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# Determination of CDT. Comparison of ion exchange columns chromatography and capillary electrophoresis

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**INTRODUCTION:** Carbohydrate deficient transferrin (CDT) is the most specific marker for harmful use of alcohol and is defined as asialo-, monosialo- and disialotransferrin isoforms. Transferrin is synthesized mainly in the liver as a glycoprotein. The two oligosaccharide chains have a bi- or triantennary structure and a different amount of bounded sialic acids determines the transferrin isoforms: from asialo- to heptasialotransferrin. Tetrasialo transferrin is the most commonly occurring isoform in human serum. Isoforms with a low rate of sialylation (asialo, monosialo, disialo) are present in a very low concentration. The presence of these isoforms is elevated in harmful use of alcohol. It is known that in average daily consumption of 60 g of pure alcohol 14 days consecutively increases the value of carbohydrate deficient transferrin. Analytical determinations and quantifications of CDT are based on differences in isoelectric points of transferrin isoforms. The most commonly used separation methods are: isoelectric focusing, capillary electrophoresis (CE), HPLC, and ion exchange columns chromatography (IEC).

**AIMS:** We compared two different methods for CDT determination: separation on ion exchange columns with immunoturbidimetric detection and capillary electrophoresis as the reference method.

**MATERIALS:** We analysed 46 human serum samples, chosen randomly, stored at –20 °C and left at room temperature till analysis. The determination of CDT with both methods was performed on the same day. The results were evaluated with linear regression analysis (least-squares method), and Pearson correlation coefficient.

**METHODS:** Ion exchange columns (Tina-quant® %CDT 2nd generation, Roche Diagnostics): The separation of transferrin isoforms with anion exchange columns is a two-step procedure with sample pretreatment and immunoturbidimetric determination. The saturation of a serum sample with iron(III) solution lasts 2-15 minutes. Afterwards the pretreated sample was transferred to an anion exchange column for CDT separation. Non-CDT fractions were eluated and discarded in the next elution step the CDT specific isoforms were collected. Total transferrin and CDT isoforms were determined on the Roche Hitachi 917 analyzer automatically after the addition of anti-transferrin antibodies. Total transferrin was determined from an aliquot of the pretreated sample with iron(III) solution. The %CDT was calculated from the CDT and total transferrin values. Proposed normal value is < 3.0 %.

Capillary electrophoresis (Capillarys CDT, Sebia): The separation of analytes with capillary electrophoresis was performed on the Sebia Capillarys 2 analyzer completely automatically. In the analyzer the pretreatment of the serum sample with iron(III) solution occurred before the application of the sample into the capillary. Transferrin isoforms were separated in alkaline buffer (pH 8.8), with constant voltage 9.2 kV, and constant temperature 40°C, and were detected at the cathodic end of the capillary at 200 nm after 8 minutes migration. The analyzer with eight parallel capillaries enables run of seven samples simultaneously. As a result the graphic pattern with separated isoforms: asialo-, disialo-, trisialo-, tetrasialo-, and pentasialotransferrin, was obtained. For this method the proposed normal value is < 1.3 %.

**RESULTS:** The comparison between both methods gave a good agreement with the following equation: IEC = 0.86 CE + 2.3, and correlation coefficient r = 0.96. The results obtained with the ion exchange columns method showed a positive deviation compared with the values of capillary electrophoresis. CDT measured by capillary electrophoresis gives a graphical review of all transferrin isoforms present in the sample and the possibility to determine the presence of asialotransferrin.

**CONCLUSION:** With both methods %CDT can be measured. With the ion exchange columns method only the percentage of all CDT specific isoforms is determined. The procedure with the capillary electrophoresis enables the quantification of all detected transferrin isoforms present in the sample and a graphical output of the results. This method is faster, with higher capacity compared to the ion exchange columns method, and gives information about the transferrin genetic variants.

**KEYWORDS:** *Carbohydrate deficient transferrin, harmful use of alcohol, Ion exchange columns, Capillary electrophoresis*

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