K15 Stability and Reproducibility Studies for Carbohydrate Deficient Transferrin Analysis Using Capillary Electrophoresis

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After attending this presentation, attendees will develop an understanding of the protein Transferrin, and gain an understanding as to its stability and reproducibility and therefore its credibility as an analytical biological marker.

This presentation will impact the forensic science community by introducing a technique that, because of its stability and reproducibility,

can be used in routine toxicological analysis concerning questions of chronic alcohol abuse.

The present research addresses the quantitative analysis of Carbohydrate Deficient Transferrin (CDT) levels in biological samples using capillary electrophoresis for reproducibility and repeatability as well as the analyte's stability *in vitro*.

Transferrin is a glycoprotein responsible for binding iron and transporting it via blood throughout the body. Multiple transferrin isoforms have been observed based on the presence of oligosaccharide chains containing acetylglucosamine, galactose, mannose and sialic acid. The sialic acid residues are in terminal positions of these chains and are the only part of the chain with a negative charge. The number of sialic residues in a transferrin molecule expresses the degree of transferrin glycosylation in an individual, which is usually:

Tetrasialio-transferrin: 75%
Pentasialio-transferrin: 15%
Trisialio-transferrin: 5%
Disialio-transferrin: 2%
Hexasialio-transferrin: 2%
A-, Mono-, Hepta-, Octa- sialo-transferrin: <1%

Carbohydrate Deficient Transferrin (CDT) refers to the sum of the disialic, monosialic, and asialic groups. Research has indicated that individuals with a pattern of consuming > 50-80 grams of alcohol (approximately > 5-8 drinks) a day for at least seven consecutive days will have an increased CDT value. This indicates sustained alcohol consumption and provides information about an individual's drinking habits (indicate a potential alcohol abuser).

One sensitive and specific instrumental method used to detect CDT is Capillary Electrophoresis (CE). CE technology employs a voltage potential to a narrow-bore silica capillary and separates components based on size and charge. CE technology can separate the different transferrin glycoforms and, by assessing peak area ratios, determine the percentage of CDT in human serum.

The control and one sample serum were run six different days, six injections per day to examine both the intra-day variability (repeatability) as well as the inter-day variability (reproducibility).

Three different storage conditions were utilized to examine the % CDT values and assess the stability of CDT in serum. Aliquot sets from four different sample sera were stored on a lab bench top at room temperature (25°C) over a nine-week period, in the refrigerator (approximately 4°C) over a ten-week period, and in the freezer (approximately -20°C) over a seventeen-week period. The % CDT was also checked every two weeks. Each serum sample along with the control was injected twice. The sample aliquots were stored in the freezer and analyzed every two weeks over an eight-week period.

The data generated during these studies indicated that CDT remains stable for extended periods of time when stored under various conditions but will remain stable the longest when stored at either 4°C or -20°C. Even if other studies are required to check the stability of the CDT related glycoproteins in serum samples over a longer span of time, the assessment of CDT under standard laboratory conditions highly supported the adoption of CDT as an indicator of alcohol abuse in the clinical and forensic environments.

CE technology proved again to be a simple and automated analytical tool producing easy reproducible and repeatable determinations of CDT in human serum, suitable for application in the daily routine of a toxicology laboratory.

Alcohol Abuse, Carbohydrate Deficient Transferrin, Capillary Electrophoresis

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