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2. Introduction

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2.1 Background and Aims

When *The Nordic Protein Project* was suggested by *The Nordic Committee on Quality Control* in 1986, it was intended to comply with two objectives:

1. to improve and control the quality of measurements of specific proteins in the Nordic countries

2. to create a general model for quality improvement for clinical chemical quantities in the Nordic countries

With support from *NORDKEM* from 1987 the project group formulated more operational objects for the work, based on the current knowledge about the situation and the possibilities within the present economic, scientific and practical limits.

It was well known that the quality of measurements of specific proteins were at a very low level in the Nordic countries as everywhere else. It was assumed that the three main reasons were (i) the heterogeneity of the plasma proteins (ii) the poor calibrators available and (iii) the different analytical principles with varying sensibility to protein heterogeneity, to the quality of calibrators and the method specific weaknesses.

(i) The heterogeneity of natural occurring, *genuine*, plasma proteins may be genetic, e.g. Haptoglobin and α_1-Protease Inhibitor, multiple forms within the single individual, e.g. Immunoglobulins and the simultaneous presence of partly metabolized proteins and changes in configuration due to binding to other molecules, whether through the transport function, e.g. Albumin and Transferrin, or by formation of complexes, e.g. with α_2-Macroglobulin or with autoantibodies. These problems cannot be solved in general for the plasma proteins as every single individual has its own pattern of variations

which for some of these will vary over time. In principle the subfractions of each specific protein can be measured separately by more specific methods, but this is not the intention of the clinical chemical specific protein measurements - at least for the time being. Therefore, the exact quantification of plasma proteins is not possible in practice, and a more pragmatic concept is needed, which is satisfactory enough to solve the clinical chemical problems. It is assumed that the presence of modified proteins and the combination of multiple forms of Immunoglobulins are rather constant in healthy adult individuals. In consequence a more modest, but also more attainable formulation is thus adopted:

To measure the specific plasma proteins in healthy adults correctly.

(ii) Commercially available calibrators have shown a variable quality. The materials were often denaturated during the preparation, e.g. by the delipidation or freeze-drying procedures, whereby, the genuine structure of proteins was changed to a great extent. Further, the *traceabilities* of assigned concentration values were unknown or questionable even for proteins where international reference preparations from WHO were available. *IFCC's Working Group on Standardization of Plasma Proteins* had produced pure preparations of three proteins, Transferrin, Orosomucoid and Transthyretin (Prealbumin), but these preparations were not approved yet. In Finland and in Denmark experiences with "liquid-frozen" serum pools for national calibrators were successful. In consequence, it was decided to produce a *Nordic calibrator* for the project and for future use. This calibrator should have assigned concentration values, which were transferred from *WHO-reference preparations* and the IFCC-purified proteins. (Later it was traced to the IFCC Reference Preparation for Human Plasma Proteins). Further, the calibrator should be produced in quantities allowing for the daily use in all the involved laboratories. Thus, the purpose was:

To produce a "liquid-frozen" Nordic Calibrator for daily use with assigned values according to WHO-reference preparations and IFCC-purified proteins. (Later changed to traceable to The IFCC Reference Preparation).

(iii) Today the main principles for analytical measurements of specific proteins are turbidimetric and nephelometric immunological methods, both kinetic and endpoint, and dye-binding methods (Albumin) and Transferrin. Further, the gel methods (RID and Rocket Electrophoresis) which dominated in the seventies are still in use a few places. It was not possible

in the project to evaluate all these principles and methods directly, but to establish a control system able to disclose the weaknesses of each individual method in order to guide the participating laboratories aiming for analytical improvements. Therefore, the third aim was:

To establish an external control system which would allow for check of traceability as well as for disclosing the weaknesses of analytical principles and methods in use and would be a basis for improvement of these methods.

During the project two other aspects of quality became clear, *Goals for the analytical Quality* and *Generating of Common Reference Intervals,* and two associated projects were established as described in chapters 4 and 7 respectively. Moreover, a number of problems had to be solved during the project as described below (2.2)

When IFCC in co-operation with BCR and CAP produced their reference preparation for plasma proteins with assigned values transferred from the same WHO and IFCC sources it was decided to transfer these values to The Nordic Calibrator as this *Secondary International Calibration Standard* should be accepted as the highest international level, and it is accepted also by the commercial producers of calibrators (cf. chapter 5). This will bring The Nordic Calibrator to the international level, and it will make the results obtained during the project and from associated projects (cf. chapter 7) available and useful for all using calibrators with concentration values traceable to the IFCC-BCR-CAP International Reference Preparation. This preparation has been released in June 1993 and it is the basis for all the concentration values presented in this book (unless other is stated).

The other objective, *to create a model for quality improvement for clinical chemical quantities in the Nordic countries* may also be fulfilled as the interest from *The Nordic EQAS-Organizers* (chapter 8) indicate. This aspect, however, must be left to the future, but the description in this book of the principles and results together with conclusions may give the elements for a concept on which a general model can be based.

Preliminary reports on the project:

1. Bjøro K, Blaabjerg O, Icén A, Irjala K, Hyltoft Petersen P. The Nordic Project. A NORDKEM-project on Analytical Quality Management. *Klinisk Kemi i Norden* 1992;vol 4,no 4:15-20.

2. Blaabjerg O, Bjøro K, Icén A, Irjala K, Hyltoft Petersen P. Goals for Analytical Quality Based on Biology in the Nordic Protein Project. *EQAnews* 1991;2:3.

3. Blaabjerg O, Hyltoft Petersen P, Icén A, Irjala K, Bjøro K. Det Nordiska Proteinproject - använda principer. *Klinisk Kemi i Norden* 1989;vol 1,no 1:21-2.

4. Blaabjerg O, Irjala K, Hyltoft Petersen P, Icén A, Bjøro K. Effect of Calibrator and Specimen Turbidity. *Moodi* 1992;2:109-11.

5. Hyltoft Petersen P, Blaabjerg O, Bjøro K, Icén A, Irjala K. The Nordic Protein Project. *Upsala J Med Sci* 1990;95:251-5.

6. Hyltoft Petersen P, Blaabjerg O, Irjala K, Icén A, Bjøro K. A Model for Quality Achievement. The Nordkem Protein Project. *Scand J Clin Lab Invest* 1993;53 suppl. 212:10-2.

7. Hyltoft Petersen P, Blaabjerg O, Irjala K, Icén A, Bjøro K. External Quality Assurance for Proteins. *Upsala J Med Sci* 1993;98:241-58.

2.2 Organization and Co-operations

The members of The Nordic Protein Project Group were *Kerttu Irjala,* Turku, *Arto Icén,* Helsinki, *Kristoffer Hellsing,* Uppsala, *Trond Reinskou,* Oslo, *Ole Blaabjerg* and *Per Hyltoft Petersen,* both from Odense. In 1990 Trond Reinskou and Kristoffer Hellsing left the group and *Kristian Bjøro,* Oslo, entered it.

In chapter 3 the concept of the project and associated projects is outlined in a logical composition describing the scientific structure according to the final concept. Details of the different projects are presented in the following chapters. Here, the presentation mainly covers the historical progress and deals with the groups and individual persons who have contributed by voluntary engagement and advises or by lighten the economical burden, the dimensions of which were not foreseen by the project group. We are very grateful for their support of many kinds. Further we want to thank the laboratories who participated in the project for their co-operation and for their patience as the project was delayed several times due to more or less anticipated problems. Thank you to all the named and unnamed contributors and helpers.

The project plan was worked out according to the objectives and the more operational specifications (cf. 2.1) with the main tasks of a) production of the calibrators, b) design of relevant models for design of control system, with control materials and relevant data presentation, c) data handling and computations, and d) ampouling and distribution of calibrator and control samples.

At the start the economical support was from *NORDKEM* (a total of 69 500 Finnish Mark), who only supported meetings - so, we had to look for organizations, groups and individuals who were willing to co-operate under these conditions, and for supporters ready to risk money in the project or even sponsor parts of the project.

Most relevant was to co-operate with the two *Nordic protein groups* at the time, *The Finnish* and *The Danish* protein groups, which have been of great value for the project, as also seen from the later contributions by co-ordinated projects on reference intervals (cf. chapter 7). Later *The Norwegian and Icelandic Societies on Clinical Chemistry* gave their principle supports. In this context the co-operation with *The Austrian Protein Group* chaired by *Thomas Endler* should be mentioned. It is evident, also, that we had tight connections to *The Nordic Committee on Quality Control* chaired by *Carl-Henric deVerdier* as long as the committee existed, and to his NORDKEM-project *Medical Need for Quality Specification in Laboratory Medicine* when it was established. Further, the project was co-ordinated with another NORDKEM-project *The Nordic Hormone Group* chaired by *Aimo Ruokonen.*

The problem of data handling and computations was solved by *Torsten Aronsson* and his computer group in Uppsala in co-operation with *Jens Rahbek Nørgaard* in Odense, and they have performed all the data processing in the project. Further, most of the typing of this book was performed by Gitte Hammershøj.

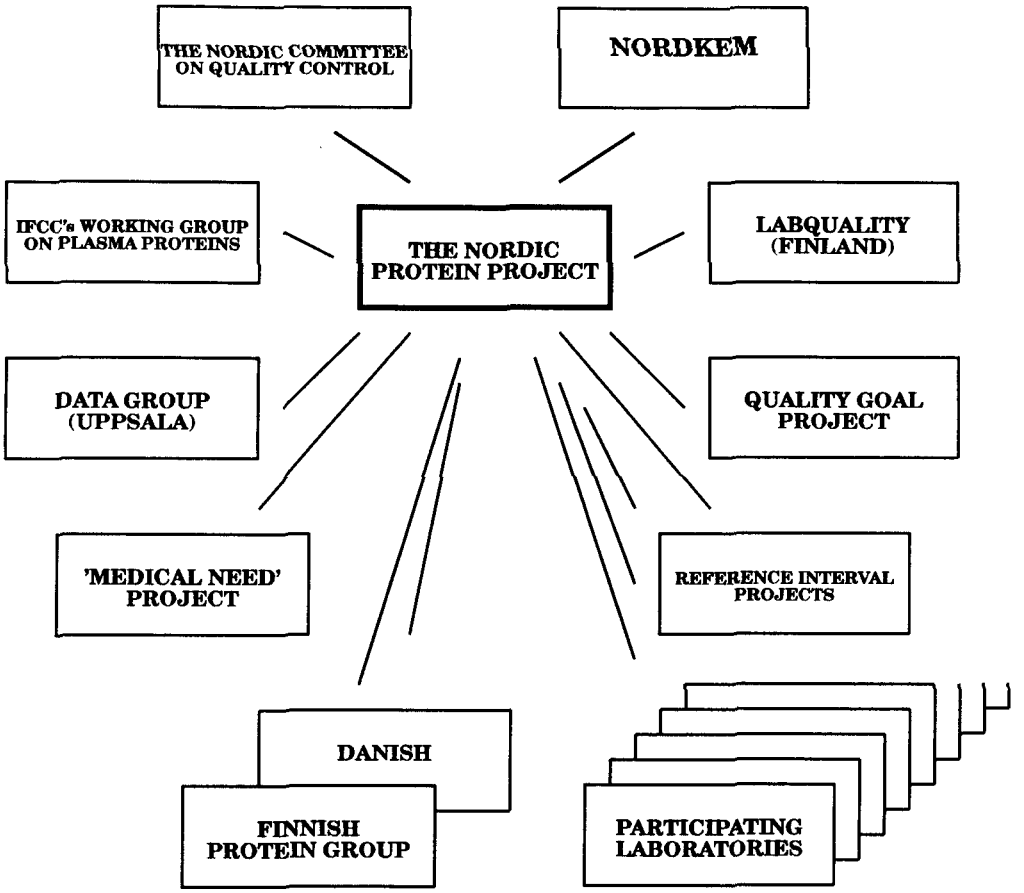

Fig. 2.2.1 *Illustration of co-operation and co-ordination between The Nordic Protein Project and relevant organization and laboratories.*

Preparation of calibrator and control samples was carried out in Odense (*Karin Jørgensen, Elsebeth Parlev,* and *Ole Blaabjerg*) in co-operation with *The Danish Protein Group* where serum specimens from blood donors were collected by *Mogens Blom*, Hjørring and *Adam Uldall*, Herlev as well. Financial support to donors for the serum to control samples was given by *The Danish Society of Clinical Chemistry.* Further, the ampouling of calibrator and control sera was performed by *Ole Riise Aps.*

The important task of financing the ampouling and the distribution of calibrator and control was organized by *LAB-QUALITY*, Helsinki, where *Raija Pikkarainen, Raimo Tenhunen,* and *Jussi Gävert* took care of this delicate matter. The investment could have been risky. The mailing of liquid frozen materials was a challenge which was solved all over Europe, but failed to Australia where it was possible, but too costly for the project.

During the project it became evident that specifications of acceptable analytical quality was needed. In 1987 *Elizabeth M.S. Gowans,* Dundee in Scotland (now *Chesters,* Shropshire in England) visited Odense as a scientific colleague and she developed the theory on which the goals/quality specifications in the project are based (cf. chapter 4).

The idea of establishing common reference intervals as a consequence of the common calibration and control of the quality came from *Ole Blaabjerg,* Odense. It became so evident for the project group that the *Finnish* and *Danish* protein groups were requested to establish the needed projects, which were accomplished as seen from chapter 7. All measurements were performed in Hjørring by *Inger Nørgaard* and *Mogens Blom* using The Nordic Protein Calibrator and controlled with the control materials from the project. The additional analyses were performed in the co-operating laboratories (B-Sedimentation Rate, ESR), in Hjørring (M-components) and at *DAKO*, (*Søren Blirup and Per Just Svendsen*) (S-CRP). Additional complementary projects seemed to complete the purpose. One project on immunoglobulin reference intervals for children (*Kerttu Irjala,* Turku) together with a project on reference intervals for non-insulin dependent diabetics (*Peter Thye-Rønn,* Odense). A special problem is caused by the genetic types of α_1-Protease Inhibitor where the Z-types may be considered either as "normal " or "abnormal". *Erik Lund,* Vejle performed the genetic typing of samples in the Danish reference interval project which made it possible to make more sophisticated evaluations for this protein. The handling of data was performed by Ane Richter.

The many projects on reference intervals are described in chapter 7.

In 1990 IFCC re-established *The Working Group on Plasma Protein Standardization,* and the group has prepared a *Reference Preparation* for plasma proteins (*The IFCC-BCR-CAP International Reference Preparation for Plasma Proteins*), which was released in June 1993. As the principle has been to refer to the highest international accepted level of standardization, these are transferred to the Nordic Protein Calibrator. The work was performed by *Kerttu Irjala and Kari Mattila,* Turku, *Aija Helin and Arto Icén,* Helsinki, *Anders Carlström,* Huddinge, *Inger Nørgaard and Mogens Blom,* Hjørring and *Tove Marseen and Ole Blaabjerg,* Odense.

Members of the IFCC-group (*John Whicher,* Leeds in England, *Siegfried Baudner,* Marburg in Germany, *Per Just Svendsen* and *Søren Blirup* both from Glostrup in Denmark) have together with the other mentioned contributed to chapter 5.

Sixty-seven laboratories joined the Nordic project from the first round, Finland: 13, Norway: 13, Sweden: 4, Denmark: 23, Iceland: 1, Austria: 11, Scotland: 1, Spain: 1.

As seen from the above written there has been a considerable co-operation with many groups and individuals, and we feel that the results have been satisfactory. Therefore, we believe that the outcome of all the efforts and results deserve a joint presentation which we hope is justified with this book, both regarding the actual protein project and as a basis for a general model for quality improvement within clinical chemistry and related fields.

Reports on related projects:

1. Baudner S. The Production and Characterization of a new International Preparation for Proteins in Human Serum. *Ann Biol clinique 1993;51:358.*

2. Blaabjerg O, Blom M, Gry H, Hyltoft Petersen P, Uldall A. Appropriate Sera for Calibration and Control of Specific Protein Assays. *Scand J Clin Lab Invest 1993;53 suppl. 212;13-5.*

3. Gowans EMS, Hyltoft Petersen P, Blaabjerg O, Hørder M. Analytical Goals for the Acceptance of Common Reference Intervals for Laboratories Throughout a Geographical Area. *Scand J Clin Lab Invest 1988;48:757-64.*

4. Hyltoft Petersen P, Gowans EMS, Blaabjerg O, Hørder M. Analytical Goals for Estimation of Non-Gaussian Reference Intervals. *Scand J Clin Lab Invest 1989;49:727-37.*

5. Just Svendsen P, Blirup S. Value Assignment to Serum Preparations. *Ann Biol clinique 1993;51:359.*

6. Whicher JT. The Development of New Standards for Plasma Proteins. *Ann Biol Clinique 1993;51:357.*

7. Whicher JT, Ritchie RF, Johnson AM, Baudner S, Bienvenu J, Blirup-Jensen S, Carlström A, Dati F, Ward AM, Just Svendsen P. New International Reference Preparation for Proteins in Human Serum (RPPHS). *Clin Chem* 1994;40:934-8.