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ORIGINAL RESEARCH

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Results of a China external quality assessment program for seminal plasma biochemistry: a 5 years experience

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Abstract

Background: This 5-year external quality assessment (EQA) study evaluated laboratory performance in analyzing seminal plasma biomarkers (zinc, citrate, fructose, neutral α-glucosidase (NAG)), assessing inter-lab variability, methodological trends, and technique-related differences. **Methods**: From 2021 to 2025, a total of 113 laboratories participated in this EQA program. Standardized lyophilized seminal plasma samples were prepared from pooled ejaculates and adjusted to target biomarker concentrations. Participants analyzed these samples using their routine methods. **Results**: Over the five-year period, zinc exhibited the lowest mean coefficient of variation (CV) at 27.0% (range: 15.1–50.5%), with the Five-Br-2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (Five-Br-PAPS) method demonstrating superior precision (mean CV: 14.5%) compared to the resorcinol monosodium salt (PAR) (21.5%) and 1-(2-Pyridylazo)-2-naphthol (PAN) (19.9%) methods, though inter-method differences were not statistically significant. For citrate, the mean CV was 41.9% (range: 16.6–62.7%), with no significant difference between the citrate lyase (mean CV: 36.8%) and Fe^{3+} complexation methods (36.3%). Fructose analysis showed the highest variability, with a mean CV of 44.7% (range: 22.7–63.9%). The hexokinase method (mean CV: 39.5%) outperformed the indole method (49.5%), though the difference was not statistically significant (p = 0.213). NAG exhibited a mean CV of 42.0% (range: 25.1–59.1%), with the kinetic method (mean CV: 25.7%) showing significantly better precision than the endpoint method (44.2%). **Conclusions**: The study highlights inter-laboratory variability in seminal plasma biochemical testing, underscoring the need for standardized methodologies and enhanced quality control. Continuous EQA participation and targeted training are essential to improve diagnostic reliability in male infertility assessments.

Keywords

Seminal plasma biochemistry; External quality assessment; Male infertility; Laboratory variability

1. Introduction

Male infertility is a significant global health concern, affecting approximately 8–12% of couples attempting to conceive, with male factors contributing to nearly 50% of cases [1]. Poor semen quality may result from abnormal accessory gland secretions, which can significantly impair male fertility [2]. Seminal plasma, constituting over 95% of semen volume, contains biochemical markers that are crucial for evaluating the functional status of male accessory glands (prostate, seminal vesicles, and epididymis) and diagnosing infertility causes. Prostate secretions include zinc, citrate, γ-glutamyl transpeptidase, and acid phosphatase [3, 4]. Seminal vesicle activity is reflected in fructose and prostaglandin levels. Epididymal function is assessed through free L-carnitine, glycerophosphocholine (GPC), and neutral α-glucosidase (NAG) [5]. These biomarkers provide essential information about semen quality, sperm motility, and overall reproductive health [6–8].

With the growing recognition of male reproductive health importance, seminal plasma biochemical testing has been increasingly adopted worldwide. However, while numerous studies have investigated the clinical correlations between seminal biomarkers and male fertility parameters [6–9], standardization and quality control of these assays remain understudied. Only limited research has systematically addressed this issue. Lu *et al.* [10, 11] made contributions by establishing standardized protocols and quality control measures for key biomarkers, such as zinc and α-glucosidase, yet comprehensive quality control remain scarce.

Hamdi *et al.*'s [12] 6-year French external quality assessment (EQA) study highlighted critical quality concerns, revealing: acceptable inter-laboratory consistency for prostate

(citrate, zinc) and seminal vesicle (fructose) markers, and significant analytical variability in epididymal biomarkers (particularly NAG) [12]. These findings underscore the urgent need for improved standardization and quality control. EQA programs are essential for standardizing laboratory performance, identifying analytical errors, and improving the accuracy of test results [13]. While EQA programs for blood biochemistry are well-established globally, similar initiatives for seminal plasma biochemistry remain limited.

This study aims to share our experience in the EQA program of four clinically relevant biomarkers (zinc, citrate, fructose, and NAG), while evaluating inter-laboratory variations and methodological performance.

2. Materials and methods

2.1 EQA program organization

The EQA program for seminal plasma biochemistry was conducted over a 5-year period (2021–2025). The program was organized by the Laboratory of Reproductive Andrology, West China Second University Hospital of Sichuan University (WCSUH-SCU), China, which is an International Organization for Standardization (ISO) 9001 certified laboratory. The study protocol was approved by the ethics review board of WCSUH-SCU (Institutional Review Board (IRB) No. 2023-072), and informed consent was obtained from the patients. Participating laboratories were required to analyze seminal plasma samples for four biomarkers: zinc, citrate, fructose, and NAG. These biomarkers were selected based on their clinical relevance in evaluating the functional status of male accessory glands (prostate, seminal vesicles, and epididymis). Annual workshops were conducted to discuss EQA findings, standardize methodologies, and promote best practices.

2.2 Sample preparation and data collection

The samples used in this study were prepared from residual specimens of semen analyses performed on patients attending the Andrology Department of our hospital, with specimens collected after 2–7 days of sexual abstinence. The mixing process of multiple samples was completed in one day. We centrifuged the samples at 3000×g for 15 minutes to remove spermatozoa and cellular debris. After adding the lyoprotectant, standard solutions were used to adjust the concentrations of the biochemical markers to the target levels. The organizer tested the prepared quality control materials through its internal testing system to roughly assess the initial levels of the samples, ensuring that the quality control materials covered different concentration ranges. The seminal plasma was then aliquoted into lyophilization vials (2 mL/vial) and flash-frozen at −80 °C to form a solid matrix. The ice crystals were then removed via sublimation in a freeze dryer (−40 °C). The homogeneity and stability of the samples were assessed according to the ISO13528 guidelines [14]. For each survey, two samples with distinct biomarker concentrations were distributed to participating laboratories. Samples were shipped at 4 °C to ensure stability during transportation. Laboratories were instructed to dissolve the sample with 1 mL pure water, process the samples using their routine methods, and return the results within two weeks of receipt. Participating laboratories submitted their results, including the analytical methods used, instrument details, and reagent brands.

2.3 Statistical analyses

Consensus values and coefficients of variation (CVs) were calculated after outlier exclusion using Tukey's criterion (1.5 × interquartile range) in accordance with ISO 13528:2022 guidelines. All statistical analyses were performed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA), with continuous data presented as means for normally distributed variables and medians for non-normally distributed data. The homogeneity of samples was evaluated by one-way analysis of variance (ANOVA). The stability of the samples was assessed using an independent sample *t*-test. Method comparisons were conducted using one-way ANOVA for zinc CV values and independent samples *t*-tests for citrate, fructose, and NAG CV values. Concentration values were compared using the Kruskal-Wallis test for zinc and Wilcoxon signed-rank test was used for the other analytes, with statistical significance set at $p < 0.05$. Due to limited data availability, CV calculations and inter-method comparisons were excluded for: (1) the 2021 Five-Br-PAPS (zinc), indole (fructose), and rate (NAG) methods, and (2) the 2022 citrate lyase method, as only single measurements were obtained for these method-year combinations.

3. Results

3.1 Basic information about participants

From 2021 to 2025, the number of laboratories participating in the seminal plasma biochemical EQA program increased year by year. By 2025, there were a total of 113 participants. Among them, the numbers of laboratories involved in zinc, citrate, fructose, and NAG testing were 112, 42, 97, and 113, respectively. The citrate program was implemented in 2022 (Fig. 1).

Hospitals participating in the EQA were predominantly tertiary-level institutions, accounting for 85%, while secondary- and primary-level hospitals made up 4% and 12%, respectively. The laboratories participating in the EQA were primarily andrology laboratories (87.5%), with clinical laboratories accounting for the remaining 12.5%.

3.2 Seminal plasma zinc

The PAR method for zinc remained dominant throughout the study, used by 66 laboratories (58.9%) in 2025 (Fig. 2A). In contrast, Five-Br-PAPS adoption rose significantly from 11.1% (2021) to 25.0% (2025, 28 laboratories). The PAN method stayed stable, with 18 users (16.1%) in 2025. The overall mean CV of the 10 samples for zinc in the whole period was 27.0% (range: 15.1–50.5%) (Fig. 2B). The highest variability was observed in 2024. Five-Br-2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (Five-Br-PAPS) showed lower variability (mean CV: 14.5%, range: 5.5–26.9%) compared to both 1-(2-Pyridylazo)-2-

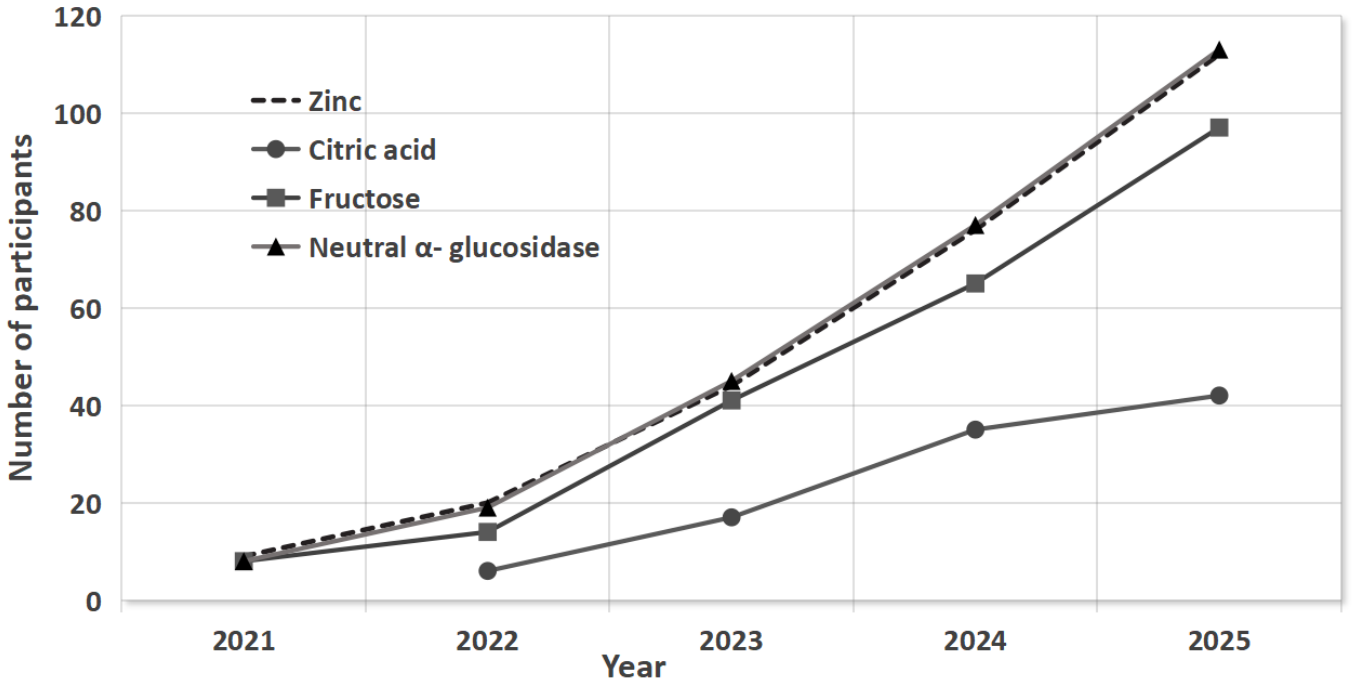

FIGURE 1. The number of participants in the EQA program for seminal biomarkers (zinc, citrate, fructose, and neutral α-glucosidase) in the period of 2021 to 2025.

naphthol (PAN) (mean CV: 19.9%, range: 4.2–39.4%) and 4-(2-Pyridylazo) resorcinol monosodium salt (PAR) methods (mean CV: 21.5%, range: 15.7–30.6%). However, no statistically significant differences were observed among the three methods ($p = 0.290$) (Fig. 2C).

Among the eight samples analyzed between 2021 and 2024, the PAR method consistently yielded slightly lower values compared to the other two analytical methods. Statistical analysis revealed significant differences ($p < 0.05$) in three specific samples: 2022-A, 2024-B, and 2025-A, with the most pronounced variations observed between the Five-Br-PAPS and PAR methods. No statistically significant differences were detected in the remaining samples (all $p > 0.05$) (Fig. 2D).

3.3 Seminal plasma citrate

From 2021 to 2025, two methods—citrate lyase and Fe^{3+} complexation—were used for citrate quantification. During this period, the Fe^{3+} complexation method was the dominant one, with 30 laboratories (70.5%) employing it in 2025 (Fig. 3A).

The overall mean CV for citrate across all methods was 41.9% (range: 16.6–62.7%) (Fig. 3B). The CV obtained by the Fe^{3+} complexation method (mean: 36.3%; range: 16.9–49.0%) showed no statistically significant difference compared to those obtained by the citrate lyase method (mean: 36.8%; range: 13.5–61%) ($p = 0.954$) (Fig. 3C).

Across the six samples analyzed from 2022 to 2024, the median values obtained by the citrate lyase method were consistently slightly higher than those from the Fe^{3+} complexation method. However, statistical significance ($p < 0.05$) was only observed in the 2025-A sample, with no significant differences detected in the remaining five samples (all $p > 0.05$) (Fig. 3D).

3.4 Seminal plasma fructose

Between 2021 and 2025, two primary methods were employed for fructose quantification: the indole method and hexokinase method. Throughout this period, the hexokinase method remained dominant with consistently high utilization rates (80–90% annually). In 2025, it was used by 80 laboratories, accounting for 82.5% of the total (Fig. 4A).

The overall mean CV for fructose across all methods was 44.7% (range: 22.7–63.9%) (Fig. 4B). The indole method demonstrated higher variability (mean CV: 49.5%; range: 22.8–63.1%) compared to the hexokinase method (mean CV: 39.5%; range: 17.0–67.7%), though this difference did not reach statistical significance ($p = 0.213$) (Fig. 4C). Particularly, the hexokinase method exhibited a consistent temporal decline in CV values.

No significant difference was observed between enzymatic and indole methods for fructose values in the 2022–2023 ($p > 0.05$) samples. However, analysis of the 2024–2025 samples revealed significantly higher fructose values obtained by the indole method compared to the enzymatic assay ($p < 0.05$) (Fig. 4D).

3.5 Seminal plasma neutral α-glucosidase (NAG)

During the study period (2021–2025), all NAG analyses were performed enzymatically, including endpoint and kinetic methods, with the endpoint method being predominant. In 2025, this method was utilized by 92 laboratories, accounting for 81.4% (Fig. 5A). The overall mean CV across all analytical methods was 42.0% (range: 25.1–59.1%) (Fig. 5B). When stratified by methodology, the kinetic method exhibited significantly lower variability (mean CV: 25.7%; range:

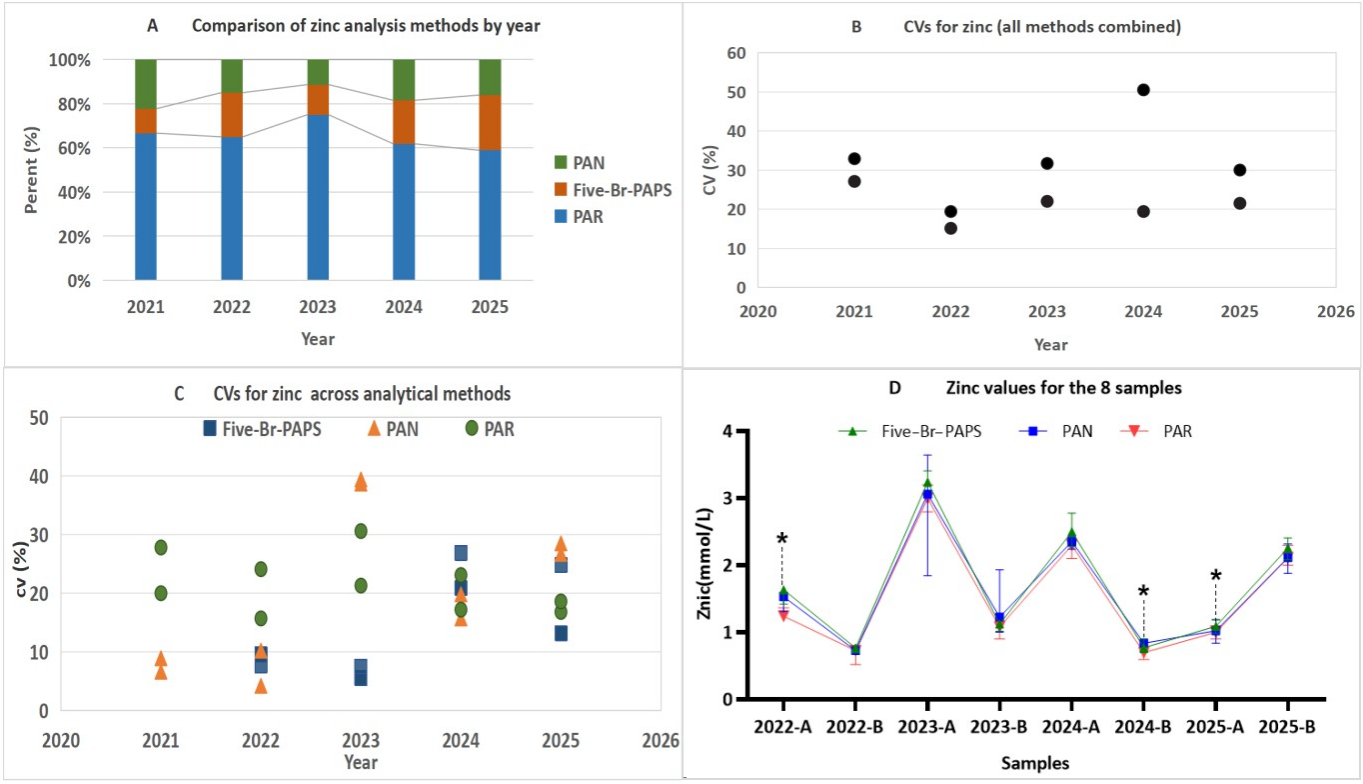

FIGURE 2. Comparative evaluation of zinc detection methodologies (2021–2025). (A) Method utilization trends, (B) Overall coefficient of variation (CV), (C) Method-specific CV comparisons, and (D) Comparison of zinc values using various analytical methods. PAN: 1-(2-Pyridylazo)-2-naphthol; PAR: 4-(2-Pyridylazo) resorcinol monosodium salt; Five-Br-PAPS: 2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol. *: $p < 0.05$ (significant difference between methods).

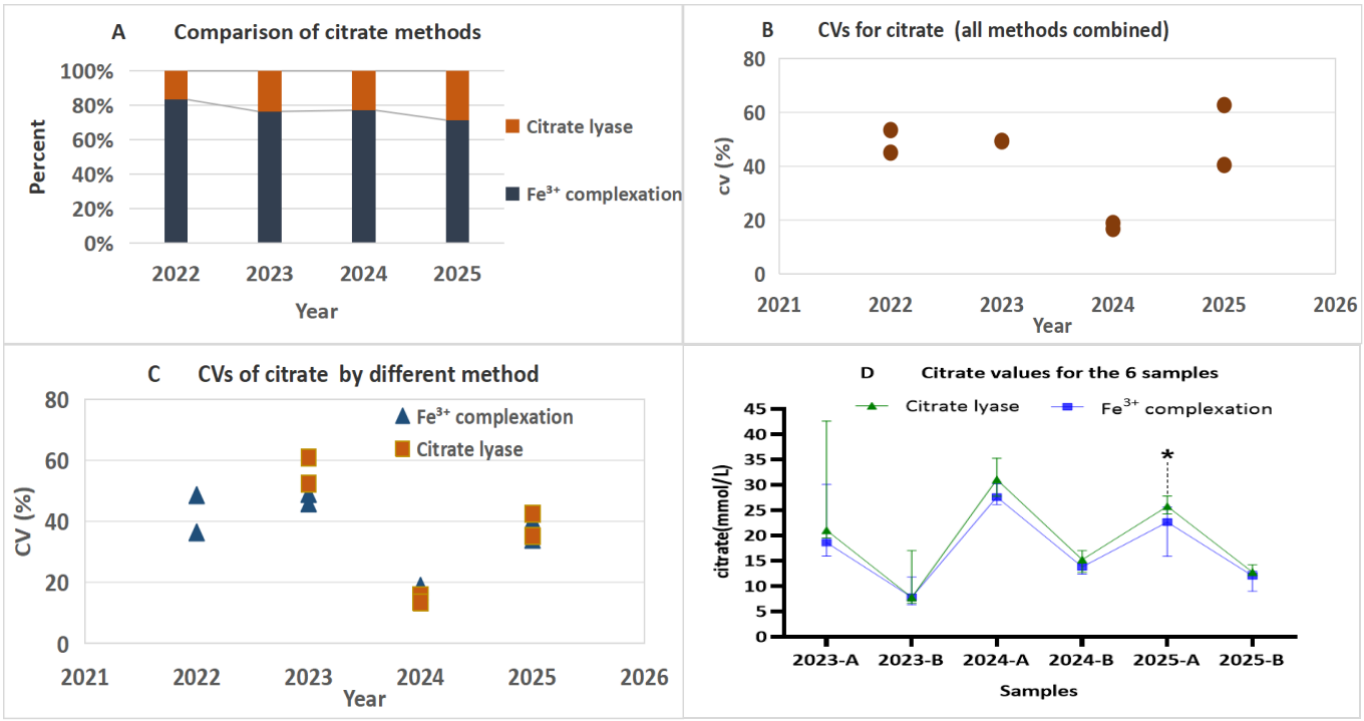

FIGURE 3. Comparative performance evaluation of citrate detection methodologies (2022–2025). (A) Method utilization trends for citrate, (B) Overall coefficient of variation (CV) across all methods, (C) Method-specific CV comparison, and (D) Comparison of citrate values using various analytical methods. *: $p < 0.05$ (significant difference between methods).

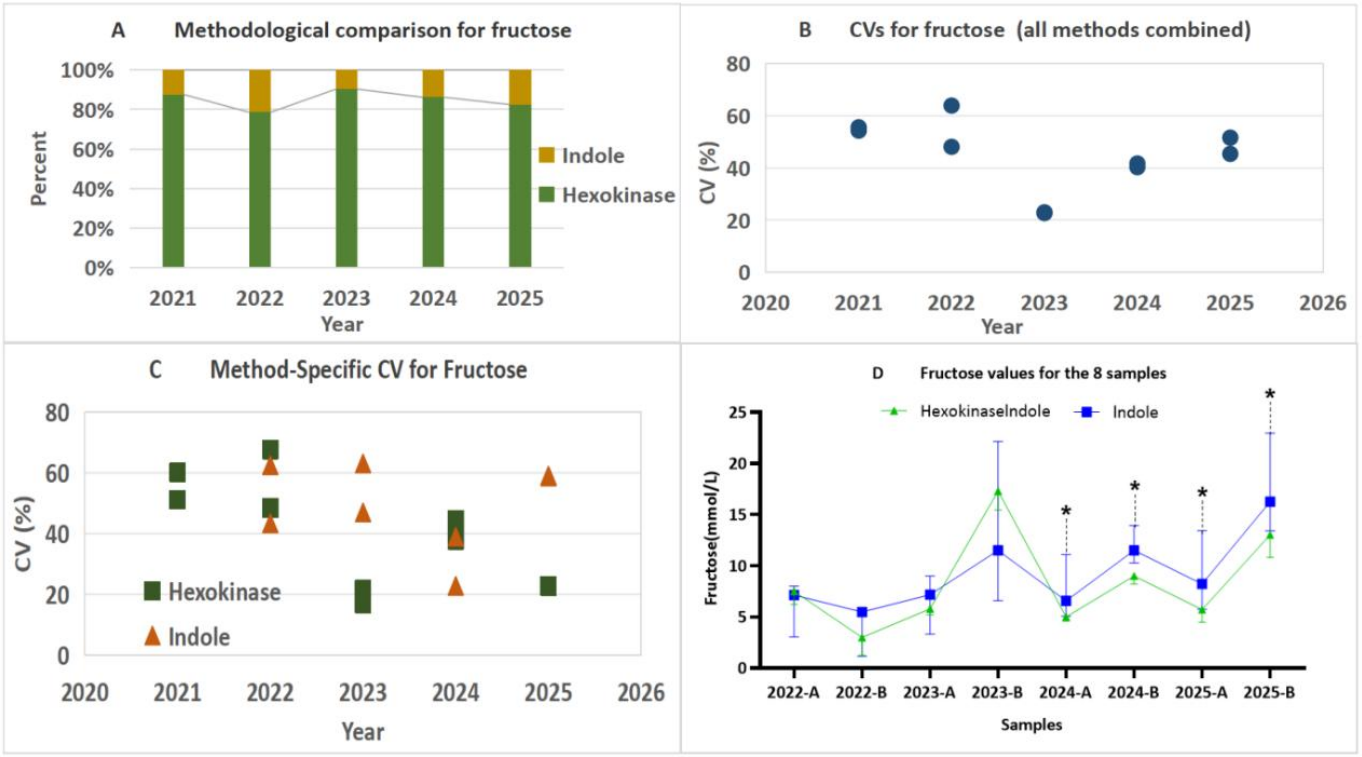

FIGURE 4. Methodological performance summary of fructose (2021–2025). (A) Method utilization trends for fructose, (B) Overall coefficient of variation (CV) across all methods, (C) Method-specific CV comparison, and (D) Comparison of fructose values using various analytical methods. *: $p < 0.05$ (significant difference between methods).

9.6–46.1%) compared to the endpoint method (mean CV: 44.2%; range: 20.4–56.6%), with this difference reaching statistical significance ($p = 0.006$) (Fig. 5C). The kinetic method exhibited a consistent year-over-year decrease in CV values.

For all but one sample (2025-B), the kinetic method demonstrated significantly higher mean NAG measurements compared to the endpoint method across the remaining seven specimens (all $p < 0.05$) (Fig. 5D).

4. Discussion

Seminal plasma biomarkers, first building upon the seminal work by Mann [15] and Lundquist [16], are now widely used to study male reproductive gland pathophysiology [17, 18] and were later adopted by the World Health Organization (WHO) [19, 20]. Despite the increasing clinical adoption, these assays face significant challenges in standardization and quality control. To our knowledge, this is the first study to systematically analyze methodological trends and inter-laboratory variability in seminal plasma biochemistry across a large cohort of laboratories. Previous reports, such as the French EQA program by Hamdi *et al.* [12], included fewer than 10 participants and focused primarily on compliance with ISO standards rather than methodological comparisons. In contrast, based on a national EQA program, our study not only evaluated the performance of 113 laboratories, but also provided longitudinal data on method utilization, CV trends, and method-specific biases.

The results of this EQA program revealed high variability, with zinc exhibiting the lowest 5-year mean CV at 27.0%, while citrate, NAG, and fructose all showed mean CVs exceeding 40% (41.9%, 42.0%, and 44.7%, respectively). These findings are inconsistent with previous reports, which suggested that NAG had the highest variability, followed by zinc, fructose, and citrate. Particularly, the temporal trends in CV differed among biomarkers: zinc, citrate, and fructose displayed significant fluctuations without a clear downward trend, whereas NAG demonstrated progressively improved precision over time. This likely reflects methodological advancements (*e.g.*, increased adoption of kinetic assays for NAG) and enhanced laboratory proficiency over time. The persistently high coefficients of variation (CVs) observed for citrate and fructose measurements may be attributed to multiple factors: (1) lack of standardized operational protocols across laboratories, leading to technician-dependent variability; (2) inherent methodological limitations of current detection techniques; (3) substantial inter-reagent variability among different commercial brands; and (4) insufficient quality control systems in routine practice.

A good seminal biomarker should demonstrate strong consistency in detection results across different analytical methods. However, our findings revealed significant discrepancies in measured values between methodologies. For zinc detection, the Five-Br-PAPS method demonstrated superior precision compared to the PAR and PAN methods, although the inter-method differences in CV did not reach statistical significance. Notably, statistically significant discrepancies were observed between Five-Br-PAPS and PAR methods for three specific samples, suggesting that methodological selection may partially account for the

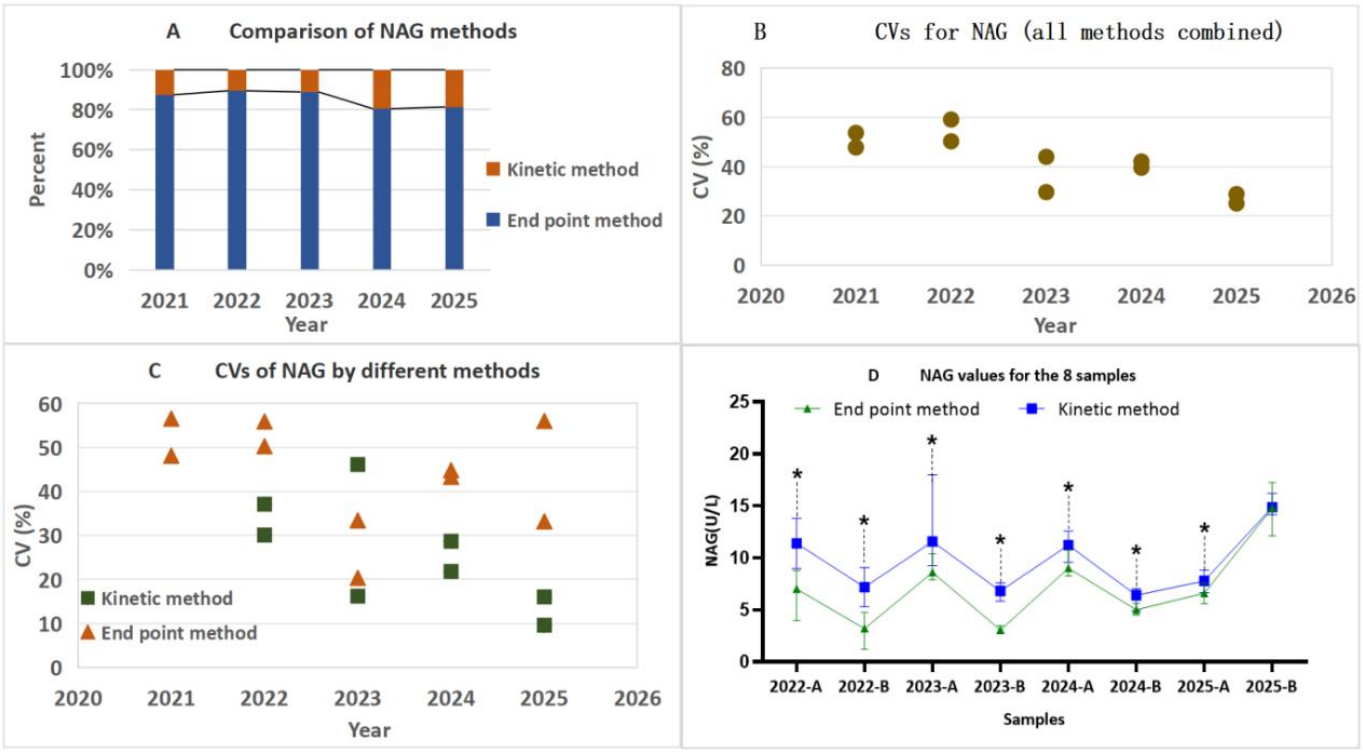

FIGURE 5. The overall implementation and performance characteristics of neutral α-glucosidase (NAG) detection during 2021–2025. (A) Comparison of analytical methods, (B) Overall coefficient of variation (CV), (C) CV stratified by analytical methods, and (D) Comparison of NAG values using various analytical methods. *: $p < 0.05$ (significant difference between methods).

observed variability. For citrate detection, both the citrate lyase method and Fe^{3+} complexation method exhibited high overall variability. No significant temporal improvement in CVs was observed. The absence of a dominant low-CV method underscores the necessity for methodological optimization to reduce inter-laboratory discrepancies. For fructose detection, both the hexokinase method and indole method demonstrated high variability, with the hexokinase method exhibiting superior precision. A progressive decline in CVs was observed for the hexokinase method over time. Strikingly, significant differences between the two methods were identified in the 2024–2025 samples, revealing method-dependent biases. For NAG analysis, the kinetic method demonstrated significantly better precision than the endpoint method, along with consistent year-over-year improvement as evidenced by progressively declining CVs. Statistically significant differences were observed between the two methods for seven out of eight samples, underscoring the critical impact of methodological selection in reducing variability.

This study has certain limitations. First, the EQA program evaluated only two samples annually, which is clearly insufficient. We have already initiated measures to increase the frequency of inter-laboratory proficiency testing rounds. Second, there is a lack of analysis on intra-laboratory technician variability. Some participating laboratories employ multiple rotating technicians, whose subtle differences in handling the details of experimental procedures may potentially affect the consistency of test results.

Given the absence of an internationally recognized gold standard for seminal plasma biochemical testing, laboratories are advised to prioritize methods demonstrating both lower CV and broader adoption, based on the following rationale: (1) methods with lower CVs have shown more stable performance in longitudinal EQA data; and (2) widely adopted methods present greater feasibility for standardization. Nevertheless, laboratories should conduct internal validation and perform regular EQA comparisons to verify method applicability, with timely protocol adjustments implemented as necessary.

To enhance the reliability of seminal plasma biochemical testing, we recommend the following measures: Proficiency testing providers should organize targeted training programs to assist participants in selecting appropriate methodologies, implementing standardized operating procedures, and establishing robust internal quality control systems, as well as conducting benchmarking and quality assessments for technicians. Concurrently, participants must heighten their awareness of the importance of EQA programs, actively utilize EQA feedback to improve overall testing quality, and maintain consistent, long-term participation in these quality evaluation initiatives.

5. Conclusions

This large-scale EQA study systematically evaluates methodological trends and quality control challenges in seminal plasma biochemical testing. The results reveal substantial inter-laboratory variability and methodological discrepancies in zinc, citrate, fructose, and NAG

measurements. These findings highlight the critical need for technical training programs covering analytical methodologies, standardized operating procedures, and robust internal quality control systems. Furthermore, laboratories should actively utilize EQA feedback to improve testing accuracy and consistency, ultimately enhancing diagnostic reliability in male infertility assessments.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

YZ, QYC, FPL—designed the research study. LJY, LY, YBW, YL, CCD, YM—performed the research. JTY—analyzed the data. YZ, FPL—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the ethics review board of West China Second University Hospital of Sichuan University (WCSUH-SCU) (IRB No. 2023-072), and informed consent was obtained from the patients.

ACKNOWLEDGMENT

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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