Sebia Capillarys 2 versus the Helena Biosciences V8 capillary electrophoresis analyser for carbohydrate-deficient transferrin measurement: comparison and analytical evaluation

T. WOOLLEY, P. ALLEN, L. FITZGERALD, L. IZZARD and E. RUTTER

Synergy Health Laboratory Service, Brecon Road, Abergavenny, Monmouthshire, South Wales, UK

Accepted: 17 December 2014

ABSTRACT

This study compares two automated capillary electrophoresis (CE) systems, the Capillarys 2 (Sebia, Surrey, UK) and V8 (Helena Biosciences, Tyne and Wear, UK) for the measurement of carbohydrate-deficient transferrin (CDT). Analytical imprecision was calculated for both platforms using internal quality control material from Sebia and Helena Biosciences, while a patient comparison was performed on 150 patient samples with CDT% levels ranging from 0.3% to 23.7%. Inter- and intra-assay imprecision between the two platforms were comparable. The correlation between platforms using patient samples was r^2=0.985. However, there was a significant proportional bias at higher CDT concentration ranges, with the Helena system showing negative bias but good correlation over the clinically significant range. Analytical performances from both CE systems have been proven as suitable for routine laboratory use. The V8 CDT results were comparable to the Capillarys 2 in human sera over the clinical range of interest.

KEY WORDS: Alcohol.
Capillarys 2.
Carbohydrate-deficient transferrin.
Electrophoresis, capillary.
V8 E Class.

Introduction

Transferrin is an important transport protein of ferric ion. The protein, with a molecular weight (MW) of 80 kDa.[1] consists of three substructural domains – a single polypeptide chain, two iron-binding sites and two N-linked glycans – each attached to one or more sialic acid residues.[2,3] These transferrin substructures show a distinct variability even under normal conditions.[4] Owing to this, transferrin is not a homogeneous molecule, but shows a distinct microheterogeneity.

There will be several transferrin isoforms in circulation at any one time. These isoforms are named in relation to their number of sialic acid residues. Theoretically, the number of sialic acid residues present on the isoform could range between zero (no oligosaccharide chain) and eight (two tetra-antennae oligosaccharide chains terminated with four sialic acid residues on each).[5]

Genetic transferrin variants are attributable to substitutions of amino acid(s) in the polypeptide chain. At least 38 transferrin variants are known; however, only four of these show a prevalence of >1%. Of these, transferrin-C1 shows the highest prevalence (>95%) in Caucasians.[4] In the common transferrin-C1 variant (others include B and D variants) the tetra-sialotransferrin molecule, containing two bi-antennary carbohydrate chains with a total of four terminal sialic acid residues, is the predominant isoform and usually accounts for approximately 75% of total transferrin found in the serum. Tri- and penta isoforms typically make up approximately 5% and 15%, respectively, whereas di- and hexa- isoforms occur at approximately 2% each. The remaining isoforms (a-, mono-, hepta- and octa-) occur at <1%.[4]

The di-sialotransferrin (DisT) molecule, subsequently known as carbohydrate-deficient transferrin (CDT) was first proposed as a maker of heavy alcohol consumption by Stibler *et al.*[6] when it was observed that individuals misusing alcohol had a higher proportion of transferrin with 0 and 2 carbohydrate side chains. DisT is the main isoform produced by excess ethanol intake.[6] An accompanying increase in the asialotransferrin (AsT) isoform is usually only observed in conjunction with considerable amounts of DisT.[7]

The formation of the transferrin side chains is understood to be controlled by two enzymes: glycosyl tranferase, which adds side-chains, and sialidase, which removes side chains.[8,9] Chronic alcohol intake, however, appears to modify the formation of both the oligosaccharide chains and terminal sialic acid residues.[10–12] Xin *et al.*[11] showed that decreased activity of these enzymes was primarily due to the oxidative by-product of ethanol intake, acetaldehyde. It has been suggested that post-translational modification of the transferrin molecule most likely takes place in the endoplasmic reticulum (ER) and Golgi apparatus of the hepatocyte.[8,13]

Correspondence to: Timothy Woolley
Email: timothy.woolley@synergyhealthplc.com

Several blood-based biological markers have been used in an attempt to monitor alcohol abuse, commonly these include asparate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), mean cell volume (MCV) and CDT. Carbohydrate-deficient transferrin is unaffected by mild alcohol-related liver disease or fatty liver (e.g., due to obesity, diabetes) which can falsely increase GGT. It is also unaffected by chronic disease or vitamin B_{12}/folate deficiencies which can increase the MCV.

A recently published study confirms that CDT has the highest sensitivity and specificity for diagnosing alcohol abuse (84% and 92%, respectively), while the MCV has the lowest (48% and 52%, respectively).[14] In addition, transferrin remains in the circulation for seven to 14 days and CDT, therefore, can give an indication of alcohol consumption over this period of time, which gives it an advantage over other blood or urine alcohol measurements, which only remain elevated for 24–48 hours after alcohol intake. Owing to its specificity, CDT is now widely accepted as the most accurate biological marker of chronic alcohol abuse[15–17] and has been particularly useful when deployed as a predictive indicator of abstinence in driving licence regranting programmes, where it is used to evaluate the physical fitness of individuals whose driving licences were confiscated for drink driving.[18–20]

An ethanol intake in excess of 50–80 g for a period of approximately two to three weeks has been shown to increase the CDT proportion of transferrin to around 3%, with 1.6% often used as the cut-off for excessive ethanol intake.[2,21]

There are several methods currently in use for the measurement of CDT. These include immunoassay,[22] high-performance liquid chromatography (HPLC)[23–26] and capillary electrophoresis.[21,27–32] Recently, HPLC has been identified as a possible reference method.[7] Of the current methods available, the fully automated Sebia Capillarys 2 lends itself to high-volume screening as it is both precise and highly correlated with the proposed HPLC reference method.[21,24,29,33] To this end, the author's laboratory currently uses the Sebia Capillarys 2. This paper reports an analytical comparison of the new V8 CDT capillary zone electrophoresis (CZE) system (Helena Biosciences, Tyne and Wear, UK) with the Capillarys 2 (Sebia, Surrey, UK).

Materials and methods

Sebia Capillarys 2

Carbohydrate-deficient transferrin was measured using the department's Capillarys 2 system (software version: 6.1.2). This technique uses a high-voltage zone electrophoretic system in alkaline buffer (pH 8.8) to separate the five major isoforms according to their sialylation level. Two carbohydrate-deficient isoforms are reported, (AsT and DisT), these being separated in silica capillaries and directly detected at an absorbance of 200 nm. The system can run seven analytical runs simultaneously, either on seven different samples or on a single sample.

Table 1. Sample demographics.

	Median age	Min age	Max age	*n*
Males	38	18	65	106
Females	32	18	59	44
All	36	18	65	150

Helena Biosciences V8

The Helena Biosciences CDT auto assay for the V8 E-Class capillary electrophoresis system (software version: 4.1.526.0) utilises onboard iron saturation for high resolution and accuracy of sample results. This assay also works under high voltage in an alkaline buffer. The assay has the ability to run eight samples when utilising all eight capillaries. Transferrin isoforms are quantified with ultraviolet (UV) protein detection at 200 nm. The Helena assay works by solely focusing on the DisT isomer for measurement of CDT, as recommended by the IFCC CDT working group. All assays were performed using the manufacturer's instructions, with no deviation from stated protocols.

Patient samples

A total of 150 patient samples were collected over a six-week period. Samples were selected on the basis of the results obtained from the current in-house platform (Sebia Capillarys 2) and covered the CDT range 0.3–23.7%. Samples were collected in Becton Dickinson (Oxford, UK) SST Vacutainer tubes and received in the department within 24 hours of collection. Samples were centrifuged as per the manufacturer's recommendations before being stored for no more than three days at 4°C(±2°C) prior to analysis (50 samples). Samples selected from storage had been frozen within three days of receipt in the laboratory and stored at –20°C (±2°C) for no more than four weeks before being re-tested (100 samples). The median age of the 150 patients selected was 36 years (age range: 18–65 years), and 44

Table 2a. Intra-batch imprecision studies: Sebia IQC material.

	Capillarys 2		V8	
	Normal IQC	Path IQC	Normal IQC	Path IQC
n	5	5	5	5
Target value	0.8±0.3	7.1±1.4	0.8±0.3	7.1±1.4
Mean	0.9	7.3	0.8	5.6
SD	0.08	0.52	0.06	0.1
CV %	9	7	8	2
95% CI (±)	0.07	0.46	0.05	0.09

Table 2b. Inter-batch imprecision studies: Sebia IQC material.

	Capillarys 2		V8	
	Normal IQC	Path IQC	Normal IQC	Path IQC
n	5	5	5	5
Target value	0.8±0.3	7.1±1.4	0.8±0.3	7.1±1.4
Mean	0.9	7.3	0.7	5.5
SD	0.08	0.43	0.09	0.3
CV %	9	6	12	5
95% CI (±)	0.07	0.38	0.08	0.26

samples were from women. Prior to the study, samples were anonymised with only patient age and gender collected (Table 1).

Internal quality control material

Precision of both platforms was determined by running both the Sebia and Helena normal and pathological internal quality control (IQC) material. The samples were tested five times on one day (intra-assay precision) and again over five separate days (inter-assay precision) on both analysers.

Statistical analysis

Analyse-it Software (Leeds, UK) was used for processing the data obtained. The results are expressed as mean (±SD) and coefficient of variation (CV%). To define the relationship and the agreement between the two methods, Passing– Bablok non-parametric linear regression and Bland–Altman plots were performed.[34,35] The null hypothesis is that there will be no difference between the results from the two analysers. The *t*-test was used for comparative purposes and the critical level of significance set at 95% (P=0.05).

Results

Imprecision

The intra-batch precision using Sebia's Normal QC material showed little difference between the two analysers (Table 2A) (mean 0.9% [±0.08] and 0.8% [±0.06] for Capillarys 2 and V8, respectively). These results are both within the assigned mean value of 0.8% (±0.3) for that IQC material. With the Sebia Pathological control the Capillarys 2 was within tolerance limits but the V8 had a negative bias (mean Capillarys 7.3% [±0.52], V8 5.6% [±0.1]; the assigned mean 7.1% [±.4]). Inter-batch precision values were again similar for the Normal IQC material (Table 2B) (mean 0.9% [±0.08] and 0.7% [±0.09] for Capillarys 2 and V8, respectively). Again, the V8 gave a lower value for the Sebia Pathological IQC material (5.5% [±0.3] for the V8 compared to 7.3% [±0.43] for Capillarys 2).

Helena Biosciences CDT IQC material was also tested on both platforms. Although the Normal IQC assigned value is similar to that of Sebia (1.1% [±0.5] *vs*. 0.8% [±0.3]), the Pathological IQC assigned value is 2.5% (±1.3), rather than Sebia's 7.1% (±1.4). Intra-batch imprecision CVs are again similar for both materials and were within the target ranges (0.95% [±0.07] and 1.0% [±0.08] for Capillarys 2 and V8, respectively; assigned value 1.1% [±0.5]).

Inter-batch precision values were within tolerance limits for both platforms (Normal IQC mean 1.0% [±0.18] and 1.04% [±0.10], and Pathological IQC mean 2.16% [±0.11] and 1.96% [±0.14] for Capillarys 2 and V8, respectively).

Patient comparison

All 150 samples were analysed on both instruments. Table 4 shows the mean, median and paired *t*-test data. There was a significant difference between the two methods (P=0.015). Passing and Bablok regression shows the correlation between the two methods (r^2 0.9336 [y=0.985x–0.557]; Fig. 1). This shows a proportional bias, with a negative bias for the Helena method. The mean bias between the methods, at different CDT concentrations, is shown in Table 5 and in the Bland Altman plot (Fig. 2).

Table 6 shows the P values at selected CDT% concentration ranges. Between the concentration range of 0.8–4.0% there was good correlation (P=0.1802). Outside of

Table 3a. Intra-batch imprecision studies: Helena IQC material.

	Capillarys 2		V8	
	Normal IQC	Path IQC	Normal IQC	Path IQC
n	5	5	5	5
Target value	1.1±0.5	2.5±1.3	1.1±0.5	2.5±1.3
Mean	0.95	2.13	1.0	2.14
SD	0.07	0.05	0.08	0.08
CV %	7	3	8	4
95% CI (±)	0.06	0.04	0.07	0.07

Table 3b. Inter-batch imprecision studies: Helena IQC material.

	Capillarys 2		V8	
	Normal IQC	Path IQC	Normal IQC	Path IQC
n	5	5	5	5
Target value	1.1±0.5	2.5±1.3	1.1±0.5	2.5±1.3
Mean	1.0	2.16	1.04	1.96
SD	0.18	0.11	0.10	0.14
CV %	17	5	10	7
95% CI (±)	0.16	0.1	0.09	0.12

Table 4. Patient sample comparison data.

	Capillarys 2	V8
n	150	150
Mean	1.66	1.85
Median	1.09	1.05
t-test (*P* value)	2.64 (0.015)	

Table 5. Mean bias values at different CDT concentration ranges.

	CDT range		
	0–0.7%	0.8–4.0%	>4%
n	51	138	13
Mean bias	–0.2	0.05	–2.8
Mean % bias	34%	0.03%	26%

Table 6. *t*-test at selected CDT ranges.

CDT range (%)	*n*	*t*-test	*P* value	Significance
0–0.7	51	8.38	0.0001	Highly significant
0.8–4.0	138	1.34	0.1802	Not significant
>4 (up to 23.4)	13	4.215	0.001	Highly significant

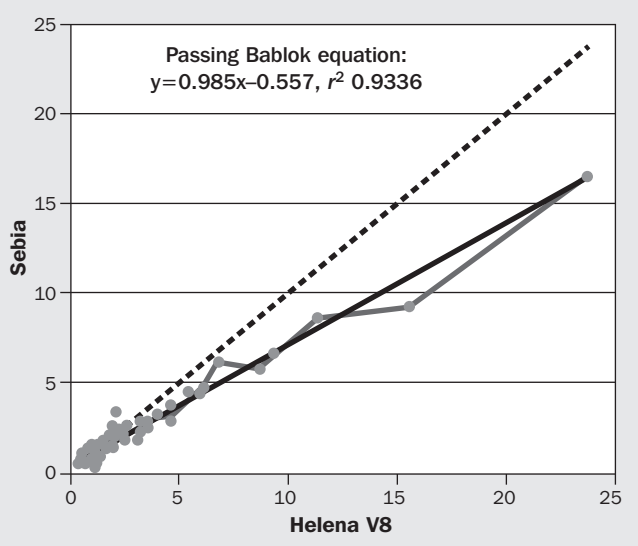

Fig. 1. Regression analysis comparing patient CDT results from the Capillarys 2 and V8.

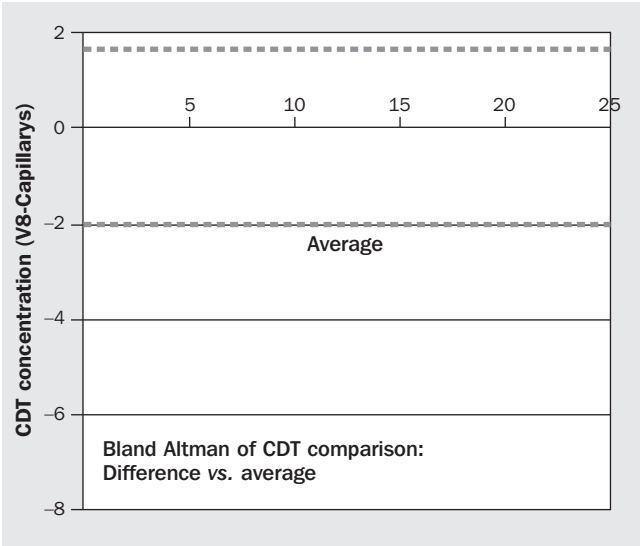

Fig 2. Bland Altman plot.

this range, however, there were highly significant differences (P=0.0001 and P=0.001 for the ranges 0–0.8 and 4.0–23.4, respectively).

Discussion

Both platforms showed good levels of imprecision when using either the Sebia or Helena IQC materials. Results were within the acceptable tolerance range on both manufacturers' Normal IQC material, but results differed from the target values on the V8 using the Sebia Pathological material. Results were comparable to those published elsewhere for the Capillarys system.[36] The V8 gave lower values at high CDT levels both for IQC material and patient samples, which can be seen in Tables 3A and 3B and in Figure 1. The Altman Bland plot identifies a distinctive proportional bias at high CDT values. Target CDT ranges for the Pathological IQC levels between the two manufacturers were vastly different (7.1% [±1.4] for the Sebia IQC material, and 2.5% [±1.3] for the Helena Biosciences IQC material).

It is felt that the lower Pathological IQC material sourced from Helena Biosciences was more relevant for the department's patient cohort, the target value sitting around the cut off of 1.6%. However, Sebia is now in the process of upgrading the Capillarys 2 software, which will allow for the use of a recently released IQC with a similar target value to that from Helena Biosciences.

Carbohydrate-deficient transferrin analysis was then performed on 150 patient samples. The results of the determinations ranged between 0.3% and 23.7% (Capillaries 2) and between 0.3% and 16.5% (V8). The median values were 1.1% on both systems, whereas the mean results were slightly lower on the V8 (1.85% Capillaries *vs.* 1.66% V8). The Capillarys 2 is stated as having linearity between 0.3% and 26.2% CDT. The overall correlation between the methods in the 150 patient samples analysed was satisfactory, displaying a correlation coefficient (r^2) of 0.985. However regression analysis and the Bland Altman plots show that the V8 has a negative proportional bias when compared against the Capillaries 2 at higher CDT levels. Over the clinical range of interest (CDT 0.8–4.0%) there was good correlation between the two methods (P=0.1802 and % mean bias 0.03%). At higher concentrations there was significant deviation (P=0.001 and mean % bias 26%).

Differences in results could be attributed to several factors related to analysis conditions, especially in the composition of the buffer or the CDT portion being measured. The V8 only measures the stable DiST CDT isomer as per the IFCC CDT Working Group recommendations, whereas the Capillarys 2 measures AsT and DisT. Although Schellenberg and Wielders[21] showed that the Capillaries 2 produces comparable results with the HPLC method, further research is required to confirm which system correlates more closely over the range seen herein. However, recent data published by the IFCC CDT Working Group on the harmonsation of CDT reporting shows a good correlation between the Sebia and Helena Biosciences methods and the IFCC candidate reference method.[37]

In conclusion, both instruments can be used equally effectively for measuring CDT levels in human serum. There is good correlation over the clinical concentration range of interest. However, further research is required to identify which platform more closely compares to the candidate HPLC reference method. □

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