kidney and is largely responsible for the marked acidosis seen in severe cases.

Patients who ingest ethylene glycol will develop a high osmolar gap as they absorb the glycol over the first few hours. Thereafter, as the ethylene glycol is metabolised to acids, the osmolar gap decreases while the anion gap increases and acidosis worsens. Patients presenting early may or may not have an acidosis, but their osmolar gap will be high. Patients presenting late with a high anion gap metabolic acidosis may or may not have an increased osmolar gap because the osmolar gap decreases as ethylene glycol is metabolised. A high anion gap metabolic acidosis is not specific to ethylene glycol ingestion and can occur following ingestion of other toxic alcohols (e.g., methanol) or with other clinical conditions including renal failure and those associated with ketoacidosis and lactic acidosis.

Ethylene glycol assays are not always immediately available. Where there is uncertainty as to poisoning or its source, an increased anion gap metabolic acidosis in the presence of an increased osmolar gap should alert to the possibility of ethylene glycol poisoning. Furthermore, the presence of an apparent lactate gap on different analysers makes this so likely that treatment for ethylene glycol poisoning may be initiated while awaiting confirmation of ethylene glycol poisoning.

In summary, this study shows that glyoxylic and glycolic acids, metabolites seen in ethylene glycol poisoning, cross-react in the lactate assay on the Roche Modular analyser to give false-positive lactate results. This interference on the Roche Modular is much less than that seen with the Radiometer ABL 835, giving rise to an 'apparent lactate gap' which may be used to indicate ethylene glycol poisoning as the cause of an increased anion gap metabolic acidosis.

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Evaluation of the Sebia Capillarys zone electrophoresis system for monoclonal paraprotein analysis

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Laboratories continue to be faced with increasing workloads and often the solution to this management problem is to automate processes. While large-scale automation has featured in 'routine' clinical chemistry and haematology departments for many years, it is only relatively recently that other 'lower volume/esoteric' sections have experienced such revolutionary developments.

Over the past few years, capillary zone electrophoresis (CZE) has emerged as a powerful automated tool for the separation of proteins and other biopolymers, including serum protein fractions,[1] offering rapid detection of monoclonal immunoglobulins and other serum protein abnormalities. In comparison to traditional electrophoretic methods (e.g., agarose gel electrophoresis), CZE offers many advantages (e.g., automation, primary tube sampling, automated data transmission and faster turnaround time).[2]

The authors recently decided to centralise their electrophoresis workload within a managed pathology network and therefore needed to consider an automated solution. Thus, it was decided to evaluate the Sebia Capillarys system (Sebia, Issy-les-Moulineaux, France) by comparing results with the department's existing Sebia Hydrasis gel system, the aim being to design a simple and practical evaluation procedure that would mirror routine methods for the interpretation and reporting of serum electrophoresis and be applicable to other district general hospitals considering implementation of this technology.

In preparation for the evaluation, a series of 242 anonymised patient serum samples were collected over a six-month period and stored at –20°C prior to analysis. The samples were from patients with paraproteinaemia, immunodeficiency and also from a limited number of cryoglobulin-positive samples (n=10) and from a single patient with α1-antitrypsin deficiency that had been received by the laboratory for routine clinical analysis. As a consequence, ethical approval was not required for the study.

The CZE system was installed in the laboratory and the authors received appropriate training from Sebia. The evaluation samples were then run in the Sebia Hydrasis and Capillarys systems and the authors read each gel track and CZE profile independently. In order to avoid bias, this was performed without knowledge of clinical information or total immunoglobulin results. Each protein fraction (excluding albumin) was commented upon and described subjectively as normal (N), increased (I), slightly increased (SI), decreased (D), slightly decreased (SD) or paraprotein

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band/s present. Subjective analysis, rather than quantitative analysis, was undertaken deliberately as this is the routine method used in the laboratory.

Where the interpreters disagreed, the profile for both methods was reviewed, along with immunoglobulin results, to arrive at a consensus opinion. The consensus results profile for each sample by gel electrophoresis was then compared directly to the consensus profiles by CZE. Where each protein fraction agreed in both methods the results for these samples were deemed to be 100% concordant. Where there were slight subjective differences (i.e., N *vs*. I or N *vs*. SI) in interpretation of some less-important protein fractions (e.g., increased α-globulins), these samples were considered to be clinically concordant.

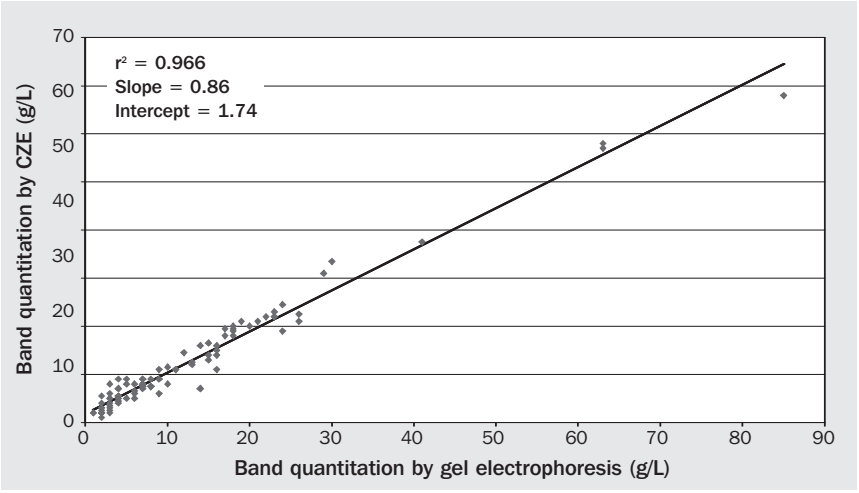

Fig. 1. Comparison of band quantitation (g/L) between gel electrophoresis and CZE.

Clinically significant differences in interpretations of patterns were noted where there were differences in terms of decreased α1, increased or decreased γ-globulin or the presence of any paraprotein band. Where a paraprotein band was identified, quantification was performed on the CZE profile by visually 'gating' the paraprotein band prior to automatic calculation as a proportion of the total protein, following the manufacturer's recommendation. Quantification was performed independently by the authors.

The original band concentrations for the gel method were found by searching band results on the pathology computer system and were calculated originally as a proportion of the total globulin level rather than total protein. Total globulin was used to quantify bands in gel electrophoresis as this is generally considered to be the most appropriate method due to the differential binding of stain by globulins and albumin, and was the laboratory's routine method.

Within-run imprecision was determined by replicate analysis (n=10) of an anonymised patient serum sample that showed a monoclonal gammopathy with mean paraprotein concentration of 22.6 g/L. Between-run imprecision was determined by replicate (n=10) analysis over 10 runs using the same reagent lot over a six-month period of an anonymised stored and aliquoted patient sample that showed a biclonal gammopathy with paraprotein concentrations of 5.4 g/L and 4.8 g/L. Paraprotein band 'gating' for these samples was undertaken independently by two different analysts and the results averaged.

Of the 242 samples analysed, 207 (85.5%) were 100% concordant by both methods. Thirty-five (14.5%) samples were not in total concordance, of which 19 (7.9% of total samples analysed) showed differences that were deemed to be clinically insignificant as outlined above. Therefore, only 16 samples (6.6%) showed what was considered to be potentially clinically significant differences in interpretation, and the details of these samples are shown in Table 1.

Within-run imprecision for CZE showed a coefficient of variation (CV) of 1.3% at 22.6 g/L. The between-run average CV for the two different analysts at 5.4 g/L and 4.8 g/L was 3.2% and 3.1%, respectively.

Band values (g/L) obtained by the two methods were compared using correlation and linear regression analysis and by difference plots (Figs 1 and 2).

Of the 242 samples tested only 16 samples (6.6%) were not deemed to show clinical concordance. Ten of these were due to perceived differences in polyclonal γ-globulin fractions and these would have been resolved if interpretation of the electrophoresis pattern had been undertaken with knowledge of the immunoglobulin results. In routine practice, immunoglobulin results are available to the clinical authoriser and therefore these 10 results would have been reported appropriately.

Of the remaining six 'discrepant' sets of results, two subsequently showed 'negative' immunofixation results. Of the other four samples, one had a slightly low α1-antitrypsin

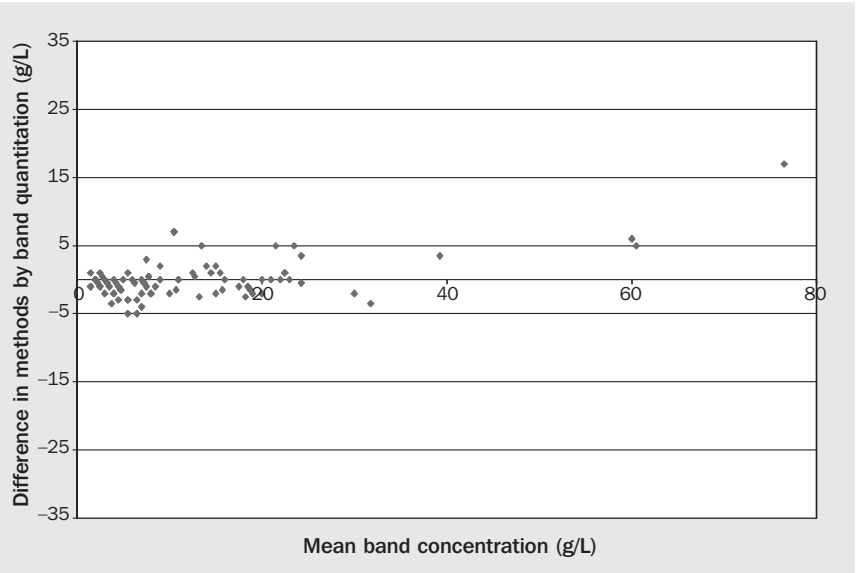

Fig. 2. Difference plot showing band quantification by gel electrophoresis and CZE.

(0.82 g/L) but was reported as 'normal' by CZE but low by gel electrophoresis. Therefore, the gel result proved more 'accurate' than the CZE result. As the α1-antitrypsin level was not markedly low, the patient is likely to be heterozygous for a deficient allele.

It is generally accepted[3,4] that heterozygotes (MZ) are at low risk of developing lung or liver disease. Moreover, CZE would never be used to screen for α1-antitrypsin deficiency and would only be detected as an incidental finding at authorisation. Therefore, this discrepancy is arguably of little clinical significance. The other 'discrepant' α1 band was 'normal' when quantified.

Of the remaining two discrepant results, one sample showed a band that was detected by CZE but not by the gel technique, and one patient had a band detected by gel (confirmed by immunofixation) but not by CZE. At clinical validation of the gel from the first sample, it is likely that immunofixation would have been requested because of the increased IgA and increased β region. The final sample was confirmed as having two tiny IgG bands by immunofixation and would have been referred for immunofixation in routine practice, even if the CZE method was used, as the immunoglobulins were severely decreased.

The authors expected to observe differences in the interpretation of protein fractions separated by gel electrophoresis and CZE, as interpretation of these profiles is subjective and will vary between interpreters. Many of the differences observed were in the α1 and α2 fractions and this would not compromise clinical care. Therefore, 93.4% of samples tested showed excellent agreement, and this compares favourably with another study that showed an overall agreement of 91%.[5]

Of the 16 samples found to be potentially significant clinically, only two had small paraprotein bands. The CZE method missed one (0.4%) sample containing two tiny paraprotein bands that were barely visible on the gel (although this sample would have had immunofixation, as described above), and one (0.4%) sample was thought to have a band by CZE which was not confirmed by immunofixation. Gel electrophoresis also missed a small paraprotein band that was detected by CZE. Therefore, the CZE method performed at least as well as the gel method during this evaluation.

Comparison of the quantification of paraprotein band values obtained by gel electrophoresis and CZE showed good correlation (r^2=0.966) and regression analysis gave a slope of 0.86 and intercept of 1.74 g/L. This finding was expected given that globulin and total protein results are used for quantification of bands using gel electrophoresis and CZE, respectively. The difference plot showed good agreement between the quantified bands, except in three cases, but the differences in band values was not more than 5 g/L in each case. There was one obvious discrepancy where one sample was quantified as 85 g/L by the gel method and 68 g/L by CZE. The sample was repeated by both methods and gave 91 g/L and 68 g/L, respectively. This difference was probably due to method differences between the direct concentration assessment by ultraviolet (UV) absorption for CZE and protein staining of the gels. These results were considered to be acceptable in terms of ongoing patient monitoring by local haematologists

The within-run imprecision of the CZE system was excellent (CV 1.3 % at 22.6 g/L). The between-run CV was calculated as an average of two different analysts using a sample that had a biclonal gammopathy with relatively low concentration of paraprotein bands (5.4 g/L and 4.8 g/L). This was performed because no single analyst will undertake all testing in routine practice; therefore, it is important to verify that reliable performance can be maintained/confirmed by multiple analysts. It was reassuring to see that the two analysts produced excellent between-run imprecision for the biclonal gammopathy sample tested during 10 analytical runs over the six-month period.

In summary, the Sebia CZE system is a suitable and precise alternative method to the Sebia Hydrasis system for serum gel electrophoresis and paraprotein band quantification. Moreover, it has several additional advantages in terms of analysis speed and throughput (78 samples/hour), is fully automated with primary tube sampling, and provides automatic data transmission.

The authors wish to thank Sebia for providing the reagents to perform this study, and Clare Del-Duca for help with the imprecision data.

Table 1. Details of the 16 samples that demonstrated differences in interpretation.

Number of samples	Gel electrophoresis interpretation	CZE interpretation	Additional information
Five	Increased γ	Normal γ	Normal immunoglobulins
Four	Increased γ	Normal γ	One IgA = 6.39 g/L (0.8–4) One IgA = 5.14 g/L One IgA = 4.17 g/L One IgM = 2.33 g/L (0.5–2)
One	Band in β, increased γ	Band in β, normal γ	Monoclonal IgA paraprotein in β region
One	Split α2, band in β	Normal	Immunofixation normal
Two	Decreased α1	Normal	One α1-antitrypsin level (A1AT) 1.6 g/L One A1AT = 0.82 g/L (0.9–2.0)
One	Normal	Tiny band in γ?	Immunofixation normal
One	Increased β	Band in β	Immunofixation identified an IgA paraprotein band in β
One	Band in γ	Decreased γ	Immunofixation identified two tiny paraprotein bands in γ

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