

**ANALYTICAL EVALUATION OF THE NOVEL HELENA V8
CAPILLARY ELECTROPHORESIS SYSTEM**

ANALITIČKA PROCENA NOVOG SISTEMA ZA KAPILARNU ELEKTROFOREZU HELENA V8

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Background: Capillary electrophoresis (CE) is used for the screening of protein abnormalities in serum and other biological fluids. Since the Helena V8 CE system has been recently commercialized, the aim of this study was to assess its analytical performance under routine laboratory conditions.

Methods: The evaluation of Helena V8 (Medical Systems S.p.A., Genova, Italy) for protein fractions (i.e., albumin, alpha-1, alpha-2, beta-1, beta-2, gamma, albumin/globulin ratio [A/G]) and monoclonal component (MC) quantification included imprecision studies, linearity, and comparison with Sebia Capillarys (Sebia, Evry Cedex, France), which was considered the reference analyzer.

Results: The imprecision of Helena V8 on all parameters was comprised between 0.8% and 15.2%; the linearity was excellent up to an MC value of 14.5% ($r=1.00$). The comparison studies showed excellent Spearman's correlations with Sebia Capillarys, with coefficients comprised between 0.84 and 0.99 (all $p<0.001$). The mean bias of Helena V8 versus Sebia Capillarys was -0.02% for G/A, -0.2% for albumin, 2.1% for alpha-1, 0.1% for alpha-2, 0.3% for beta-1, -0.2% for beta-2, -2.2% for gamma and 1 g/L for MC.

Conclusions: The results of this evaluation attest that Helena V8 is a suitable alternative for routine separation and quantification of protein fractions and monoclonal component.

Keywords: protein separation, capillary electrophoresis, analytical evaluation

Kratak sadržaj

Uvod: Kapilarna elektroforeza (KE) koristi se za otkrivanje abnormalnih nalaza proteina u serumu i ostalim biološkim tečnostima. Pošto je sistem za KE Helena V8 nedavno postao dostupan, cilj ovog rada bio je da se ispituju njegove analitičke performanse u rutinskim laboratorijskim uslovima.

Metode: Evaluacija Helene V8 (Medical Systems S.p.A, Đenova, Italija) za proteinske frakcije (tj. albumin, alfa-1, alfa-2, beta-1, beta-2, gama, odnos albumin/globulin [A/G]) i kvantifikaciju monoklonalne komponente (MK) obuhvatila je studije nepreciznosti, linearnosti i poređenja sa sistemom Sebia Capillarys (Sebia, Evry Cedex, Francuska), koji važi za referentni sistem.

Rezultati: Nepreciznost Helene V8 na svim parametrima bila je između $0,8\%$ i $15,2\%$; linearnost je bila odlična, do vrednosti MK od $14,5\%$ ($r=1,00$). Poređenja su pokazala odlične Spearmanove korelacije sa sistemom Sebia Capillarys, uz koeficijente između $0,84$ i $0,99$ (sve $p<0,001$). Srednja vrednost odstupanja za Helenu V8 u poređenju sa Sebia Capillarys bila je $-0,02\%$ za G/A, $-0,2\%$ za albumin, $2,1\%$ za alfa-1, $0,1\%$ za alfa-2, $0,3\%$ za beta-1, $-0,2\%$ za beta-2, $-2,2\%$ za gama i 1 g/L za MK.

Zaključak: Rezultati ove procene potvrđuju da je Helena V8 odgovarajuća alternativa za razdvajanje proteina i kvantifikaciju proteinskih frakcija i monoklonalne komponente.

Ključne reči: razdvajanje proteina, kapilarna elektroforeza, analitička procena

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Introduction

The separation and quantification of different protein fractions in serum and other biological fluids provides an unquestionable contribution for the diagnosis of various disorders, including paraproteinemias, hemoglobinopathies, immune deficiency and genetic abnormalities, offering also valuable information for the diagnosis and monitoring of multiple myeloma, other malignancies, autoimmune and neurological disorders. Suggestive variations in the relative concentration of protein fractions also allow reliable screening and monitoring of pathological disorders associated with nephrotic syndrome, inflammatory and hepatic diseases (1, 2). Capillary electrophoresis (CE) is a relatively innovative technique, whose diffusion in clinical laboratories has remarkably increased over the past decade (3). As compared with high-pressure liquid chromatography (HPLC) and high-resolution electrophoresis on agarose gel, which have been the gold standards for routine plasma protein separation for long (1), the leading advantages of CE are represented by the opportunity of full automation, positive identification of patient samples, small amounts of sample and reagents, lower costs and high versatility for assessment of a large panel of analytes beside traditional plasma protein fractions, including hemoglobin variants, carbohydrate-deficient transferrin (CDT) and therapeutic drugs among others (1–4). The only two available choices in the CE diagnostic market have been represented for long by the Capillarys[®] systems (Sebia, Evry, France) and Paragon CZE 2000[®] (Beckman Coulter, Inc., Fullerton, CA, USA) (5). The latter instrument is no longer commercialized, thus leaving Sebia as the exclusive manufacturer of CE analyzers in several countries. Nevertheless, since the new instrument V8[®] from Helena has recently become commercially available, the aim of this study was to assess its analytical performance under routine laboratory conditions.

Materials and Methods

Study protocol

The Helena V8[®] (commercialized in Italy by Medical Systems S.p.A., Genova, Italy) is an eight-channel automated CE system, with full walk-away automation, multi-assay functionality and rapid reflex testing for fast and continuous diagnosis of multiple disease states, whose technical performance has been previously described elsewhere (6). Additional features include fully automated buffer switching, on-board reagent block for user-defined reagents and random-access sample handling, complete audit trail accountability, uninterrupted high throughput analysis, as well as automated sample preparation for off-board gel analysis. The evaluation of analytical performance of Helena V8 for routine protein fractions

(i.e., albumin, alpha-1, alpha-2, beta-1, beta-2, gamma, albumin/globulin ratio [A/G]) and monoclonal component (MC) quantification included imprecision studies, assessment of linearity for MC, and test results comparison with Sebia Capillarys (Sebia, Evry Cedex, France), which is routinely used in our laboratory and is considered as the reference instrument for separation and quantification of protein fractions in this study (5). The identification and quantification of MC in patients' electrophoretograms obtained with both Sebia Capillarys and Helena V8 were performed by the same experienced laboratory professional. Results of protein fractions and MC were finally reported as percentages of total electrophoretogram area.

Imprecision studies

One normal serum sample, one serum specimen with hypogammaglobulinemia and one with MC were assessed for albumin, alpha-1, alpha-2, beta-1, beta-2, gamma and MC in 10 sequential runs (within-run imprecision), and for 10 consecutive working days (between-run imprecision), by using an identical reagent lot. Final results were expressed in terms of coefficient of variation (CV).

Linearity studies

A serum with an MC value of 14.5% was serially diluted at fixed ratios (i.e., 1:2; 1:4; 1:8; 1:16; 1:32 and 1:64) with an additional serum without MC, to cover the most clinically significant range of concentrations for this parameter. Serial dilutions were analysed in duplicate and theoretical values were finally calculated from the measured values of the undiluted specimens. Linearity was assessed with calculation of linear regression analysis and Spearman's correlation coefficient (r).

Comparison studies

The comparison studies of protein fractions quantification were based on all consecutive inpatient serum samples received in the clinical chemistry and hematology laboratory of the Academic Hospital of Parma during a typical working day, and for which a routine protein electrophoresis was requested by hospital physicians. The comparison studies of MC quantification were based on all consecutive inpatient and outpatient serum samples received in the same laboratory during a typical working day, for which routine quantification of MC was requested by hospital physicians or general practitioners (GPs). All samples were centrifuged, separated and divided in two aliquots immediately after arrival in the laboratory. The first aliquot was assessed on Sebia Capillarys, whereas the second was simultaneously tested on

Helena V8. Test results were analyzed with Spearman's correlation, whereas the mean bias with 95% Confidence Interval (95% CI) was estimated by Bland-Altman plot analysis.

Results

Imprecision studies

The within-run imprecision was comprised between 1.3% and 2.5% for albumin, 2.8% and 6.0% for alpha-1, 3.6% and 6.7% for alpha-2, 5.2% and 7.3% for beta-1, 3.3% and 7.8% for beta-2, 2.2% and 7.1% for gamma, and was 1.5% for MC (Table I). The between-run imprecision was comprised between 0.8% and 2.0% for albumin, 3.7% and 10.8% for alpha-1, 4.2% and 5.7% for alpha-2, 4.3% and 11.4% for beta-1, 3.8% and 15.2% for beta-2, 2.6% and 6.2% for gamma, and was 1.5–1.6% for MC (Table I).

Linearity studies

The linearity of MC quantification on Helena V8 was excellent, in the range between 0.3% and 14.5%, as mirrored by the equation of the linear regression

analysis plotted against a theoretical MC concentration ($y=1.01x - 0.2$; $r=1.000$; $p<0.001$). The detection limit of MC, as calculated according to this study protocol, was proven to be 0.3%.

Comparison studies

The comparison studies of protein fractions and MC quantification finally included 48 and 28 routine serum samples, respectively. An excellent Spearman's correlation between Helena V8 and Sebia Capillarys was observed for all measures, being 0.96 for A/G, 0.97 for albumin, 0.90 for alpha-1, 0.95 for alpha-2, 0.84 for beta-1, 0.95 for beta-2 and 0.99 for MC and gamma, respectively (all $p<0.001$). The mean bias of Helena V8 against Sebia Capillarys was -0.02% (95% CI, -0.0 to 0.02; $p=0.32$) for G/A, -0.2% (95% CI, -0.7 to 0.3%; $p=0.42$) for albumin, 2.1% (95% CI, 1.7 to 2.4%; $p<0.01$) for alpha-1, 0.1% (95% CI, -0.2 to 0.5%; $p=0.39$) for alpha-2, 0.3% (95% CI, 0.1 to 0.6%; $p<0.01$) for beta-1, -0.2% (95% CI, -0.4 to 0%; $p=0.06$) for beta-2, -2.2% (95% CI, -2.4 to -2.0%; $p<0.01$) for gamma and 1 g/L (95% CI, 0. to 1 g/L; $p=0.02$) for MC, respectively (Figure 1).

Table I Results of imprecision of albumin, alpha-1, alpha-2, beta-1, beta-2, gamma and monoclonal component (MC) on one normal serum sample and two serum specimens with hypogammaglobulinemia and MC with the novel Helena V8 capillary electrophoresis system. All values are expressed as percentage (%).

	Normal		Hypogammaglobulinemia		MC	
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV
Within-run						
Albumin	62.1±1.0	1.7	63.3±0.8	1.3	50.2±1.3	2.5
Alpha-1	6.9±0.3	4.5	8.8±0.2	2.8	6.8±0.7	6.0
Alpha-2	10.1±0.4	4.5	11.4±0.4	3.6	11.0±0.7	6.7
Beta-1	5.9±0.3	5.6	5.8±0.4	7.3	6.4±0.3	5.2
Beta-2	3.6±0.1	3.3	4.5±0.2	4.0	3.3±0.2	7.8
Gamma	11.4±0.4	3.5	6.3±0.4	7.1	22.3±0.5	2.2
MC	-	-	-	-	19.9±0.3	1.5
Between-run						
Albumin	60.0±1.2	2.0	60.9±0.5	0.8	54.4±0.8	1.5
Alpha-1	5.8±0.6	10.8	5.6±0.2	3.7	5.7±0.6	10.8
Alpha-2	8.9±0.4	4.5	13.2±0.7	5.7	10.2±0.4	4.2
Beta-1	7.2±0.5	6.7	7.9±0.9	11.4	6.5±0.3	4.3
Beta-2	4.3±0.2	5.8	5.3±0.8	15.2	5.0±0.2	3.8
Gamma	13.7±0.7	5.3	6.9±0.4	6.2	18.2±0.5	2.6
MC					20.0±0.4	1.6

SD, Standard Deviation; CV, Coefficient of Variation.

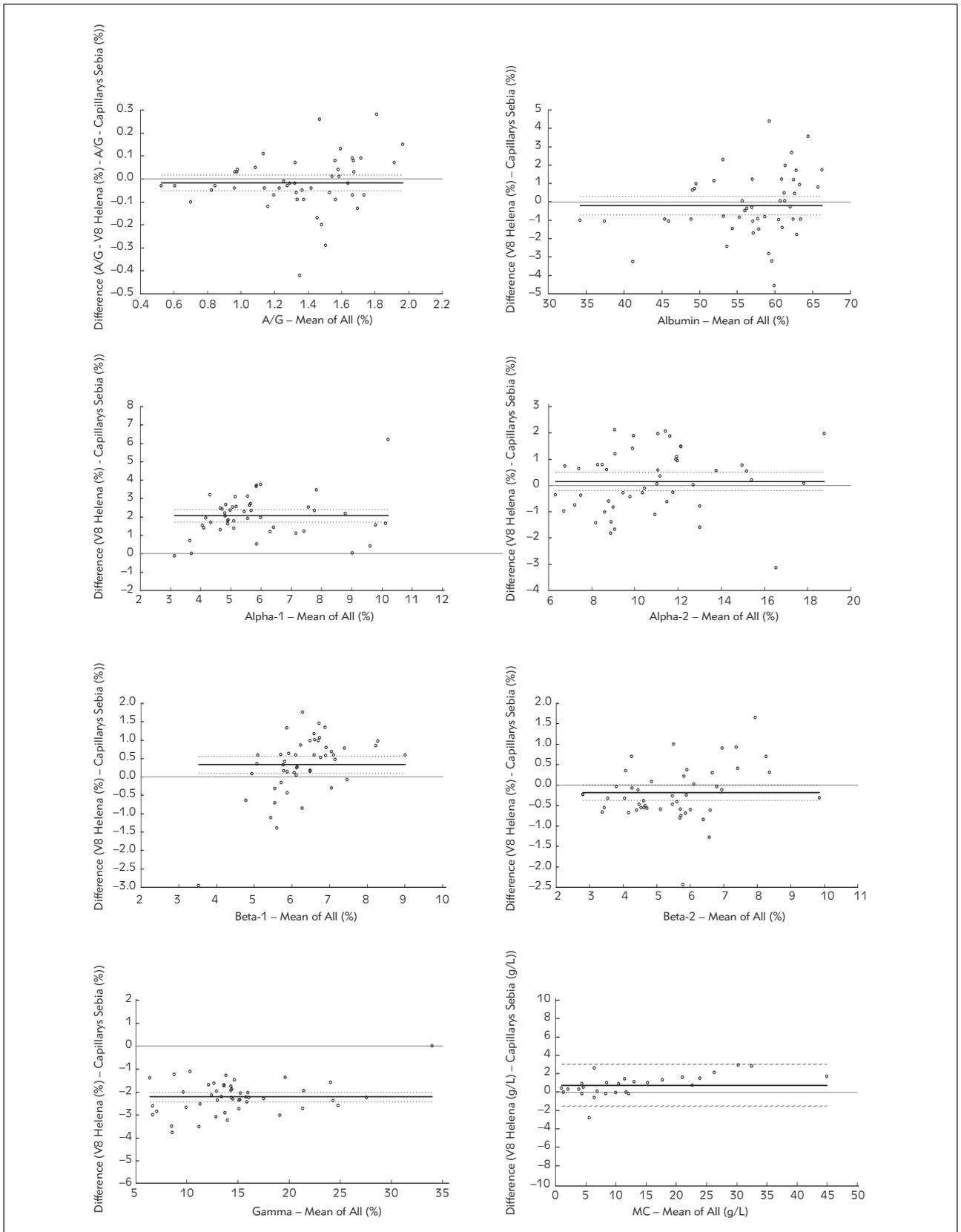


Figure 1 Bland & Altman plots of albumin, alpha-1, alpha-2, beta-1, beta-2, gamma, albumin/globulin ratio (A/G) and monoclonal component (MC) quantified in routine samples with Sebia Capillary and the novel Helena V8 capillary electrophoresis system. The continuous lines designate the mean bias, whereas the dotted lines delimitate the 95% confidence interval (95% CI).

Discussion

Protein electrophoresis is an important laboratory technique, which is conventionally used for the screening of protein abnormalities in serum as well as in other biological fluids such as urine and cerebrospinal fluid (CSF) (1–2). The recent development of CE techniques suitable for routine laboratory use has represented a major breakthrough in the separation and quantification of protein fractions, since this technique allows to obtain highly accurate results, contextually saving precious human and economical resources (5). These unquestionable advantages have, however, been counterbalanced by the relative lack of alternatives in the *in vitro* diagnostic market, wherein the only CE systems commercially available in some countries in recent times have been represented by the Sebia Capillarys systems (5). After its recent development and commercialization, the Helena V8 represents a new perspective for routine separation and quantification of protein fractions and MC. In our evaluation, under typical laboratory conditions, the instrument exhibited satisfactory imprecision (CV comprised between 0.8% and 15.2%) and optimal linearity (i.e., a correlation coefficient of 1.00). The comparison of routine serum samples between Sebia Capillarys and Helena V8 also gave excellent results, with correlation coefficients comprised between 0.84 and 0.99, as well as mean biases always lower than $\pm 2.2\%$ for protein fractions and 1 g/L for MC. A larger bias was observed for alpha-1 (2.1%) and gamma (-2.2%), but this is not an alarming find-

ing since the excellent correlations found for both measures with Sebia Capillarys (0.90 and 0.99, respectively) would only require a readjustment of reference ranges on a local basis. This is in ideal agreement with the previous findings of Chartier et al. (6), who identified similar bias and thus different reference ranges between the two CE systems for both alpha-1 (3.3–4.0% for Helena V8 versus 2.6–3.2% for Sebia Capillarys) and gamma (7.5–10.2% for Helena V8 versus 8.9–11.9% for Sebia Capillarys). Our results are also globally in agreement with those of Poisson et al. (7), who reported that Helena V8 and Sebia Capillarys were analytically equivalent to immunofixation electrophoresis for identification of IgG MC >3 g/L, a value that is also equivalent to the detection limit of MC identified in our study. Chartier et al. also previously reported within- and between-run imprecision for Helena V8 lower or equal to 5.2% and 7.7%, along with good correlation between this CE system and agarose gel electrophoresis, with correlations comprised between 0.81 and 0.96.

Taken together, the results of this analytical evaluation attest that Helena V8 is a suitable alternative for routine separation and quantification of protein fractions and MC in clinical laboratories.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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