. QC included running both the test and reference reagents with control samples, and only results within predefined QC limits were accepted. All sample data were anonymized to protect participant privacy and included no personally identifiable information. During the experimental process, all eligible samples were randomly assigned to the two detection systems according to a predetermined protocol, and operators were blinded to sample identity to maintain objectivity.

Detection systems and data analysis methods

Automated test system

This test system fully automated erythrocyte osmotic fragility analysis system produced by Shenzhen Pumen Technology Co., Ltd. as the test system⁶. This system operates based on the principle of scattering nephelometry and features automatic sampling and rapid result output. Figure 1 presents a schematic representation of scatter nephelometry, wherein a laser light source emits a stable beam focused through a lens and directed into a blood sample. Red blood cells scatter the incident beam as the light traverses the sample. A photosensitive sensor then detects the resulting scattered light, which converts the optical signal into an electrical output for subsequent analysis. For each measurement, 200 μ L of thoroughly mixed whole blood was added to 2.5 mL of erythrocyte fragility test reagent, following the manufacturer's Instructions for Use. The analyzer performed automated mixing and incubation at room temperature, followed by real-time scattering detection. Reagents were stored between 2 and 30 °C and verified to be within their expiration dates.

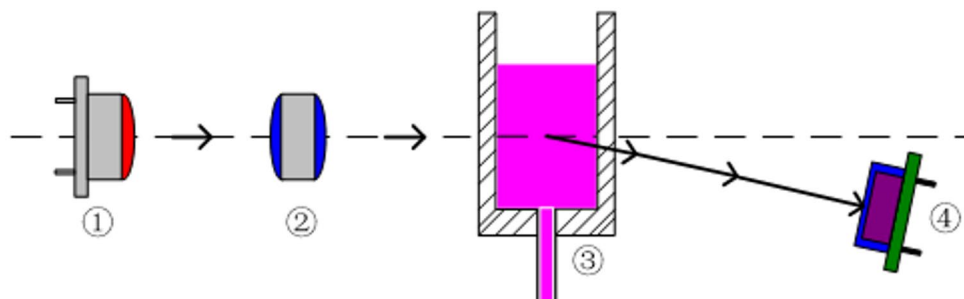


Fig. 1. Schematic diagram of scattering nephelometry. ①Laser light source ②Focusing lenses ③Reaction cup ④Light-sensitive sensors.

Research centers	Gender	Male	Female
Sun Yat-sen Memorial Hospital, Sun Yat-sen University	Number of cases(N, %)	58, 51.79%	54, 48.21%
	Age mean \pm standard deviation	28.43 \pm 24.66	
	median	16.5	
	Min ~ Max	0 ~ 83	

Table 1. Overall age distribution of the Sample.

Reference system

The reference system employed Changchun Huili Biotechnology Co., Ltd.'s traditional erythrocyte osmotic fragility kit combined with Shimadzu's UV-2450 spectrophotometer for detection⁹. According to the kit instructions, each measurement required 100 μ L of whole blood, sequentially mixed with 100 mL of solution A, 100 mL of solution B, and 50 mL of solution C. After thorough mixing and incubation at room temperature, absorbance was measured at the specified wavelength on the spectrophotometer. All reagents were stored at 2–8 $^{\circ}$ C and used within their validity period. Calibration verification of the spectrophotometer was performed daily before testing.

Analytical comparison and validation protocol

To achieve an unbiased comparison between the automated system and the reference method, all specimens were analyzed simultaneously under standardized environmental conditions (25 ± 1 $^{\circ}$ C). Both analyzers were operated by experienced laboratory staff to reduce operator-dependent variability. Method performance was examined through linear regression to characterize the correlation between systems (R^2 , slope, and intercept), Kappa statistics to evaluate agreement in categorical classifications, and Bland–Altman analysis to assess measurement bias and the limits of agreement. Acceptable bias limits were defined in accordance with the technical specifications of the EOF assay (scatter nephelometry method) provided by Shenzhen Pumen Technology Co., Ltd., which specify that the permissible deviation corresponds to one-half of three times the assay's coefficient of variation (CV).

Statistical analysis

Data were analyzed using SPSS 26.0 and MedCalc (version 19.6). Continuous variables are presented as mean \pm standard deviation (SD). Categorical variables are expressed as percentages and compared using χ^2 tests. Method agreement between the automated system and the reference method was evaluated using Kappa statistics for categorical data, linear regression (R^2 , slope, intercept) for correlation, and Bland–Altman analysis to assess bias.

Results

Sample characteristics

A total of 112 valid samples were included in this study, as shown in Table 1. The gender distribution of the sample was 58 males (51.79%) and 54 females (48.21%). In terms of age, the age range of the samples ranged from 0 to 83 years, with a median age of 16.5 years. The sample includes healthy individuals and patients with different degrees of anemia, ensuring the sample is representative and the data is widely applicable. The osmotic fragility of red blood cells in patients with anemia is usually significantly different from that in the normal population, which makes the sample selection have strong clinical practical value. All samples were randomized to avoid selection bias and ensure the objectivity and reliability of the study results.

Linear regression analysis

We used linear regression analysis to further evaluate the relationship between the two systems. The results showed an extremely high correlation between the assessment and reference systems. Equations 3–1 below is the regression equation.

$$Y = 1.0168X - 0.0138 \quad (3-1)$$

where Y represents the measurement value of the assessment system, and X represents the measurement value of the reference system. The slope (1.0168) in the regression equation is close to 1, and the intercept (-0.0138) is close to 0. The 95% confidence interval (CI) for the slope was 0.9881–1.0455, and the 95% CI for the intercept was -0.0886 – 0.0610 , confirming that both parameters are statistically consistent with ideal values. Figure 2 presents a scatter plot of the test results of the assessment system and the reference system, indicating that the measurement results between the two systems are almost identical. The correlation coefficient of regression analysis $r=0.989$, which is very close to 1, further verifies the high consistency of the two systems. This shows that the assessment system can accurately reflect the measurement results of the reference system, and the error between the two is minimal, which meets the clinical application requirements.

Kappa consistency analysis

To further verify the consistency of the two systems, we used the Kappa coefficient to evaluate the correlation of the test results. The value of the Kappa coefficient ranges from 0 to 1, and the closer the value is to 1, the higher the agreement between the results of the two systems. According to the results of this study, the Kappa coefficient is 1.00, indicating that the test results of the assessment system are in full agreement with the reference system. Statistically, a Kappa value of 1.00 shows no errors or biases, which further supports the reliability of the assessment system in clinical applications, especially in different clinical scenarios where the consistency of test results is excellent. Equations 3-2 shows the formula for calculating the Kappa coefficient:

$$Kappa = \frac{(PA - Pe)}{(1 - Pe)} \quad (3-2)$$

Among them, PA is the actual consensus rate, and Pe is the theoretical consensus rate. In this study, PA is calculated as Eqs. 3-3:

$$PA = \frac{(a + d)}{(a + b + c + d)} = 100\% \quad (3-3)$$

The formula for calculating Pe is Eqs. 3-4:

$$Pe = \frac{(a + b)(a + c) + (c + d)(b + d)}{(a + b + c + d)^2} = 53.13\% \quad (3-4)$$

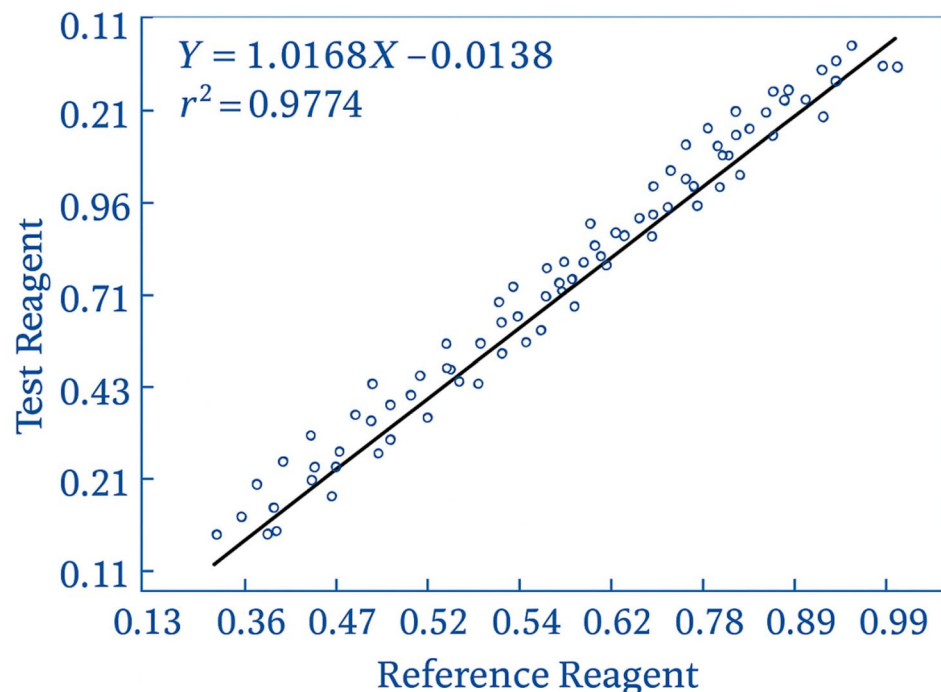


Fig. 2. Scatter plot of the test results of the assessment system and the reference system.

Substituting the formula yields $Kappa=1.00$. Since the Kappa value was greater than 0.75, it indicated that the classification results of the two systems were completely consistent and had the acceptability of clinical application.

Analysis of bias

Analysis of absolute bias

We employed a Bland-Altman analysis to further evaluate the difference in measurements between the two systems. The Bland-Altman analysis was used to compare the differences between the two measure groups and to assess the acceptability of bias with a 95% agreement interval (± 1.96 standard deviation). Equations 3-5 are the calculation process for absolute bias analysis, where Y is the measurement of the assessment system, and X is the measurement of the reference system. The distribution of bias showed that the two systems could produce similar measurements under the same sample conditions, and the differences in their measurements were manageable.

$$bias=Y - X \quad (3-5)$$

Using the Bland-Altman analysis, we assessed the absolute bias between the assessment system and the reference system, and the results are shown in Fig. 3. The mean bias was -0.00429 , and the standard deviation (SD) of the bias was 0.02358. Accordingly, the 95% Limits of Agreement (LOA) were -0.05050 to 0.04192 (mean bias $\pm 1.96 \times SD$). The figure shows that 99.11% of the data points fall within the ± 1.96 SD range, indicating that the test results of the two systems are highly consistent and the bias is clinically acceptable.

Analysis of relative bias

We further used relative bias analyses to assess the two systems' relative consistency. Relative bias analysis assesses the agreement between the assessment system and the reference system by calculating the relative value of bias, particularly whether the measurement difference is within acceptable limits. Equations 3-6 are the formula for calculating relative bias, where Y represents the measurement of the assessment system, and X represents the measurement of the reference system. The bias between the measurement results of the assessment system and the reference system is within the clinically acceptable range, so the difference does not affect the clinical diagnosis.

$$Relative\ bias = \frac{Y - X}{X} \times 100\% \quad (3-6)$$

As shown in Figs. 4 and 99.11% of the data points fell within the ± 1.96 standard deviation (SD) range, which proved that the measurement difference between the two systems was minimal and had good clinical application potential.

Analysis of medical decision-level bias

The medical decision level bias analysis aimed to assess the acceptability of the assessment system versus the reference system in clinical decision-making. In clinical practice, the level of medical decision refers to the

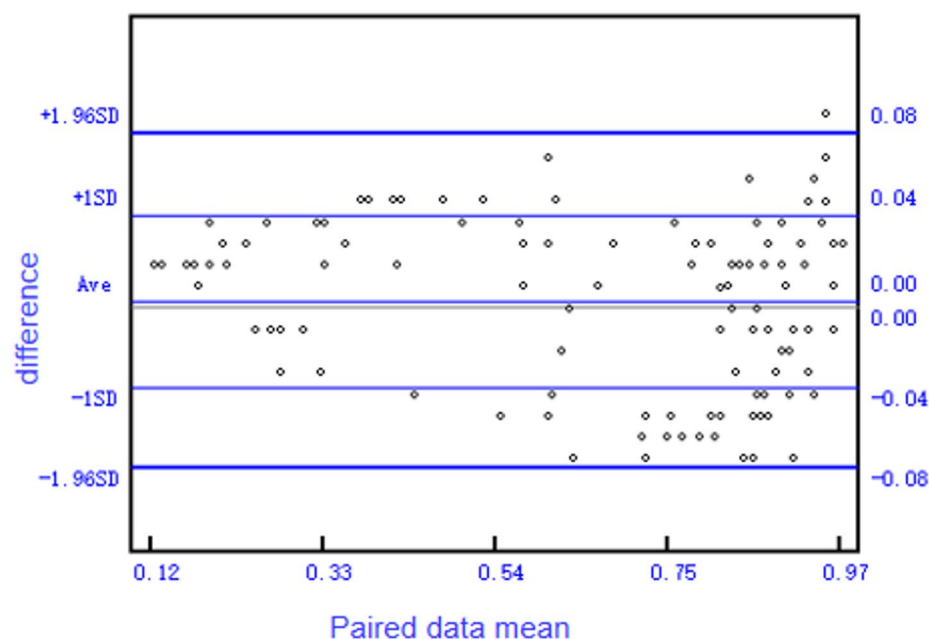


Fig. 3. Statistical analysis of absolute bias between the assessment system and the reference system.

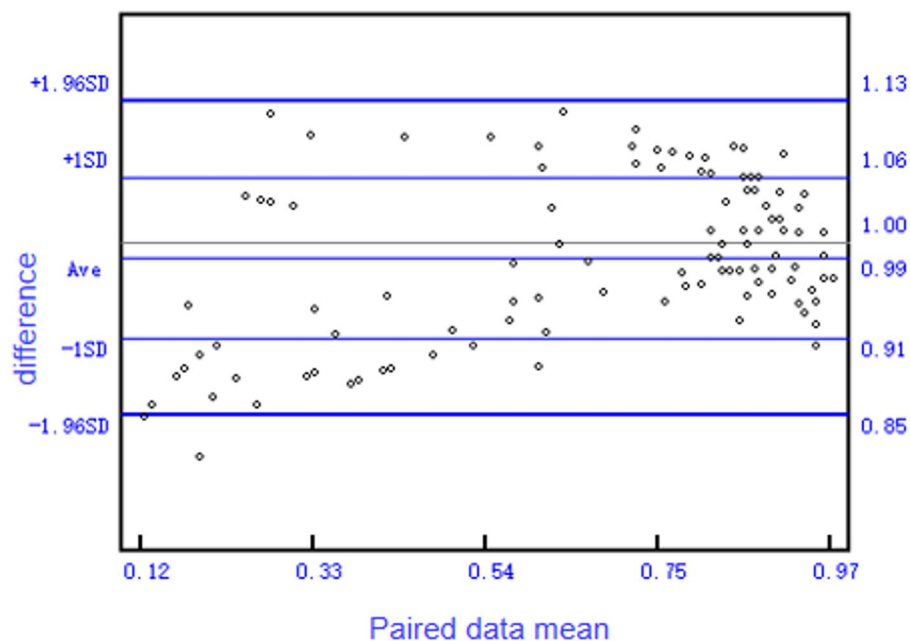


Fig. 4. Statistical analysis chart of the relative bias between the assessment system and the reference system.

specific threshold (decision point) that a physician uses when making a diagnosis or treatment decision based on test results. We selected two clinical decision points ($X_c=65\%$ and $X_c=55\%$) for analysis by calculating the expected bias values for these two points and their 95% confidence intervals and comparing them with clinically allowed bias ranges. The allowable bias values were determined based on the technical requirements of the EOF assay (scatter nephelometry method) from Shenzhen Pumen Technology Co., Ltd., in which the permissible deviation is defined as half of three times the coefficient of variation (CV) of the assay. Accordingly, the allowable bias was 7.8% at $X_c=65\%$ and 6.6% at $X_c=55\%$. At $X_c=65\%$, the confidence interval for expected bias was $[-0.011, 0.005]$, which is substantially lower than the allowable bias. At $X_c=55\%$, the confidence interval for expected bias was $[-0.034, 0.025]$, also within the allowable bias. These results indicate that the bias between the assessment and reference systems at these two decision points is consistent with clinical requirements and can be considered clinically acceptable.

This analysis shows that the automatic erythrocyte osmotic fragility analysis system based on scatter turbidimetry can completely replace the traditional reference system in clinical decision-making, and the bias of the two will not affect the diagnostic results in practical application. Through this validation, the clinical feasibility of the system in the osmotic fragility test of red blood cells, especially in diagnosing anemia and hemolytic anemia, provides information consistent with traditional methods and has the potential for a wide range of applications.

Discussion

To our knowledge, this is the first study evaluating the performance and clinical applicability of a fully automated erythrocyte osmotic fragility analysis system based on scatter turbidimetry. This study demonstrates the application of a fully automated erythrocyte osmotic fragility analysis system based on scatter turbidimetry, representing a significant innovation compared with traditional detection methods. Although widely used in clinical practice, conventional techniques, such as multi-tube and single-tube, involve extensive manual operations¹⁰. These processes are not only cumbersome but also yield results susceptible to operator experience and environmental factors, thereby reducing the accuracy and reliability of detection. Due to their biconcave shape, red blood cells (RBCs) exhibit distinct osmotic behavior in hypotonic solutions, leading to hemolysis and measurable changes in light scattering¹¹. The rate of this change correlates with the degree of osmotic fragility, which varies across hematological disorders. Elevated fragility is observed in conditions such as hereditary spherocytosis and autoimmune hemolytic anemia, whereas decreased fragility is typical in thalassemia and iron deficiency anemia^{4,12}. Traditional methods are limited by manual variability. This study uniquely integrates scatter turbidimetry into a fully automated system, enabling precise, high-throughput evaluation of RBC osmotic fragility in clinical diagnostics.

The fully automated detection system, based on scatter turbidimetry, enables automated sample injection, data collection, and analysis through precise light scattering technology, significantly reducing human error. A further key innovation lies in its high-throughput detection capability, making it particularly suitable for processing large samples in clinical settings. Through full automation, the system can produce accurate results in a short timeframe, thereby enhancing the efficiency of clinical laboratories especially in anemia screening and the diagnosis of hemolytic diseases. Implementing such automated systems in large-scale clinical screening programs can substantially improve testing efficiency and reduce operational costs. The findings of this study

indicate that Pumen Technology's fully automated erythrocyte osmotic fragility analysis system yields reliable measurements with high consistency, underscoring its clinical value in anemia screening. Compared to traditional methods, the automated system minimizes operator-dependent variability and enhances the stability and accuracy of results. In the early screening of anemia—particularly in patients with hereditary red blood cell morphological abnormalities or immune hemolytic anemia—the system delivers rapid and precise data, aiding clinicians in making more accurate diagnoses^{6,13}.

The system uses light-scattering technology to integrate sample injection, measurement, and data analysis. This automation reduces human error, minimizes inter-operator variability, and improves measurement consistency and reliability. In our study, the system supported high-throughput analysis, processing approximately 60–80 samples per hour. The average time required for analyzing a single sample was approximately 8 min, compared with 15–20 min using the reference method, reflecting improved operational efficiency. These results indicate that the system can facilitate faster and more consistent analysis in clinical testing.

Analytically, the system demonstrated strong agreement with the reference method. Linear regression analysis showed a correlation coefficient of 0.989, a slope of 1.0168, and an intercept approximating zero, indicating high concordance. Kappa consistency analysis yielded a value of 1.00, reflecting perfect agreement without misclassification. Bland-Altman analysis further confirmed the robustness, with 99.11% of data points falling within the ± 1.96 standard deviation limits and the observed bias remaining within clinically acceptable thresholds.

The system's automation minimizes human error and enhances detection stability, making it particularly advantageous for screening and diagnosing anemia, hemolytic anemia, and related disorders. The fully automated erythrocyte osmotic fragility analysis system based on scatter turbidimetry holds considerable promise as a standardized diagnostic tool, potentially improving both the efficiency of hematological disease diagnosis and the overall quality of medical services. Moreover, the erythrocyte osmotic fragility test method employed in this study can be completed within a short timeframe while delivering accurate results, meeting modern laboratories' need for efficient diagnostics. Its speed, precision, and simplicity offer significant advantages in anemia screening and provide robust technical support for future research on red blood cell function and the clinical diagnosis of hemolytic disorders. The system's automated nature makes it especially suitable for high-throughput testing in high-demand environments, such as hospitals and large medical examination centers. With growing clinical demands particularly in public health programs—the widespread use of automated erythrocyte osmotic fragility analysis systems may significantly enhance the quality of healthcare services while yielding substantial social and economic benefits^{13–15}. Therefore, Pumen Technology's automated erythrocyte osmotic fragility analysis system exhibits great potential and clinical value, aligning with the demands of modern clinical laboratories and providing more efficient and accurate testing services. With continued development and broader adoption, this technology is expected to become a routine tool for anemia and related disease screening, contributing to improved global health outcomes^{16–18}.

Nonetheless, several limitations should be acknowledged. Although the study includes a clinically diverse cohort, the sample size may not encompass rare pathological variants or borderline cases that could challenge the system's resolution. Additionally, while this study focused on analytical and categorical concordance, further validation is necessary to assess clinical impact such as diagnostic accuracy, therapeutic decision-making, and cost-effectiveness through prospective multicenter trials. Moreover, as a single-center study, the results may not fully capture variability across different laboratory settings. The potential for different behavior in rare RBC disorders not tested should be considered, and future studies should also evaluate reagent lot-to-lot variability to ensure consistent performance.

Conclusion

This study demonstrates that the fully automated erythrocyte osmotic fragility analysis system Shenzhen Pumen Technology Co., Ltd. provides accurate, consistent, and reliable clinical measurements. The system enhances detection efficiency and reduces operator variability, making it particularly valuable for anemia and hemolytic disease screening. Given its strong performance and operational advantages, this technology has significant potential for routine clinical application, improving diagnostic workflows and contributing to better patient care.

Data availability

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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References

- Emilse, L. A. M. et al. Cryohemolysis, erythrocyte osmotic fragility, and supplementary hematimetric indices in the diagnosis of hereditary spherocytosis. *Blood Res.* **53**, 10–17. <https://doi.org/10.5045/br.2018.53.1.10> (2018).
- Armbruster, D. A., Overcash, D. R. & Reyes, J. Clinical chemistry laboratory automation in the 21st Century - Amat Victoria Curam (Victory loves careful preparation). *Clin. Biochemist Reviews.* **35**, 143–153 (2014).
- Brestoff, J. R. Full spectrum flow cytometry in the clinical laboratory. *Int. J. Lab. Hematol.* **45** (Suppl 2), 44–49. <https://doi.org/10.1111/ijlh.14098> (2023).
- Zheng, S., Li, Q., Ou, T., Li, Y. & Wu, S. Clinical performance study of a new fully automated red blood cell permeability fragility analyzer. *J. Healthc. Eng.* **2022**, 5642907. <https://doi.org/10.1155/2022/5642907> (2022).
- Park, Y., Best-Popescu, C. A., Dasari, R. R. & Popescu, G. Light scattering of human red blood cells during metabolic remodeling of the membrane. *J. Biomed. Opt.* **16**, 11013. <https://doi.org/10.1117/1.3524509> (2011).

6. Shenzhen Keishi Biotechnology Co. L. Erythrocyte osmotic fragility determination method and kit thereof. <https://patents.google.com/patent/CN110530824B/en>
7. Beltrán, A. et al. Flow cytometry as a new accessible method to evaluate diagnostic osmotic changes in patients with red blood cell membrane defects. *Biomedicines* **12** <https://doi.org/10.3390/biomedicines12071607> (2024).
8. Ciepela, O. Old and new insights into the diagnosis of hereditary spherocytosis. *Annals Translational Med.* **6**, 339. <https://doi.org/10.21037/atm.2018.07.35> (2018).
9. Shimadzu UV-2450 Manuals. Molecular Spectroscopy. <https://www.manualslib.com/products/Shimadzu-Uv-2450-13945830.html>
10. Nobre, C. S., Silva, J. A., Jácomo, R. H., Nery, L. F. A. & Barra, G. B. Flow Cytometric Analysis of Erythrocytes Osmotic Fragility in Hereditary Spherocytosis: A Case-Controlled Study Evaluating the Best Anticoagulant, Sample Pre-Treatment and NaCl Concentration for Reliable Screening of this Red Blood Cell Membrane Disorder. *Cytometry Part B, Clinical cytometry* **94**:910–917. (2018). <https://doi.org/10.1002/cyto.b.21733>
11. Hoffman, J. F. Biconcave shape of human red-blood-cell ghosts relies on density differences between the rim and dimple of the ghost's plasma membrane. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 14847–14851. <https://doi.org/10.1073/pnas.1615452113> (2016).
12. Andres, O. et al. Hereditary spherocytosis is associated with decreased pyruvate kinase activity due to impaired structural integrity of the red blood cell membrane. *Br. J. Haematol.* **187**, 386–395. <https://doi.org/10.1111/bjh.16084> (2019).
13. Fucharoen, G., Sanchaisuriya, K., Sae-ung, N., Dangwibul, S. & Fucharoen, S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. *Bull. World Health Organ.* **82**, 364–372 (2004).
14. Fucharoen, S. et al. Prenatal diagnosis of thalassaemia and hemoglobinopathies in thailand: experience from 100 pregnancies. *Southeast Asian J. Trop. Med. Public Health.* **22**, 16–29 (1991).
15. Sanchaisuriya, K. et al. A reliable screening protocol for thalassaemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. *Am. J. Clin. Pathol.* **123**, 113–118. <https://doi.org/10.1309/fuf9evgq24v1pktp> (2005).
16. Sirichotiyakul, S., Maneerat, J., Sa-nguansermisri, T., Dhananjayanonda, P. & Tongsong, T. Sensitivity and specificity of mean corpuscular volume testing for screening for alpha-thalassaemia-1 and beta-thalassaemia traits. *J. Obstet. Gynaecol. Res.* **31**, 198–201. <https://doi.org/10.1111/j.1447-0756.2005.00280.x> (2005).
17. Angastiniotis, M. & Lobitz, S. Thalassemsias: an overview. *Int. J. Neonatal Screen.* **5**, 16. <https://doi.org/10.3390/ijns5010016> (2019).
18. Lai, K., Huang, G., Su, L. & He, Y. The prevalence of thalassaemia in Mainland china: evidence from epidemiological surveys. *Sci. Rep.* **7**, 920. <https://doi.org/10.1038/s41598-017-00967-2> (2017).

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None.

Author contributions

L. Ma, Y. Huang, T. Chen, F. Yang, X. Wang, F. Xiong, J. Tang, S. Zhang, and Y. Gao designed the study, performed the experiments, analyzed the data, and interpreted the results. L. Ma, Y. Huang, T. Y. Gao wrote the manuscript text and prepared all figures. All authors reviewed and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Chenjiaqiao Hospital (No. 2021-35). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was obtained from the participants in accordance with the national legislation and the institutional requirements.

Additional information

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