ORIGINAL ARTICLE

Evaluation of analytical performance of Sebia Capillarys 3 Octa electrophoresis method for serum protein electrophoresis

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ABSTRACT

Introduction: Protein electrophoresis is a crucial test in clinical diagnostic laboratory, aimed for evaluation of plasma protein distribution. With its capability for high-resolution protein separation, rapid analysis and automated features, capillary electrophoresis (CE) has emerged as a valuable alternative to the traditional gel-based methods that are widely used in our country. This study aimed to evaluate the analytical performance of Sebia Capillary 3 Octa CE system in our laboratory setting.

Materials and Methods: The study was conducted at the Protein Diagnostic Laboratory, Hospital Pakar Universiti Sains Malaysia. Within-run and between run precision was assessed using Sebia Capillarys 3 Octa CE system with commercially available normal and pathological control sera. Accuracy was evaluated by comparing results from the Sebia Capillarys 3 Octa with those from the Sebia Hydrasys 2 Scan gel electrophoresis analyzer using both healthy and patient serum samples. Reference interval verification involved testing serum from healthy volunteers. Statistical analyses included mean, standard deviation, coefficient of variation (CV), linear regression, and Bland-Altman analysis.

Results: Sebia Capillarys 3 Octa demonstrated good precision across all serum protein fractions, with within-run CVs for normal serum ranging from 0.97% (albumin) to 7.04% (alpha-1), and between-run CVs below 7.22%. Pathological serum showed CVs from 0.60% (gamma) to 5.09% (alpha-1), and from 0.89% (albumin) to 10.32% (alpha-2) for within-run and between run CV, respectively. CE correlated strongly with gel electrophoresis for albumin and gamma globulin (r > 0.95), with alpha 1, alpha2 and beta showed good correlation (r >0.80) between the two methods. There was minimal bias (–1.1 to +2.1) noted. Reference interval verification confirmed compatibility with manufacturer-provided ranges.

Conclusion: Sebia Capillarys 3 Octa provides reliable, automated analysis for serum protein fractions, offering performance comparable to the conventional agarose gel electrophoresis analyzer with enhanced operational benefits for routine laboratory use.

KEYWORDS:
Capillary electrophoresis, Sebia Capillarys 3 Octa, protein electrophoresis, analytical evaluation, method verification

INTRODUCTION

Capillary electrophoresis (CE) is an analytical technique that separates charged molecules in solution based on their electrophoretic mobility under an electric field.[1] Its efficiency, sensitivity and high resolution have led to its significant impacts in recent years for analyzing complex biological mixtures, especially in healthcare diagnostics.[2] In particular, CE has been increasingly applied in protein electrophoresis for the separation of major serum protein fractions i.e. albumin, alpha-1, alpha-2, beta, and gamma globulins.[3] Protein electrophoresis plays a crucial role in the diagnosis and monitoring of plasma cell dyscrasias through the detection of monoclonal immunoglobulins and related electrophoretic abnormalities indicative of conditions such as amyloidosis.[4-6]

The foundational principles of CE date back to the 1950s, with significant technical advances made in the 1980s by Jorgenson and Lukacs[7], who pioneered the concept of high-efficiency separations in capillary formats. Modern CE systems offer enhanced analytical performance, shorter turnaround times, and automation capabilities compared to traditional gel-based methods.

In compliance with ISO 15189:2022, clinical laboratories are mandated to verify a method's analytical performance prior to clinical implementation. This includes precision studies encompassing within-run and between-run variability according to CLSI EP15-A3 guidelines for method verification and error management.[8] Precision verification is essential, as analytical imprecision may result in misleading results that can impact patient management.[9-11] Accuracy, defined as the closeness of a measurement to the true value, is assessed via method comparison with a validated reference method using patient specimens, as outlined in CLSI EP09.[12] Such evaluations help identify potential biases and assess the clinical suitability of a new method. In addition, CLSI EP28-A3c provides guidance for verifying reference intervals, ensuring that the laboratory reference ranges are appropriate for the local patient population.[13]

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The SEBIA Capillarys 3 Octa system is a fully automated CE platform optimized for routine clinical serum protein electrophoresis, offering high-resolution profiles with over 95% concordance with gel electrophoresis results.[14,15] Nevertheless, gel electrophoresis remains widely practiced in Malaysian laboratories due to its cost-effectiveness, operational familiarity, and visual interpretability.[16] Despite its analytical performance that has been validated in other countries, the utility of CE in Malaysia remains limited. The study on Sebia Capillarys 3 Octa system in Malaysia is important as it provides valuable data for the implementation of this method in the country. The analytical performance verification using our population sample strongly supports its applicability, to suit our local laboratory workflow and healthcare system. Hence, our study aims to evaluate this by performing precision, accuracy studies, and reference interval verification of the system in our centre.

MATERIALS AND METHODS

The experimental study was conducted in the Specialized Diagnostic Protein Laboratory, Department of Chemical Pathology, Hospital Pakar Universiti Sains Malaysia (HPUSM), Kubang Kerian, Kelantan.

Sample collection

The determination of sample size followed international standards, specifically the Clinical and Laboratory Standards Institute (CLSI) guidelines and ISO 15189:2022 Medical Laboratory Accreditation requirements for method verification.[11-13] These standards were used to guide the minimum number of replicates and samples needed for evaluating analytical performance, including precision, accuracy, and reference interval verification. For the precision study, commercially available quality control materials i.e. normal and hypergamma controls were utilized. The accuracy study involved 40 fresh serum samples of healthy and diseased subjects, ensuring a representative range of electrophoretic protein patterns. For the reference interval verification study, at least 20 serum samples were collected from healthy adult subjects. For both accuracy study and reference interval verification studies, individual sample from every study subject were analysed.

For experiments which required serum sample collection, study subject recruitment was done according to specific inclusion and exclusion criteria. For healthy subjects, volunteers were recruited through advertisement posters displayed in hospital areas accessible to the general public, including patients' family members and hospital staff. For diseased group, data was retrieved from existing laboratory records. The study was approved by the Human Research Ethics Committee USM (HREC), and written informed consent was obtained from all subjects.

Laboratory measurement

Laboratory analysis involved total protein measurement using the biuret method on the Roche Cobas E701 analyzer. Serum protein electrophoresis was conducted using Sebia Capillarys 3 Octa. For method comparison, Sebia Hydrasys 2 protein electrophoresis analyzer were used.

Statistical analysis

Data was analysed using MedCalc version 23.2.0 software. The mean, standard deviation (SD), and coefficient of variation (CV) were calculated to assess within-run imprecision (repeatability) and between-run imprecision (reproducibility). The CVs obtained were compared with the manufacturer's claims and, if necessary, against the upper verification limits to evaluate acceptability. Linear regression and Bland-Altman plots were used to evaluate both the correlation coefficient (r-value) and the degree of bias between methods. A correlation value closer to +1 indicates a stronger positive correlation, while values approaching -1 indicates a strong negative correlation; a value of 0 indicates no correlation. For reference interval verification, the number of outliers was assessed according to CLSI EP28-A3 guidelines, with two or fewer outliers among 20 samples considered acceptable for verification of the manufacturer's reference interval.

RESULTS

Precision study

Table I and II display the precision study for both normal serum and pathological serum in 8 capillaries, including within-run and between-run CV. For the normal serum, all coefficients of variation (CV) values for the six protein fractions were within acceptable ranges, as determined by either the manufacturer's claim or the upper verification limit. The within-run precision was highest for albumin, which had the lowest CV 0.97%, while Alpha-1 had the highest within-run CV 7.04%. Similarly, between-run precision remained within acceptable limits, with albumin averaging 0.88%, while Alpha-1 ranged from 4.59% to 7.22%.

In the pathological serum, all CV values were also within acceptable limits. The within-run CV was lowest for Gamma of 0.6%, indicating excellent precision, while alpha-2 showed the highest within-run variability 10.32%. Between-run CV values for albumin ranged from 0.89% to 2.07%, whereas Alpha-2 varied between 4.91% and 10.32%. This analysis demonstrates the reliability of capillary electrophoresis in measuring serum protein fractions, with all fractions falling within acceptable CV limits.

Accuracy study

Table III represent the comparison between the Sebia Capillarys 3 Octa and Sebia Hydrasys 2 Scan gel electrophoresis systems. The correlation coefficient (r) indicates excellent correlation for albumin and gamma globulin (r>0.95), while other fractions showed good correlation (r>0.80). The slope and intercept values for albumin, alpha 2 and gamma supported the strong linear relationship between the two systems for the analytes. However, minor discrepancies were observed for alpha-1 and beta regions.

Bland-Altman analysis showed biases ranging from -1.1 to 2.1 for all protein fractions, with 95% differences within ±1.96 standard deviations. These findings demonstrate high agreement between the two methods. The small observed biases are within the clinically acceptable limit, thus unlikely to affect the test interpretation and medical decision.

Table I: Precision study for normal serum using 8 capillaries for each protein fraction

Fraction	Run	Mean (%)		Standard Deviation (%)		Coefficient of variation (%)		Manufacturer's claim CV (%)
		Lowest	Highest	Lowest	Highest	Lowest	Highest	
Albumin	Within run	60.80	61.69	0.02	0.11	0.97	1.33	2.00
	Between run			0.61	1.12	0.99	1.85	
Alpha-1	Within run	4.09	4.33	0.14	0.31	3.24	7.04*	7.00
	Between run			0.19	0.31	4.59	7.22*	
Alpha-2	Within run	8.74	9.09	0.29	0.47	3.19	5.34	7.00
	Between run			0.31	0.46	3.58	5.10	
Beta-1	Within run	6.39	6.62	0.14	0.34	2.16	5.29	7.00
	Between run			0.25	0.35	3.78	5.51	
Beta-2	Within run	4.73	4.90	0.14	0.26	2.96	5.38	7.00
	Between run			0.14	0.26	2.89	5.26	
Gamma	Within run	14.05	14.41	0.18	0.24	1.27	1.73	4.00
	Between run			0.22	0.30	1.54	2.08	

*Upper verification limit was applied

Table II: Precision study for pathological serum using 8 capillaries for each protein fraction

Fraction	Run	Mean (%)		Standard Deviation (%)		Coefficient of Variation (%)		Manufacturer's claim CV
		Lowest	Highest	Lowest	Highest	Lowest	Highest	
Albumin	Within run	48.81	49.70	0.40	0.79	0.81	1.62	2.00
	Between run			0.44	1.01	0.89	2.07*	
Alpha-1	Within run	3.32	3.58	0.02	0.30	5.09	8.28*	7.00
	Between run			0.21	0.35	6.29	9.93*	
Alpha-2	Within run	6.86	7.34	0.23	0.58	3.50	7.84*	7.00
	Between run			0.36	0.74	4.91	10.32*	
Beta-1	Within run	4.97	5.34	0.14	0.24	2.68	4.80	7.00
	Between run			0.18	0.36	3.61	7.26*	
Beta-2	Within run	3.42	4.82	0.10	0.19	2.90	4.92	7.00
	Between run			0.18	0.30	4.20	7.57*	
Gamma	Within run	31.49	31.76	0.19	0.70	0.6	2.49	4.00
	Between run			0.45	0.72	1.36	2.27	

*Upper verification limit was applied

Table III: Comparison between Sebia Octa 3 Capillary and Sebia Hydrasys 2 Scan Gel electrophoresis for each protein fraction

Protein fraction	Correlation coefficient (r value)	Slope	Intercept	Mean bias
Albumin	0.99	0.873	3.071	2.10
Alpha-1	0.80	0.840	1.215	0.93
Alpha-2	0.94	0.721	1.194	0.78
Beta	0.81	1.136	-0.375	-0.7
Gamma	0.99	0.973	1.45	-1.1

Correlation coefficient (r), slope, intercept, and mean bias comparing protein fraction measurements between two electrophoresis methods

Table IV: Comparison of observed serum protein fraction with manufacturer's reference interval

	Observed Mean (%)	Observed Upper limit (%)	Observed Lower limit (%)	Manufacturer's reference interval n=246 (%)
Albumin	58.9	55.1	65.1	55.8 - 66.1
Alpha-1	3.5	2.9	4.2	2.9 - 4.9
Alpha-2	8.4	7.2	9.9	7.1 - 11.8
Beta-1	5.9	5.3	6.5	4.7 - 7.2
Beta-2	5.2	3.9	5.7	3.2 - 6.5
Gamma	18.2	12.0	18.8	11.1 - 18.8

Observed mean, lower, and upper limits were calculated based on the study population. Verification was considered acceptable if ≥95% of observed values fell within the manufacturer's reference intervals

Reference interval study
Table IV presents the reference interval verification study. The observed data, expressed as percentages, fall within the manufacturer's reference intervals for all protein fractions. Albumin met the criteria with 18 out of 20 data points within the range, while other fractions were fully verified. This demonstrates the reliability of the reference intervals for the local population studied.

DISCUSSION

Our study aimed to evaluate the analytical performance of serum protein electrophoresis (SPE) using the Capillarys 3 Octa system by Sebia. Our findings collectively support the reliability and clinical applicability of capillary electrophoresis (CE) as an effective methodology for routine protein fractionation in a clinical laboratory setting.

Precision study
The within-run and between-run demonstrated excellent reproducibility for albumin and gamma globulins, with coefficients of variation (CVs) ranging from 0.81% to 2.29%. The finding was aligned with previous research finding by Bossuyt et al.[17] who reported CVs between 0.3% and 3.5% for the Capillarys system, affirming the high reproducibility of CE-based SPE systems across various laboratory settings. However, imprecision was notably higher in the alpha-1 and alpha-2 globulin regions, with CVs ranging from 5.34% to 10.32%. This is consistent with previous findings by Lebricon et al., who observed lower precision in these zones, with CVs reaching 10–25%, attributed to protein heterogeneity and challenges in automated integration.[18] Capillarys, in particular, showed higher inter-day CVs in the alpha-2 globulin region (>6%) compared to intra-day CVs (<4%).[17]

RICOS biological variation data provides desirable specifications for imprecision, bias, and total allowable error, derived from intra- and inter-individual biological variation.[19] For albumin, the desirable CV for imprecision is set at 1.6%, aligning closely with the manufacturer's specifications. However, for α1- and α2-globulin fractions, the biological variation data suggests higher acceptable CVs, reflecting the inherent challenges in resolving these fractions due to overlapping peaks and lower signal intensity.[19] Nonetheless, all observed CVs were within acceptable thresholds, particularly when compared against the upper verification limit (UVL). The usage of UVL for CVs exceeding the manufacturer's specification is critical in ensuring analytical reliability, especially in pathological samples where variability may exceed expected thresholds. To limit false rejection due to chance, the UVL for repeatability was calculated and compared against imprecision estimates. This approach reduces the likelihood of erroneous result rejection and strengthens the robustness of the precision verification process.[20]

The good precision observed may be attributed to the high level of automation and standardized separation environment offered by the Capillarys 3 Octa platform. Unlike gel electrophoresis, which involves manual sample loading, staining, and densitometry, the capillary system minimizes human handling and environmental variation. Furthermore, the use of internal temperature control, buffer stability, and real-time quality monitoring within the Capillarys system may enhance analytical consistency. The system's standardized separation environment, including temperature-controlled buffers and RFID-tracked reagents, ensures consistent performance in high-throughput laboratories.[21]

Accuracy study
The study findings demonstrated high agreement between CE and gel electrophoresis system, supporting their reliability for protein fraction analysis. However, for the slope and intercept, minor discrepancies were observed for alpha-1 and beta regions, which may be attributed to differences in dye-binding affinities or algorithmic peak recognition. These findings are consistent with previous study which reported similar correlation coefficients using the Capillarys 2 Flex Piercing system.[22] Likewise, Berth et al.[23] demonstrated comparable accuracy between CE and AGE, highlighting the improved automation and reproducibility in CE systems. The inherent strengths of CE features such as automation, precise sample injection, and consistent temperature control contribute to enhanced reproducibility and reliability. Favresse et al. supported these advantages in their evaluation of the Helena V8 system, where albumin and gamma globulin showed minimal bias and strong correlation.[24] Bossuyt et al. also emphasized the high comparability of CE with gel-based methods but noted increased bias in more complex protein fractions like alpha and beta globulins.[25] These findings suggest that while CE systems like Capillarys 3 Octa offer strong overall agreement and practical advantages, certain fractions particularly alpha-1 and beta may still present limitations in inter-method comparability. Further refinement in peak integration algorithms and improved zone discrimination may enhance performance in these regions.

Reference Interval
It is known that manufacturer's RI may not be representative of all populations. This could be due factors such as demography or methodology used for the establishment of the RI. Thus, it is important to carry out RI verification procedure based on the established guideline to ensure that it is applicable for our population. Our findings demonstrated that all observed values for major serum protein fractions albumin, alpha-1, alpha-2, beta-1, beta-2, and gamma globulins were within the manufacturer's specified reference ranges, affirming the analytical consistency and suitability of of the Sebia Capillarys 3 Octa system for routine clinical application in the studied population. These findings are consistent with prior literature supporting the reliability of capillary electrophoresis in protein fraction analysis. Favresse et al. (2021) showed that capillary electrophoresis yields reproducible and robust results across varied populations, reinforcing its utility in RI establishment and clinical interpretation.[24] It is known that population-specific considerations are important. Bossuyt et al. (2001) provided age-specific RIs for Caucasian paediatric subjects across four developmental age groups, highlighting significant age-dependent shifts in serum protein patterns that warrant dedicated RI evaluation for children.[25,26] Gender-based differences in serum protein fractions have also been

documented. Lichtinghagen et al. (2010), using the Sebia Capillarys II system, analyzed serum from 428 healthy donors and found significantly higher albumin levels in males, while females exhibited elevated levels of alpha-2 and gamma globulins. These findings emphasize the value of gender-specific RIs to enhance diagnostic accuracy.[27] Furthermore, RIs are known to vary across electrophoresis platforms. Chartier et al. (2011) compared the Sebia Capillarys 2 and Helena V8 systems and found statistically significant differences in most protein fraction RIs, except for beta globulins.[28] Similarly, Howard et al. (2021) identified notable discrepancies in protein fraction patterns between the Hydragel 30 gel system and the Capillarys III Tera, underlining the necessity of analyzer-specific validation before adopting RIs in clinical settings.[29]

National initiatives have also recognized the importance of standardization in capillary electrophoresis. Albert et al. (2010) described a quality control program in France that emphasized harmonizing protein quantification and SPE interpretation using the Sebia Capillarys 2 system. Their efforts underscored the value of local RI verification and method harmonization to enhance inter-laboratory consistency and result comparability.[30]

CONCLUSION

In conclusion, the findings from this study support the Sebia Capillarys 3 Octa capillary electrophoresis system as a reliable, precise, and clinically valid method for serum protein fractionation. The automation, throughput capacity, and reproducibility offered by CE support its adoption in high-volume clinical laboratories.

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CONFLICT OF INTEREST

The sponsor involved in the technical support for the study. However, the sample collection, data analysis and interpretation were done independently by the researchers. The authors declared no other conflict of interest.

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