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5.4 Transfer of Values from BCR 470 to the Nordic Protein Calibrator

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A protein calibrator must contain the measurants in genuine form, be stable, consist of a matrix which very closely resembles the serum samples from patients, and the target concentration values must be traceable to the highest level of trueness in the hierarchy, whether a reference method or a reference preparation. The Nordic Calibrator fulfils the three first conditions (chapter 5.3) and with the IFCC/CAP/BCR 470 preparation (chapters 5.1 and 5.2 and ref. 1) available in 1993, the task was to transfer the values from this reference preparation to the Nordic Protein Calibrator.

Transfer of concentration values

IFCC has set up a protocol for correct transfer of values from IFCC/CAP/BCR 470 to any secondary calibrator (chapter 5.2) and the transfer was performed in five Nordic laboratories according to this protocol.

The five laboratories were

1. Department of Clinical Chemistry, Odense University Hospital, DK-5000 Odense C, Denmark, using a turbidimetric method (Cobas Fara®, Roche).
2. Department of Clinical Chemistry, Hjørring Sygehus, DK-9800 Hjørring, Denmark using a turbidimetric method (Cobas Fara®, Roche).
3. Laboratory, Helsinki University Central Hospital, SF-00290 Helsinki, Finland using a turbidimetric method (Hitachi 911®,).
4. Central Laboratory, University Hospital of Turku, SF-20520 Turku, Finland using a nephelometric method (BNA®, Behringwerke).
5. Department of Clinical Chemistry, Danderyd Hospital, S-182 88, Danderyd, Sweden using a (Cobas Fara®, Roche).

All measurements were performed according to the IFCC recommendations and antisera from Behringwerke were used for the BNA-measurements whereas, the other laboratories used antisera from DAKO.
The data from all measurements were computed by Søren Blirup and Per Just Svendsen (both DAKO) according to the IFCC protocol and the results for individual laboratories together with mean and standard deviation are shown in table 5.4.1.

Table 5.4.1 Target concentration values for the Nordic Calibrator.
Values from 5 laboratories and their mean and standard deviation.
Measurements and calculations according to the IFCC protocol.

Protein	Dand	Odens	Hjør	Hels	Turk	Mean	SD	CV %
Prealbumin	0.3219	0.3360	0.3355	0.3146	0.3329	**0.3282**	0.0095	2.89
Albumin	44.137	42.161	43.184	43.453	45.202	**43.627**	1.1311	2.59
Orosomucoid	0.7773	0.7791	0.7685	0.7914	0.7887	**0.7810**	0.0092	1.18
α_1-Antitryps.	1.1150	1.1677	1.2101	1.1883	1.4250*	**1.1703**	0.0407	3.48
Haptoglobin	1.0273	1.0191	1.0201	1.0317	1.0206	**1.0238**	0.0055	0.54
Transferrin	2.5668	2.5488	2.5357	2.5682	2.5709	**2.5581**	0.0152	0.60
IgG	10.088	9.473	9.641	10.041	10.129	**9.874**	0.2974	3.01
IgA	2.0860	2.0600	2.0768	2.1103	2.2019	**2.1070**	0.0561	2.66
IgM	0.8354	0.8402	0.8269	0.8471	0.8879	**0.8475**	0.0238	2.80

*: This value is approx. 20 % higher than the others, and has been omitted from the calculations of mean

The measurements of target values for Haptoglobin and Transferrin are very precise with CV-values close to 0.5 %, and for Orosomucoid with approx. 1 %. Most of the proteins have CV-values between 2 and 3 % which is considered sufficient for the assignment of the values, but could be investigated further. For Prealbumin, Helsinki has a lower value than the rest. For Albumin Turku has the highest value. For IgG, Odense and Hjørring have the lowest values. For IgA and IgM, Turku has the highest values. However, there is no clear picture, except from the highest values for IgA and IgM for nephelometry (Turku). Regarding α_1-Antitrypsin, there is a discrepancy between Turku, with the nephelometric method and antiserum from Behringwerke, and the rest, with turbidimetric methods and antiserum from DAKO, and even among the latter group, Danderyd has a value approx 6 % lower than the rest. The nephelometric value has been omitted from the calculation of the mean and the cause of the difference is investigated further in section 5.5.

Discussion

The transfer of values has been performed according to the IFCC protocol and the procedures have been followed with painstaking accuracy, so the results must be considered the best obtainable. Except from α_1-Antitrypsin, the results resemble the assignment of values to IFCC/CAP/BCR 470 and the outcome must be considered as the level of the state of the art, although, we hoped for CV-values below 2 % for all the proteins. The immunoglobulins are heterogeneous with more than 1,000,000 forms, so the compositions of the pools may be different - although the number of individuals used for the production of the calibrators is very large. This might explain the higher values for IgA and IgM by nephelometry. But for Prealbumin and Albumin there is no simple explanation.

α_1-Antitrypsin is, by all means, a real problem, and the fraction of Z-phenotypes is too small to give such an effect (of 20 %). The explanation must be looked for in the degree and type of denaturation of the protein in the two pools. The procedures for delipidation are different and the storage conditions (freeze dried and liquid frozen) are also different. Both may result in varying types and degrees of denaturation which may result in different reactions with antibodies in the measurements, and thereby different types and sizes of immunocomplexes, which may be detected differently by turbidimetry and nephelometry. If so, then the target values cannot be used for the both types of analytical principles. The value assigned to the Nordic calibrator, thus, is only relevant for turbidimetric methods. Other problems with α_1-Antitrypsin are discussed in chapter 6.

From another point of view the acceptability of the assigned values, is to compare the dispersions to the analytical quality specifications for using common reference intervals for the plasma proteins. If we look at the CV-values, the problem seems to be serious only for Albumin, where the acceptable analytical CV is approx. 4 % for using common reference intervals, which should be compared to the obtained of approx. 3 %. This doesn't leave much to the laboratories' analytical imprecision. The CV of the transfer, however, is just an estimate of the dispersion of bias-values among the transfer-laboratories and the estimated bias between the two most diverging laboratories is approx 7 %, which is far outside the acceptable 2 %. The same contemplations should be made for Prealbumin and for IgG, where the acceptable bias is approx. 5 %, but less serious than for Albumin.

Except from α_1-Antitrypsin, the transferred values, however, give the basis for the use of common reference intervals, which in spite of the weak points, is far advantageous to the current situation (cf. chapter 7).

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