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SUPPORTING INFORMATION

# ACCURATE DETERMINATION OF TOTAL SERUM TRANSFERRIN AND TRANSFERRIN GLYCOFORMS IN HUMAN SERUM

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**Abstract**

The Supporting Information details the full uncertainty budget calculated for Fe by post column-IDA, Species-Specific-IDA and exact matching-IDA for all the samples measured as well as the coverage factor and the number of unique peptides of Tf obtained for all the fractions.

## Uncertainty calculations

The measurement uncertainty in the post column-IDA, Species-Specific-IDA and exact matching-IDA measurements were calculated using the following equations; (1), (2) and (3) respectively:

$$U = k\sqrt{u_s^2 + u_{SC}^2 + u_p^2 + B_{Cal}^2 + B_{Disc}^2 + B_{DT}^2 + B_{Bkg}^2 + B_w^2} \quad (1)$$

$$U = k\sqrt{u_s^2 + u_{SC}^2 + u_p^2 + B_{Cal1}^2 + B_{Cal2}^2 + B_{Disc}^2 + B_{DT}^2 + B_{Bkg}^2 + B_w^2} \quad (2)$$

$$U = k\sqrt{u_s^2 + B_{Cal}^2 + B_{Disc}^2 + B_{DT}^2 + B_{Bkg}^2 + B_w^2} \quad (3)$$

where:
$u_s$ is the uncertainty for replicate measurements
$u_{sc}$ is the uncertainty for spike calibration mixes
$u_p$ is the uncertainty for pump flow calibration
$B_{Cal}$ is the estimated standard uncertainty of the primary calibrant
$B_{Disc}$ is the estimated standard uncertainty for the instrument mass discrimination
$B_{DT}$ is the estimated standard uncertainty for the instrument dead-time correction
$B_{Bkg}$ is the estimated standard uncertainty for the instrument background correction
$B_w$ is the estimated standard uncertainty of the weighing measurements
$k$ is the coverage factor

## Glycoform identification by ESI-MS

To carry out the identification of each Tf isoform, 25 µL of 909c previously saturated with iron was injected several times into the HPLC system; the peak which corresponded to each isoform was collected, pre-concentrated and de-salted (using 10 KDa filters). Before carrying out the identification of the peptides by ESI-MS, each isoform was digested and de-salted (using C18 spin-columns). Raw data were submitted for query against a FASTA human protein database (released 4/3/2013, ftp.uniprot.org) using Proteome Discover software (version 1.3, Thermo Scientific). The following parameters were used during the database search with SEQUEST: variable oxidation of methionine and static carbamidomethylation of cysteine residues. The coverage factor as well as the number of unique peptides from the SEQUEST result file of Tf obtained in each glycoform collected in listed in the following table.

| **Glycoform** | **Coverage factor (%)** | **# unique peptides** |
|---|---|---|
| Asialo-Tf | 82 | 32 |
| Disialo-Tf | 95 | 45 |
| Trisialo-Tf | 90 | 41 |
| Tetrasialo-Tf | 79 | 54 |
| Pentasialo-Tf | 94 | 43 |
| Hexasialo-Tf | 86 | 41 |