Application Information

BECKMAN COULTER®

Purification of Transferrin Isoforms from Serum prior to Analysis with Capillary Electrophoresis

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1. INTRODUCTION

CDT (Carbohydrate Deficient Transferrin) is a major biomarker for the diagnosis of alcohol abuse and alcohol dependence. Transferrin is a glycoprotein which may have one or two branched glycan chains. In serum electrophoresis it migrates in the beta zone after the gamma (Fig. 1). The glycan chains have a terminating sialic acid ranging from zero to 8 sialic acids. Usually 2-, 3-, 4-, 5-, and 6-sialo transferrin are observed. The less sialylated isoforms, 0-sialo and 2-sialo transferrin, increase in relation to excessive alcohol intake (Fig. 2). For this reason it is used in clinical and forensic medicine including occupational and traffic medicine.

Several analytical techniques may be used, such as isoelectric focusing, immunochemical determination, HPLC and CE. The technique should be reproducible and precise, avoiding false positive or negative results, because it has medical as well as legal implications.

CE is recognized as a reference for this type of test because it combines high separation power, easy sample preparation and a fast result. The CEofix™ CDT kit was developed to run on P/ACE™ MDQ capillary electrophoresis system. Sample preparation simply involves saturation with a Fe solution. It uses a patented (5,611,903) dynamic double coating of the capillary that allows reproducible migration and avoids proteins sticking to the capillary wall. It also allows analysis of CDT in presence of transferrin variants and is also used for CDG (Carbohydrate Deficiency of Glycosylation) which is a test for some metabolic diseases. The kit was developed to avoid interfering peaks, such as high level of CRP. However, in some rare cases, interference of unknown peaks may be observed. For this reason, a method was developed to purify transferrin isoforms from serum before analysis.

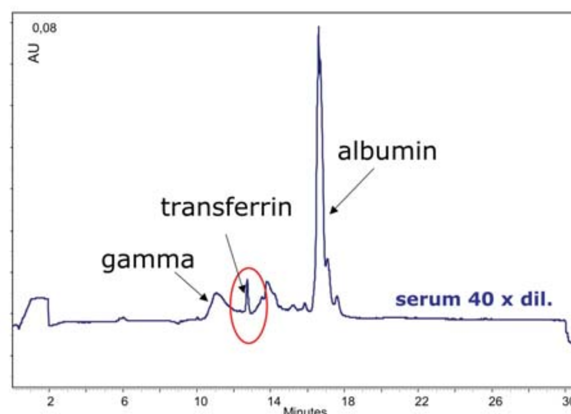


Figure 1: *Serum analyzed with CEofix nTMP buffer. Transferrin migrates after the gamma zone in the beta zone.*

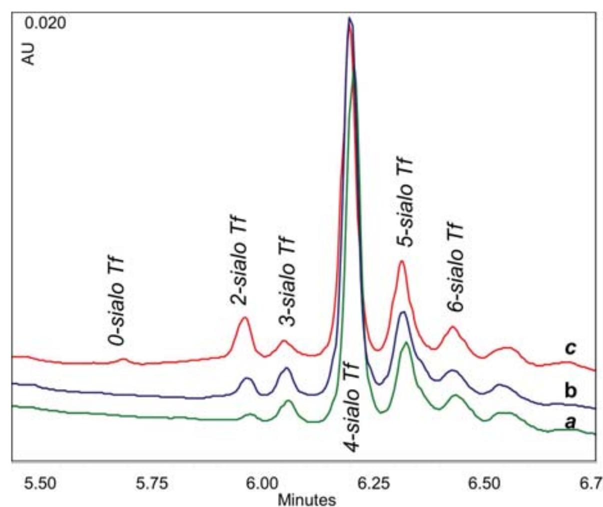


Figure 2: *Transferrin in serum analyzed with CEofix CDT buffer: (a) normal profile, (b) profile with increased 2-sialo transferrin and (c) increased 2-sialo transferrin and appearance of 0-sialo transferrin.*

2. METHOD

The purification takes place via an affinity column using avian antibody IgY-antigen interaction. After capture, the transferrin is stripped off and concentrated with an ultrafiltration spin column. Finally, the transferrin isoform analysis is performed with a CE.

3. MATERIAL

	Kit
Purification	ProteomeLab™ IgY-Transferrin spin column kit (Beckman Coulter, Fullerton, CA, U.S.A.) Part number A25523
Concentration	Vivaspin 500 column 30,000 MWCO (Vivascience, Hannover, Germany, Part number VS0122)
Total Transferrin	CEofix™ NTMP Nitrilotri(methyl-phosphonic) acid buffer 50 mM, pH 7.2 (Analis, Suarlée, Belgium, Part number 10-004780)
CDT	CEofix CDT TRiS/borate buffer pH 8.5 (Analis, Suarlée, Belgium, Part number 10-004760)
Instruments	P/ACE™ MDQ with UV detector at 200nm and 32Karat 7.0 Software (Beckman Coulter, Fullerton, CA, U.S.A.) Paragon™ CZE 2000 (Beckman Coulter, Fullerton, CA, USA, Part number 465500) with CZE IFE/s 50 Test Kit (Part number 446290)
Capillary	50 µm ID x 375 µm OD x 50 cm total length (Beckman Coulter, Fullerton, CA, USA, Part number 338451)

4. IMMUNOCAPTURE OF TRANSFERRIN FROM SERUM

400 µL of serum is diluted with 1600 µL of dilution buffer and applied in 4 times 500 µL on the spin column. After washing unbound material off the column, the bound transferrin is eluted using Stripping Buffer. The proteins are further concentrated and exchanged into TBS/BSA buffer using a Vivaspin ultrafiltration spin column. CDT is further analyzed using the standard method of the CEofix CDT kit (Fig. 3).

5. OPTIMIZATION OF IMMUNOCAPTURE

For optimization, a solution of Apo-Transferrin (Sigma T4382) was added at 3 mg/mL in TRIS buffered saline (TBS-BSA (Sigma A4503) and further diluted with the Dilution Buffer of the ProteomeLab kit as described in the instruction manual. The Proteomelab Spin Column was sequentially loaded with 500 µL, and the concentration of the flow-through fraction was measured with the CEofix NTMP buffer (Fig. 4).

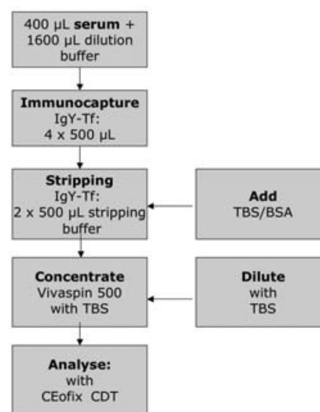


Figure 3: *Purification of transferrin (Tf) in serum in IgY-Tf spin column, followed by concentration by ultrafiltration and analysis of CDT.*

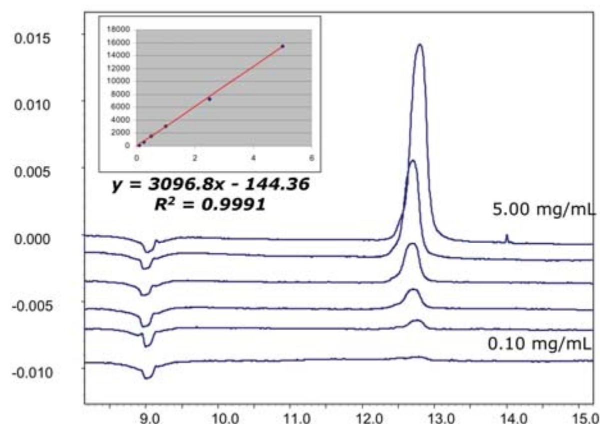


Figure 4: *Linearity of apo-transferrin analyzed with CEofix nTMP buffer and 50 µ x 60 cm capillary.*

After 4 x 500 µL, the column is totally saturated and transferrin is found in the flow through fraction (Fig. 5).

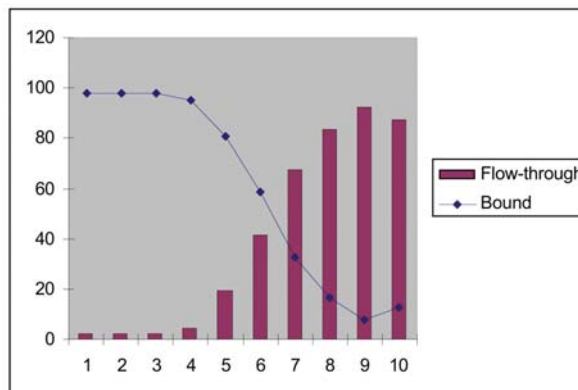


Figure 5: *Percentage of apo-transferrin that is immunocaptured on the IgY-Tf spin column. application of transferrin in steps of 500 µL.*

6. RESULT

When analyzing serum that does not have interfering peaks (Fig. 6 and 7), we observe a reduction of the gamma zone. A small difference in value may be observed.

Problematic samples (Fig. 8, 9 and 10) showed specific removal of the interference allowing a perfect interpretation of the transferrin isoform profile.

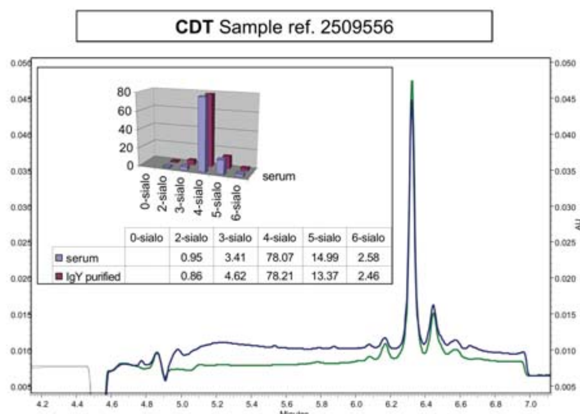


Figure 6: *Normal sample before and after immunocapture on IgY-Tf spin column.*

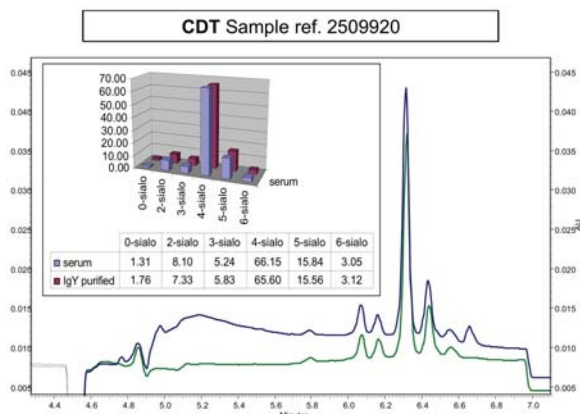
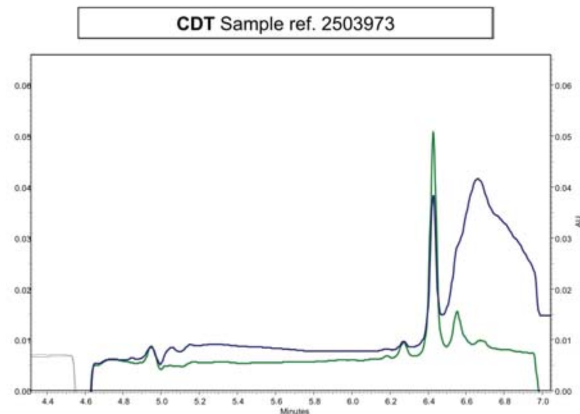
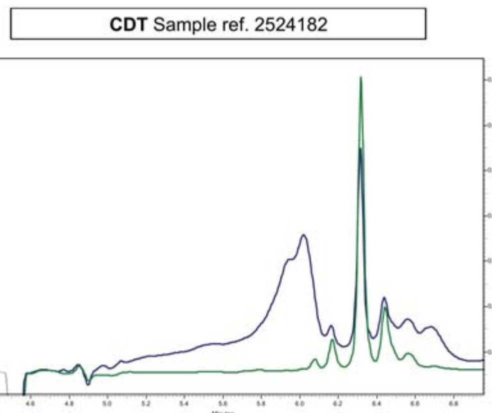


Figure 7: *Similar but for a positive sample.*





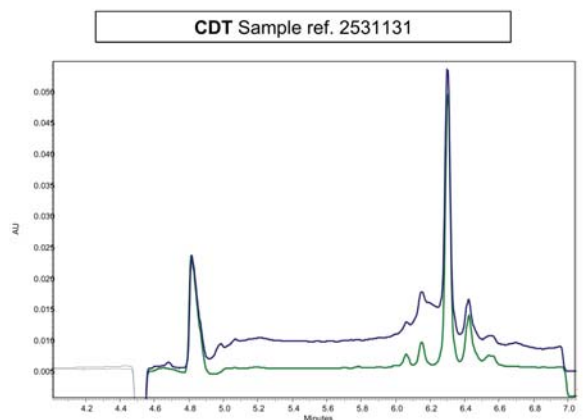
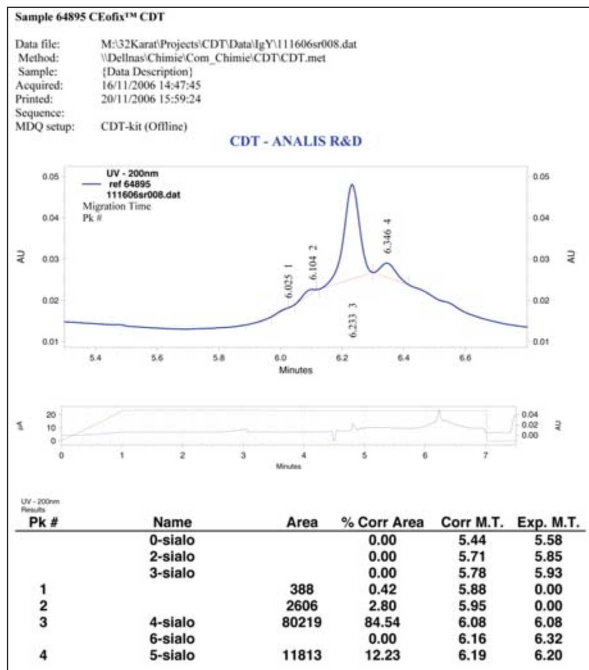


Figure 8, 9 and 10: *Examples of removal of interfering peaks with IgY-Tf spin column.*

7. EXAMPLE

A problematic serum sample was received that showed interference between the gamma and beta zones (Fig. 11). This profile did not allow us to determine a value for the CDT. An immunofixation after electrophoresis on Agarose (Fig. 12) was performed on the serum showing the presence of an IgA-lambda monoclonal antibody. After purification, a normal profile (Fig. 13) was observed allowing a result to be determined.



Pk #	Name	Area	% Corr Area	Corr M.T.	Exp. M.T.
	0-sialo		0.00	5.44	5.58
	2-sialo		0.00	5.71	5.85
	3-sialo		0.00	5.78	5.93
1		388	0.42	5.88	0.00
2		2606	2.80	5.95	0.00
3	4-sialo	80219	84.54	6.08	6.08
	6-sialo		0.00	6.16	6.32
4	5-sialo	11813	12.23	6.19	6.20

Figure 11: *Problem sample (ref. 64895) with interference between the gamma and beta zone.*

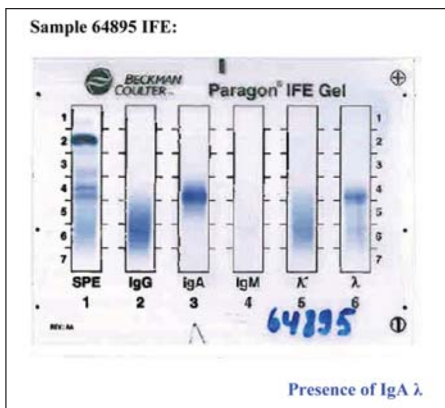
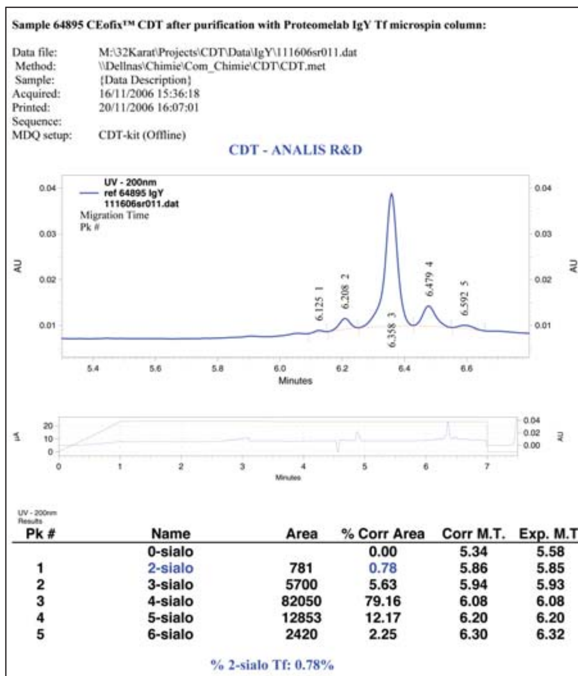


Figure 12: *Immunofixation of sample 64895 showing an IgA lambda.*



Pk #	Name	Area	% Corr Area	Corr M.T.	Exp. M.T.
	0-sialo		0.00	5.34	5.58
1	2-sialo	781	0.78	5.86	5.85
2	3-sialo	5700	5.63	5.94	5.93
3	4-sialo	82050	79.16	6.08	6.08
4	5-sialo	12853	12.17	6.20	6.20
5	6-sialo	2420	2.25	6.30	6.32

% 2-sialo Tf: 0.78%

Figure 13: *Same Sample analyzed after immunocapture on IgY-Tf spin column.*

8. CONCLUSION

More work will be performed to correlate the results of normal samples before and after immunoaffinity purification. We also want to accumulate additional data on problematic samples to validate this method. The same technique may be used to purify transferrin from serum, plasma or cerebrospinal fluid before analysis with CE-MS.

REFERENCES

1. CEofix Application Note 040605, Analis (Belgium)

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