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Comparison between a Second Generation Automated Multicapillary Electrophoresis System with an Automated Agarose Gel Electrophoresis System for the Detection of M-Components

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Abstract

During the last decade, capillary electrophoresis (CE) has emerged as an interesting alternative to traditional analysis of serum, plasma and urine proteins by agarose gel electrophoresis. Initially there was a considerable difference in resolution between the two methods but the quality of CE has improved significantly. We thus wanted to evaluate a second generation of automated multicapillary instruments (Capillarys, Sebia, Paris, France) and the high resolution (HR) buffer for serum or plasma protein analysis with an automated agarose gel electrophoresis system for the detection of M-components. The comparison between the two systems was performed with patients samples with and without M-components. The comparison included 76 serum samples with M-components > 1 g/L. There was a total agreement between the two methods for detection of these M-components. When studying samples containing oligoclonal bands/small M-components, there were differences between the two systems. The capillary electrophoresis system detected a slightly higher number of samples with oligoclonal bands but the two systems found oligoclonal bands in different samples. When looking at resolution, the agarose gel electrophoresis system yielded a slightly better resolution in the alpha and beta regions, but it required an experienced interpreter to be able to benefit from the increased resolution. The capillary electrophoresis has shorter turn-around times and bar-code reader that allows positive sample identification. The Capillarys in combination with HR buffer gives better resolution of the alpha and beta regions than the same instrument with the ß1-ß2+ buffer or the Paragon CZE2000® (Beckman) which was the first generation of capillary electrophoresis systems.

Introduction

Serum or plasma protein electrophoresis has a long tradition in clinical laboratories. Today, the majority of these tests are performed by agarose gel or cellulose acetate electrophoresis but capillary electrophoresis is being increasingly used (1,2). The assays are routinely used to screen for and monitor dysproteinemias (3,4). Another important application for electrophoresis is to monitor inflammatory responses (acute phase reaction), following tissue injury, infarction, infection or immune-related diseases. The two most widely used assays for the acute phase response in humans are C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR).

Key words: agarose gel electrophoresis, capillary electrophoresis, human, nephelometry, plasma

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Other markers of the acute phase response are α_1-antitrypsin, α_1-acid glycoprotein, haptoglobin, fibrinogen and procalcitonin (PCT). Except for procalcitonin these proteins can all be observed in a high-resolution capillary electrophoresis electropherogram (5,6).

The electrophoretic methods used have to be able to reliably detect and estimate M-components and acute phase response. A test report from a routine examination of a sample with suspected M-component should include evaluation of both the size of the M-component and the background immunoglobulin levels. For routine purposes the methods should be able to detect M-components well below 1 g/L in regions where there are no other interfering bands and M-components of ≤1g/L in areas with other bands, according to the recommendations of the Swedish expert group on protein analysis (www.equalis.se). The decision limits for complete remission evaluation of myelomas are even lower. The laboratory should also be able to distinguish between hypo- normo- and hyper-gammaglobulinemia based on the electrophoresis alone or in combination with nephelometric determination of immunoglobulins. The methods should also provide a good separation of albumin and the acute phase proteins α_1-antitrypsin, α_1-acid glycoprotein and haptoglobin. For laboratories that analyse plasma samples the methods should allow estimation of the fibrinogen peak. These requirements can usually not be achieved by paper electrophoresis or nitrocellulose electrophoresis, which has led to the replacement of these methods with agarose gel electrophoresis.

During the last decade, capillary electrophoresis (CE) has emerged as an important alternative to traditional analysis of serum and plasma proteins by agarose gel electrophoresis. CE analysis of plasma proteins can be fully automated with bar-code identification of samples, preseparation steps and direct postseparation quantification of individual peaks permitting assay times of less than 10 min and high throughput. We have analysed serum and plasma samples with the Capillarys® instrument as a representative for the second generation of capillary electrophoresis instruments. The new HR (high resolution) application for the Capillarys® capillary electrophoresis system yields an improved resolution in comparison with the previous buffer system (ß1-ß2+ reagent) and allows a good separation of plasma samples, thus making it possible to quantify individual peaks from the electropherogram (6). We used the albumin concentrations in the samples, measured by nephelometry, to assign protein concentrations to each peak.

Materials and methods

Samples

Consecutive plasma samples sent to the laboratory for routine analyses were included in the study. The samples were collected in gel tubes with lithium-heparin (LH PST™ II, BD Vacutainer Systems, Plymouth, UK). The study was approved by the local Ethical Board at Uppsala University (01-167).

Instrument

Capillarys™

Capillary electrophoresis was performed using Capillarys™ Capillary Electrophoresis System (Sebia, Paris, France) and the ß1-ß2+ buffer and the new high resolution (HR) buffer. The instrument is equipped with 8 capillaries allowing a throughput of approximately 60 samples per hour. An automatic dilution of 40 µL sample to a final volume of 200 µL in the migration buffer is performed in dilution segments. Samples are then hydrodynamically injected for 4 s by anodic depression (injected volume approximately 1 nL) The separation is obtained by applying a voltage of 7 kV for 4 min in the 8 fused-silica capillaries (total/effective length 17.5/15.5 cm; inner diameter 25 µm). The temperature is controlled by a Peltier element. The protein separation is performed at pH 9.9 and the proteins are detected at an absorbance of 200 nm. Weekly cleaning of the capillaries by a washing solution is recommended by the producer when analysing serum samples and daily cleaning is recommended when analysing plasma samples. The instrument automatically defines peaks in the chromatogram and the area for the peak in percentage of the total absorbance. If albumin or total protein concentration is provided, the instrument will automatically calculate the protein concentration of each peak (peak area). The instrument default setting is for baseline subtraction when calculating peak areas. When measuring individual peaks (e.g. M-components) it is also possible to use valley to valley subtraction, which can be used to subtract background immunoglobulins. In this study, M-components were quantified by visual estimation of the M-component size after subtraction of background immunoglobulins. The evaluator had access to quantitative data on albumin, α_1-antitrypsin, α_1-acid glycoprotein, haptoglobin, IgA, IgG and IgM when evaluating the electropherogram. Oligoclonal bands were defined as more than one peak with estimated protein concentration of <1 g/L per peak.

Hydrasys

High-resolution agarose gel electrophoresis was carried out on the semiautomated Hydrasys instrument (SEBIA) with Hydragel 15 ß1-ß2+ HR electrophoresis gels (SEBIA) according to the manufacturer's instructions. Serum was loaded on the applicator, which was placed in the instrument together with buffer strips and a gel plate. Sample application time was set to 45 s and separation was performed at 20W for 12 min. After electrophoresis, the gel was removed and stained with a mixture of 120 mL amidoblack, 360 mL staining solution, 225 mL acid violet and 1380 mL distilled water (reagents included in the kit) for 4 min followed by destaining in the gel-processing module of the instrument and drying. M-components were quantified by visual estimation of the M-component size after subtraction of background immunoglobulins. The evaluator had access to quantitative data on albumin, α_1-antitrypsin, α_1-acid glycoprotein, haptoglobin, IgA, IgG and IgM when evaluating the agarose gel. Oligoclonal bands were defined as more than one band with estimated protein concentration of <1 g/L per band.

Analysis of albumin

Albumin and α_1-antitrypsin were analyzed on a BN Prospec™ nephelometer (Dade Behring, Deerfield, IL, USA) with reagents from the same manufacturer, including a calibrator traceable to CRM 470. The albumin assay had a total CV of 1.7% at 38 g/L and the α_1-antitrypsin assay had a CV of 1.8% at 1.7 g/L. The albumin value was used to assign values for individual peaks in the chromatogram.

Results

Coefficient of variation (CV) for Hydrasys and Capillarys (ß1-ß2+ buffer)

The Hydrasys gels were scanned with a Hyrys densitometer (version 4.02, Sebia). A total of 30 samples assayed in duplicates in two separate runs were evaluated. The CV of the intensity of individual peaks were calculated. The total CVs (%) of the individual fractions were 1.05 for albumin, 5.78 for alpha$_1$, 1.72 for alpha$_2$, 3.35 for beta$_1$, 4.78 for beta$_2$ and 3.32 for the gamma fraction.

Pools of serum and plasma samples were analysed in all 8 capillaries on day 0-4. On day 0 the samples were tested in triplicates while in duplicates on day 1–4 for a total of 11 runs.

The CVs of the individual peaks in percentage were all below 5% both for serum and plasma samples. These CVs were lower than the CV for the peak absorbance that was above 5%.

Detection of M-components

498 consecutive samples referred for routine analyses were analysed with Hydrasys and Capillarys (utilizing the HR buffer). The Capillarys HR buffer was chosen for this comparison as it yields better resolution than the ß1-ß2+ buffer (Figs 1b, 1c). The samples were initially run on the Hydrasys and interpreted as part of the routine work with several interpreters. The Hydrasys gels were then carefully reexamined by one of the authors (L.O.H.) and the samples were analysed on the Capillarys (A.L.). Both evaluations were blinded. M-components were quantified by

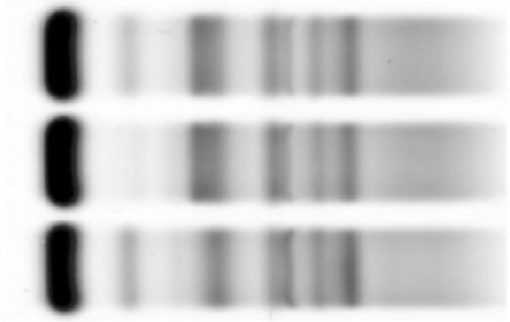

Fig 1a. Densitometric scanning of three samples separated by Hydrasys agarose gel electrophoresis.

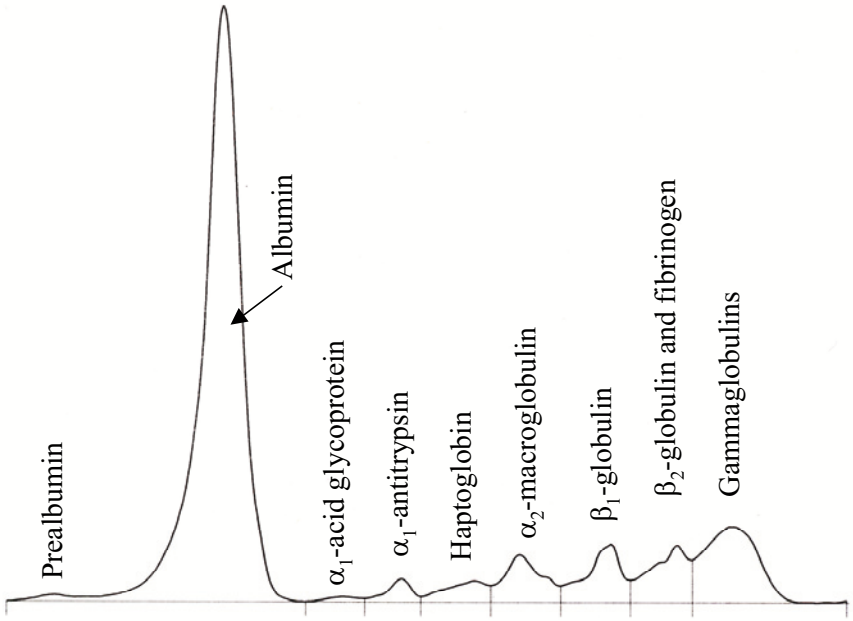

Fig 1b. Separation of plasma sample on the CAPILLARYS™ Capillary Electrophoresis System with the HR buffer.

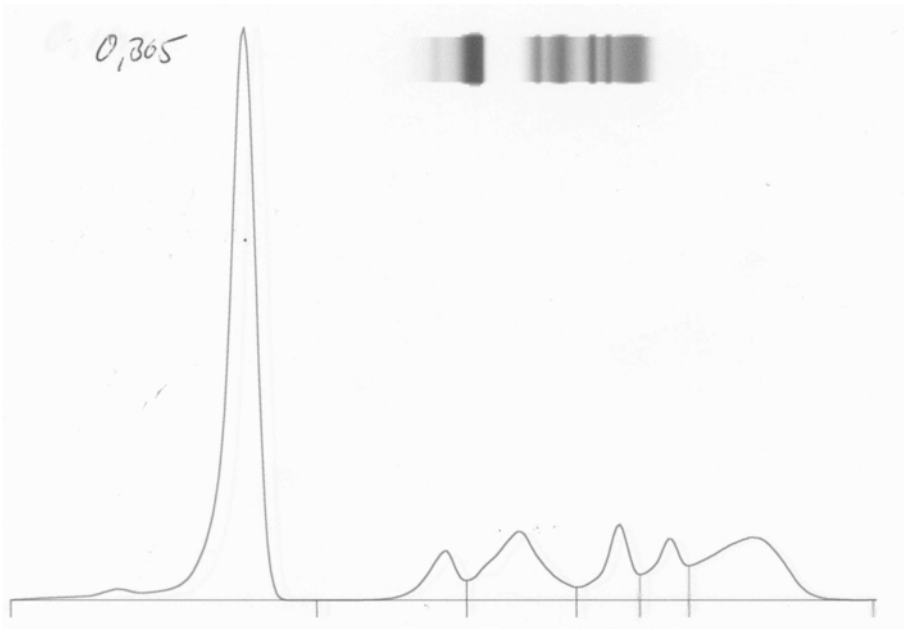

Fig 1c. Separation of serum sample on the CAPILLARYS™ Capillary Electrophoresis System with the ß1-ß2+ buffer.

Table 1. Detection of M-components and oligoclonal bands with Hydrasys and Capillarys

M-component > 1 g/L			
Detected by	Both	Only Capillarys	Only Hydrasys
	76	0	0

Oligoclonal band/M-component ≤ 1 g/L			
Detected by	Both	Only Capillarys	Only Hydrasys
	17	33	21

visual estimation in relation to other peaks/bands and quantitative data. There was a very good agreement between the two methods for the detection of M-components > 1 g/L (Table 1). Both methods detected M-components in the same 76 samples. The number of oligoclonal bands with each method was also studied. In 17 cases the evaluators found oligoclonality with both methods, while in 33 cases oligoclonality was demonstrated only by the Capillarys and in 21 other cases only by Hydrasys. The samples that differed had very weak bands usually close to the level of detection. The initial routine examination had not commented on the presence of oligoclonality in any of the samples for which there was a difference between the two methods.

Discussion

The resolution of capillary electrophoresis has improved significantly over the last decade. We have previously shown that the Capillarys® capillary electrophoresis system allows a good separation of α_1-antitrypsin, α_1-acid glycoprotein and haptoglobin in serum and plasma samples, thus making it possible to quantify these proteins from the electropherogram (6). Nephelometric determination of these proteins and capillary electrophoresis in combination with quantification of albumin showed a good correlation but with slopes deviating from 1.0. This is similar to staining of agarose electrophoresis where proteins stain with varying intensity. Thus, if the band intensity or peak area is used for quantification of protein concentration it is important to perform comparisons between the visual estimation of the band and nephelometric or turbidimetric quantification of the corresponding protein. The capillary electrophoresis system does not allow analysis of the HDL or LDL fractions in plasma and serum in contrast to agarose gel electrophoresis.

Both the capillary electrophoresis system and the agarose system provide acceptable separation for most routine work. Small M-components may be more easily detected if quantitative data are used in combination with the electrophoretic

method. Also, immunofixation can be used to detect small M-components. However, the sensitivity of both systems for detection of M-components is less than the sensitivity that can be obtained with immune fixation methods that have been optimized to detect very small M-components (0.05 g/L or less, personal communication, Prof G. Merlini, Padua, Italy). The Myeloma Subcommittee of the European Group for Blood and Marrow Transplant recommends that immunofixation (IF) of M-components is required for evaluating disease response in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation regardless if the routine electrophoresis (EP) was negative or not (7). This recommendation is based on studies showing that highly sensitivity IF is superior for detection of very small M-components (8).

Non-secretory/low-secretory myelomas produce too low concentrations of monoclonal antibodies or antibody fragments to be detected by agarose or capillary electrophoresis systems. Apart from this source of negative results, both agarose electrophoresis and capillary electrophoresis has been reported to yield occasional method specific false negative results (9–11). The frequency of false negative results is low and it requires very large comparisons to verify if one system is superior in this aspect. The result of such a comparison may very well be dependent on the laboratory as preanalytical handling will be laboratory dependent (e.g. cryoglobulins).

Abnormal peaks may also be due to interfering substances (12). The authors reported the detection of a peak in the electropherogram from a Paragon capillary electrophoresis system after infusion with 62 g of iopamidol (Jopamiron, Bracco, SPA).

Serum free light chain determination is an interesting alternative to electrophoretic detection of M-components, especially for monitoring of non-secretory/low-secretory myelomas (13). However, there is a discrepancy between serum free light chain and electrophoresis when quantifying M-components. Thus, agarose and capillary electrophoresis are still the most widely used methods for detection of M-components.

Conclusion

The resolutions of the two systems are fairly similar. The quality of the two methods will mainly be dependent on the experience of the interpreter with the specific system and this will be the main decider of which of the systems that yields the highest resolution. An experienced interpreter will be able to obtain more information than an unexperienced regardless of the system used. An expert interpreter of test results from one of the system is not automatically an expert interpreter on the other system. It is thus recommended that the interpreters evaluate a large number of test results with the new system prior to reporting actual patient results.

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