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5.1 External Analytical Quality Assurance for Proteins

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ABSTRACT

In the Nordic Protein Project an external control scheme (external quality assessment) was combined with the two other indispensable aspects of analytical quality, i.e. standardization (with a common high quality calibrator) and specification of needed analytical quality for sharing common reference intervals for nine serum proteins in the Nordic countries. The quality specifications are reliable for the purpose and given in clinical chemical terms - ready for application to the control systems. Further, a control design for disclosing external and internal errors, separately, is designed with respect to calibration and robustness towards analytical interference from turbid patient samples.

SITUATION

The Nordic Protein Project was initiated by The Nordic Committee on Quality Control in 1986. The background was the miserable quality of specific protein measurements as appraised from external quality assessment schemes. The objectives of the project was, therefore, 'to improve and control the quality and measurements of specific proteins in the Nordic Countries'. The project was supported by NORDKEM from 1987 allowing for meeting activities.

It was found that the main cause for the poor quality of specific protein measurements was the insufficient quality of commercial calibrators with no documentation for traceability to WHO-reference preparations for specific plasma proteins. Based on experiences in the Finnish and the Danish protein groups it was decided to produce a liquid frozen (-80°C) serum calibrator for daily use in the Nordic laboratories (4). This calibrator should be cleared by ultracentrifugation and, where possible, the concentration values should be

transferred from WHO's reference preparations. Two years later IFCC established a working group for an international reference serum. This serum should serve as a tool for standardization of commercial calibrators, but not as calibrator for use in routine laboratories.

IFCC's reference serum is now established and concentration values are being transferred to The Nordic Protein Calibrator.

The second main cause for the poor quality of specific protein measurements was the unspecific signals from turbid patient samples in several measurement procedures. This problem, however, was related to individual analytical principles and procedures and had to be investigated in detail by means of a special design of the control system.

It was, further, decided to look at the individual laboratories' performance, focusing on calibration functions and intermediate reproducibility within- and between-standard deviations.

Other quality problems like molecular heterogeneity of individual proteins (haptoglobin, α-Proteinase inhibitor, α_1-antitrypsin, M-components, etc) were deliberately disregarded in the project.

In consequence, the overall purpose of the project was defined 'to improve and control the quality in order to measure nine specific proteins correctly in healthy individuals'. The question, however, was to specify the quality needed for this objective (see below).

CHARACTERISTICS OF THE ANALYTICAL PROCEDURES

The analytical principles (procedures) used by the laboratories in the project were:

1. Immunological
    a. Electroimmunoassay (rocket electrophoresis)
    b. Nephelometric (kinetic or end point)
    c. Turbidimetric (end point)
2. Non-immunological
    a. Albumin (dye-binding)
    b. Transferrin (Fe-binding)

MODELS FOR EVALUATION OF QUALITY SPECIFICATIONS

There are three main approaches on which analytical quality specifications can be based:

A. The state of the art

B. Biological

C. Clinical

where 'the state of the art' is the simplest and easiest to apply, 'The biological' is the most unambiguous and universal, and 'the clinical' is the most relevant for the use of laboratory results.

Re. A. In 'the state of the art' concept there are no relations to the usefulness of clinical chemical data and the specifications derived will fluctuate with the current analytical procedures in use.
Therefore, this approach was rejected.

Re. B. The 'biological' approaches are attractive as they are based on nearly constant data on within- and between-subject biological variations and there are many detailed publications (e.g. Costongs (5), Fraser (7)). The proposed goals (available at the start of the project), however, were only specified for analytical imprecision with very unclear relations to analytical bias (1, 6, 8, 16). Later Harris (10) published an elegant model combining imprecisions and bias, but based on statistical variances which weaken the bias concept in relation to individual laboratories. Further, a model with clear specifications of the two aspects of analytical quality was developed in relation to the project (9, 12). This model was adapted to the purposes of the project and therefore accepted.

Re. C. The 'clinical' approaches were considered the most relevant, but lack of proposed goals and lack of specific informations about the clinical use of the nine proteins would demand several projects to evaluate the needed specifications for precision and bias.
Therefore, the 'clinical approach' was not taken up in the project, and no relevant quality specifications for the nine proteins based on clinical strategies has been published until now.

The overall purpose of the project was then defined as 'establishing the basis for sharing common reference intervals in the Nordic countries for nine specific proteins where the populations are homogeneous for the quantities'. The theoretical concept was established in co-operation with Elizabeth M.S. Gowans from Dundee, Scotland (9, 12).

It was based on the IFCC recommendation for reference intervals (15) where, a minimum of 120 individuals is recommended for establishing reference intervals. This should give an acceptable confidence interval for each of the limits, as increasing sample size reduces this uncertainty. The idea is to establish a common reference interval based on a large number (e.g. 800) of individuals, whereby, the uncertainty becomes negligible - and then allow for analytical variation instead. Thus, each laboratory fulfilling the criterion for this acceptable uncertainty can use the common reference interval - and, thereby, be better than if they had established their own reference interval according to the IFCC recommendations (9).

This concept will - when common reference intervals are established - secure transferability of data and reduce the costs in each laboratory. Further, it makes it worth doing, also to establish reference intervals according to age and sex as well as for different ethnic groups.

A prerequisite, however, is a common and reliable standardization and subsequent control, which makes it possible to guarantee the analytical quality over time and geography. These are the objectives of The Nordic Protein Project.

According to the IFCC recommendations the 0.90 confidence interval for each reference limit (n = 120) allows for 1.3 to 4.4. percent of the population outside the limit, instead of the assumed 2.5 percent (9). If the reference interval is estimated with negligible uncertainty (n >800) Fig. 1, then maximum allowable analytical imprecision and bias can be estimated for the value where they cause the same percentages outside the reference limits. The combination of the two analytical deviations is complex but, for negligible bias the maximum allowable imprecision, s_A, is 0.55 times the biological standard deviation, ($s_B = \sqrt{s^2_{\text{within-subject}} + s^2_{\text{between-subject}}}$) and for negligible s_A the maximum allowable bias, B_A, is + or -0.24 · s_B

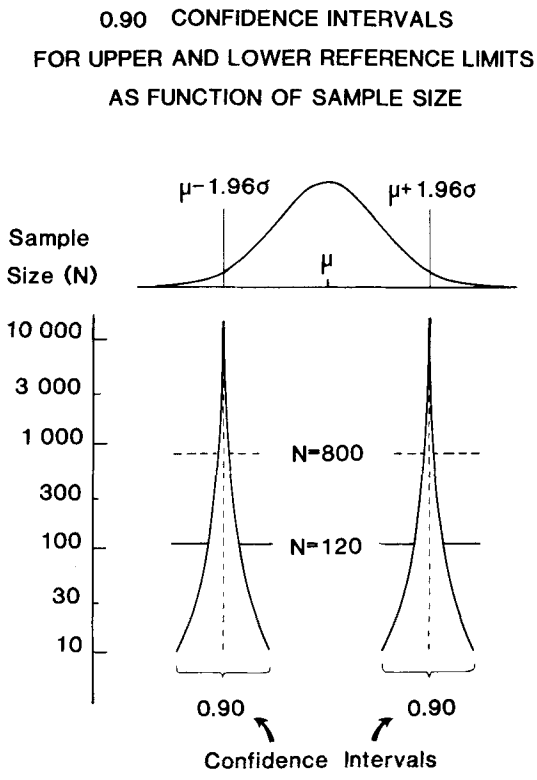

Figure 1. Influence of sample size on the 0.90 confidence intervals for the reference interval limits. From Gowans et al. (9) with permission.

Here, s_A and B_A should also include preanalytical variation and bias, but for practical reasons this is included in the biological terms, as they will usually be part of this, both during collection of reference samples and in routine. If, however, a laboratory has problems with collection of blood specimens and handling of the laboratory samples, then the allowable s_A and B_A should be reduced correspondingly. But this aspect has not been considered in the project.

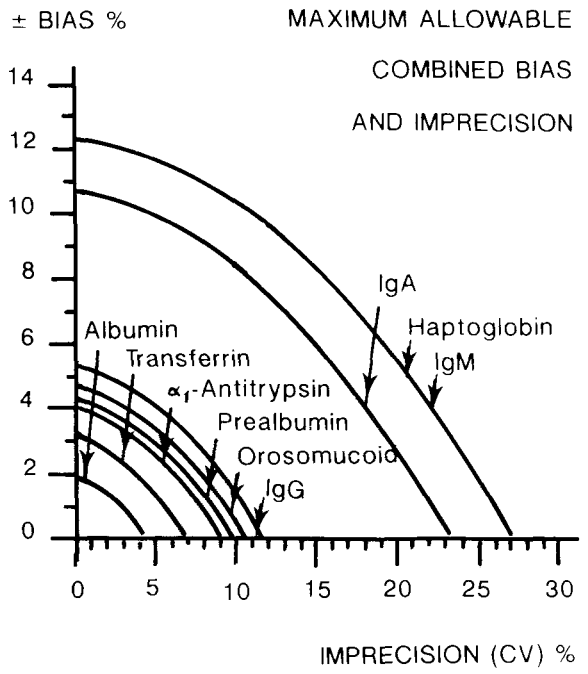

Figure 2. Maximum allowable percentual combined bias and analytical coefficient of variation for measurements of the nine specific proteins. From Hyltoft et al. (14) with permission.

Most of the nine proteins investigated in the project are distributed close to log-Gaussian, so the quality specifications are derived accordingly (14). The quality specifications used for the nine proteins in the project are shown in Fig. 2. For simplicity, the maximum allowable values have been used separately in the project. This is correct for IgA, IgM, and haptoglobin with low s_A-values but is more doubtful for the other proteins.

DESIGN OF CONTROL SYSTEM

Only external control (external quality assessment) has been applied in the project. Here, the control system was designed related to the following problems:

A. Bias at three concentrations

B. Method influence of turbidity in samples

C. Calibration functions

D. Reproducibility

Control samples: As for the calibrator it is essential to have genuine proteins in the control samples. Four control samples with a well defined relationship between concentrations are produced from one untreated serum pool with its natural turbidity (control T). The three others are produced by ultracentrifugation of this, where the lipid layer is sucked off and they are reconstituted with the clear protein free infranatant (intermediate fraction) to concentrations for all nine proteins in fractions of 1.0 (control A), 1.5 (control B), and 0.5 (control C) compared to control T (Fig. 3).

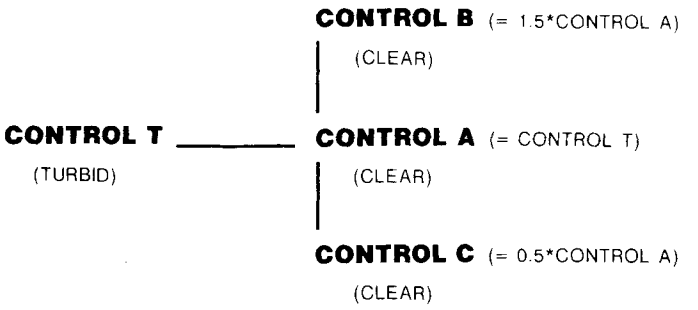

Figure 3. The four control samples used in the project. Concentrations for the nine specific proteins are indicated as fractions of the turbid Control T. From Hyltoft et al. (13) with permission.

Theoretical aspects:

A. Sources of factors involved in analytical quality

In order to establish an 'error directed' control system the types of errors and the sources of both quality - and error-generating factors must be specified individually and combined. These general factors are illustrated in *Scheme A*, where external and internal factors are combined with the permanent and variable factors. The producer is responsible for the external factor mainly bias of the measurement procedure, δ (analytical principle, standardization, method, and equipment) which are outside the laboratories' influence (unless the laboratory makes its own method or changes the procedure compared to the instructions - or chooses another producer/instrument, but then the new producer is responsible). The laboratory is responsible for the implementation and the daily performance including maintenance mainly laboratory component of bias, B, and an intermediate standard deviation, $\delta yi,I$.

SCHEME A

Sources of Factors involved in Analytical Quality

Sources	External factors	Internal factors	
Permanent factors	Analytical principle, standardization, method, equipment	Implementation, laboratory instructions	
Variable factors	Variation in batches (e.g. calibrator and reagents)	Variation in performance and regular errors	
	Producer responsibility	Laboratory responsibility	

The principal sources of bias are the standardization and the specificity. Standardization may be 'method defined' but, in this project the standardization is based on calibration, solely. In *Scheme B* the factors involved in calibration are outlined. The producer of calibrator is responsible for the calibration material and the traceability and the reproducibility of both. The laboratory is responsible for the choice of calibration function and for the reproducibility of this function and the whole procedure.

Thus, the permanent factors will result in a permanent and common bias for all measurements in a laboratory, whereas, the variable factors will cause deviations which may be persistent within periods of a batch or a technician etc. or intermittent related to single runs.

Specificity (see Appendix) can be categorized accordingly as shown in *Scheme C* but, here, the bias, aberrantsample bias, s will be individual for patient (and control) samples according to the composition (matrix effect).

SCHEME B

CALIBRATION

	External factors	Internal factors	
Permanent factors	Traceability, material and matrix	Calibration function	
Variable factors	Variation in production and assignment of values	Variation in actual calibration and performance	
	Common for labs using same calibrator	Deviation from labs using same calibrator	

B. Control of external factors

In reality, the external control of external factors often results only in a registration, because the follow up with changes of procedures in the individual laboratories may be costly, and cannot be effectuated before the procedure should be renewed anyway. But it can classify the producers and - in the long run - improve the producers' products.

Therefore, in the project, the external control focused on calibration and handling of turbid samples. This was done simply by histogram plots of control results according to analytical principles.

The clear controls are close to ideal samples and will control the calibrators, whereas, the turbid control will control the combined effect of calibrator and sensibility to turbid samples. An example from transferrin results is shown in Fig. 4, illustrating (as expected) that calibration can be considerably improved by use of a common high quality calibrator, and by solving the problems for some analytical principles to handle turbid samples.

Batch-to-batch variation in calibrators and reagent are not considered in this project, as a more frequent and detailed design is needed as illustrated for hCG (15).

SCHEME C

SPECIFICITY

	External factors	Internal factors	
Permanent factors	Analytical principle including time factors and temp.	Permanent deviation from the defined conditions	
Variable factors	Variation in production of reagents	Variable deviation from agreed performance	
	Common for labs using same procedures	Deviation from labs using same procedures	

C. Control of internal factors

The internal factors to be controlled in the project are calibration function (stable and variable) and imprecision (within- and between-run). The laboratories are asked to measure the four controls in duplicates in four different runs in three surveys over a three years period. In each survey the statistics for each laboratory was performed by Torsten Aronsson, Uppsala, and a computer group. The results for the three clear controls were normalized by factorisation (1 · control A, 2/3 · control B, 2 · control C) and a two-way analysis of variance with replicates was applied, with cross-classification according to controls and runs, and the coefficients of variation were computed:

CV (stable calibration):	Between calibrator variation
CV (variable calibration):	Interaction component
CV (between-run):	Between-run variation
CV (within-run):	Replicates component

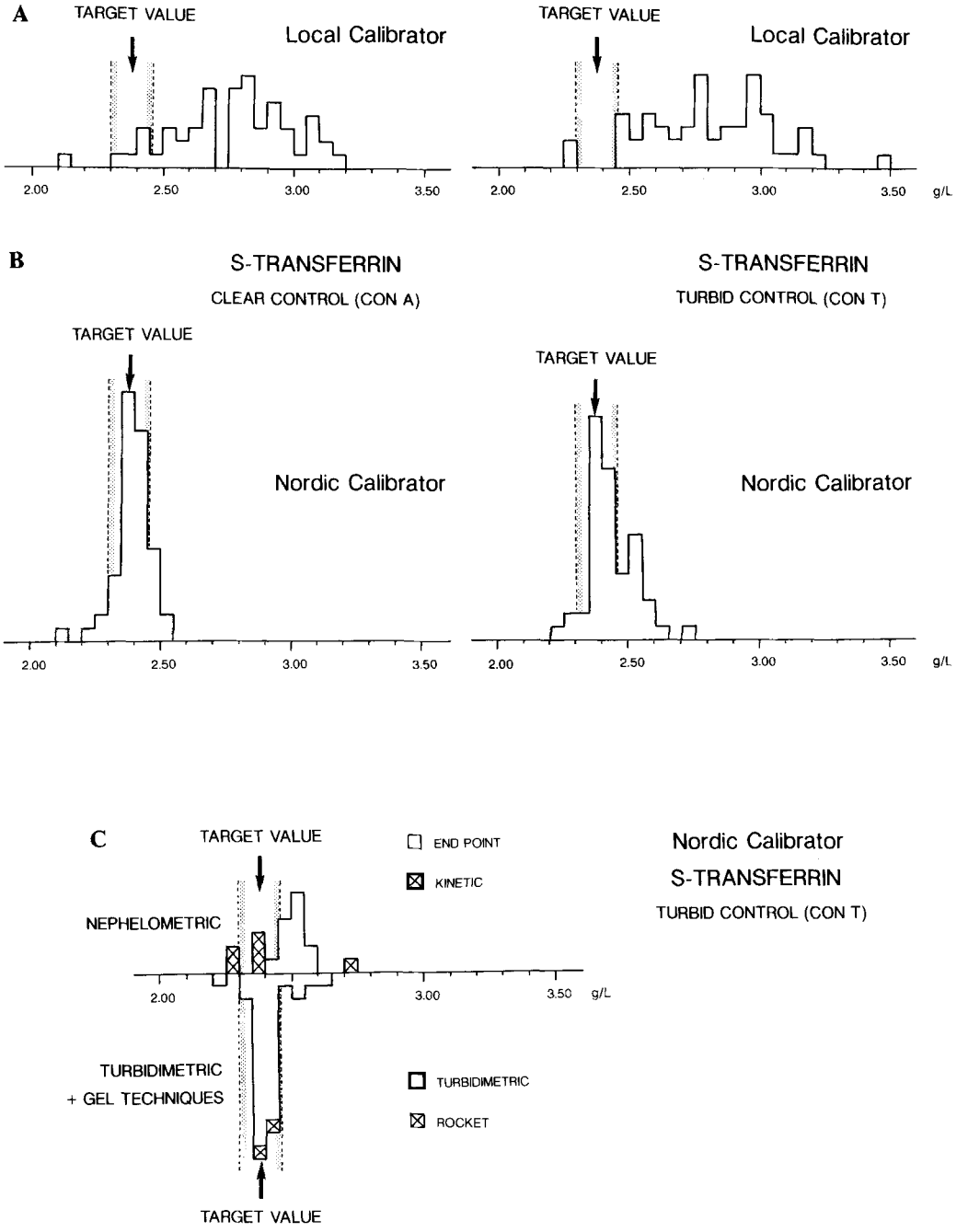

Figure 4. Histograms showing the results for transferrin as an example

A. Control A. Effect of use of commercial calibrators (upper) and the Nordic calibrator (lower).

B. Control T. Controlling the combined effect of calibrator and sensibility to turbid samples (upper) and main effects of turbidity (lower).

C. Control T. Main effects of turbid samples as related to analytical principle. Target values with maximum acceptable bias are indicated. From Blaabjerg et al. (14) with permission.

Based on these components the individual laboratories were advised, about the weak points regarding their calibration function and procedure.

The total calibration of the individual laboratory was, further, illustrated in a bias-plot for controls A, B, and C with confidence intervals and indications of maximum allowable bias (Fig. 5). Combined illustration of quality of calibration and of handling of turbid samples is given in Fig. 6. Here, the quality of calibration is shown for control A and the effect of turbidity as the difference (control T - control A) which will be zero if turbidity correction is optimal. If not, a better clearing procedure of turbid samples is needed.

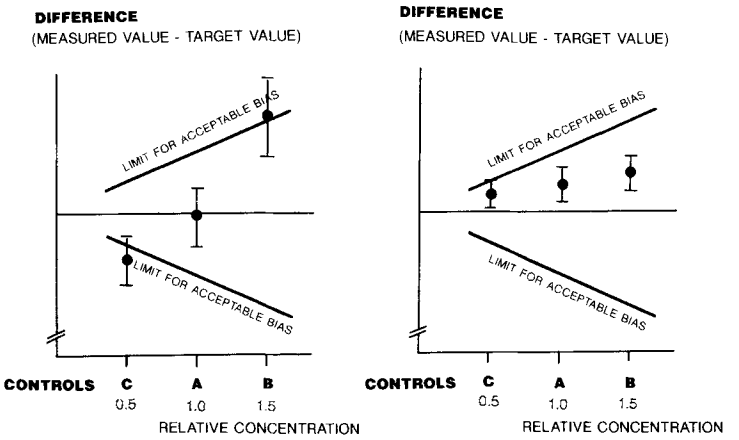

Figure 5. Bias-plot illustrating calibration in an individual laboratory at three concentrations of the analyte, the acceptance limits are indicated and results for each control are shown as mean ±0.90 confidence interval. Left, a correct calibration for Control A but wrong calibration function. Right, acceptable calibration and calibration function (which might be improved further if desired - but not needed. From Hyltoft et al. (13) with permission.

D. Practical aspects and communication

A project like the Nordic Protein Project with so many aspects of quality involved needed cooperation with many organizations and individuals. The complexity may be illustrated in Fig. 7.

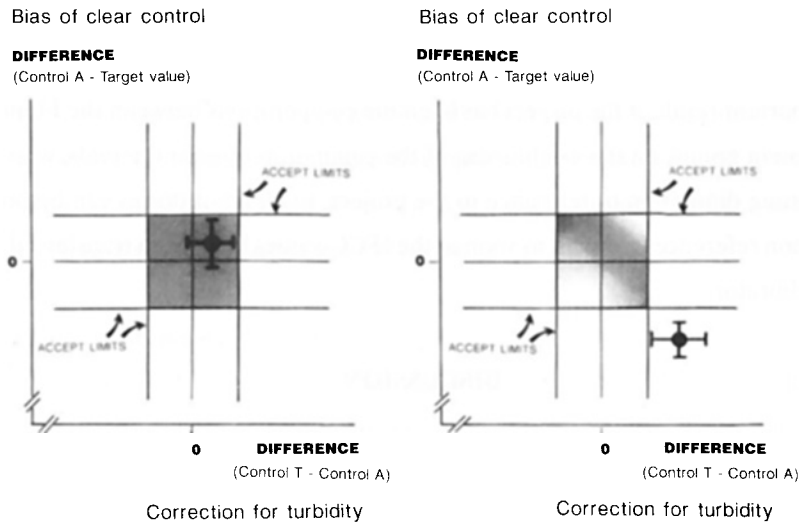

Figure 6. Double bias-plot illustrating quality of calibration (ordinate: Control A - target) and handling of turbidity (abscissa: Control T - Control A). The acceptance limits are indicated. Left, a laboratory with acceptable quality. Right, a laboratory with problems regarding both calibration and handling of turbid samples.

A special problem with an untraditional control project where many new principles are introduced is the communication with the participants. Here, the communication was performed through short presentations of the steps and related theory given through 'blue folders' and individual comments with advices for changes and trouble-shooting in the evaluations to each laboratory. Further, copies of publications on the project were sent to the participants.

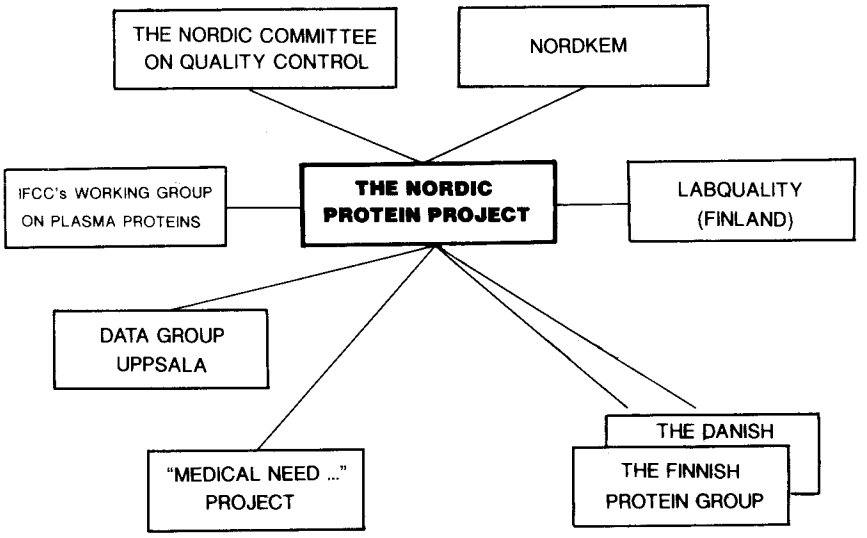

Figure 7. Illustration of co-operation and co-ordination between The Nordic Protein Project and relevant organizations and laboratories.

Other relevant informations

A very important result of the project has been the co-operations between the Finnish and Danish protein groups on the establishing of the common reference intervals, which have added a strong dimension of relevance to the project, so all laboratories can be provided with common reference intervals, as soon as the IFCC-values have been transferred to The Nordic Calibrator.

DISCUSSION

As seen from the view-point of the 'Medical need for quality specifications in laboratory medicine' -project, The Nordic Protein Project has covered the relevant aspects:

(i) The clinical situation

Here, we have chosen the general situation of reference intervals for nine specific proteins, as informations on clinical strategies (and derived quality specifications) are too scanty for the purpose of the project and the time available.

Consequently, the theoretical basis for analytical quality needed for sharing common reference intervals has been evaluated in relation to the project (9, 12).

(ii) The analytical components

Nine specific proteins are investigated in the project (prealbumin, albumin, alfa-proteinase inhibitor (α_1-antitrypsin), orosomucoid, haptoglobin, transferrin, IgA, IgG and IgM) and quality specifications have been given for all, including both analytical coefficient of variation and analytical bias.

(iii) The model

The model is general and completely valid for the aspect of clinical chemistry related to general interpretation of laboratory data.

(iv) The evaluations

The evaluation of quality specifications are performed independent of the project as a general theory, but this theory has been inspired from The Nordic Protein Project - and may be considered completely relevant for this. Further, the specifications are expressed in clinical chemical terms and are directly applicable to control systems.

(v) The control system

In order to cover several aspects of quality, the control system must be designed according to the objectives, regarding both control materials and evaluations of control data. This has been done in practice in the project - and the concept may serve as a model for other control schemes.

(vi) Creation of quality

An indispensable prerequisite for the whole project has been the high quality calibrator (4). Without standardization the whole project would have no real possibility to solve the problems - and it might not had been relevant to ask most of the questions. Common reference intervals for example would had been an illusion and the quality specifications pure theory, and it would have been a question whether target value for the controls and been reliable.

(vii) The three main aspects of analytical quality

The Nordic Protein Project has confirmed the importance of including goals for quality, creation of quality, and control of quality in any project on analytical quality - as non of these has been dispensable in the project (Fig. 8).

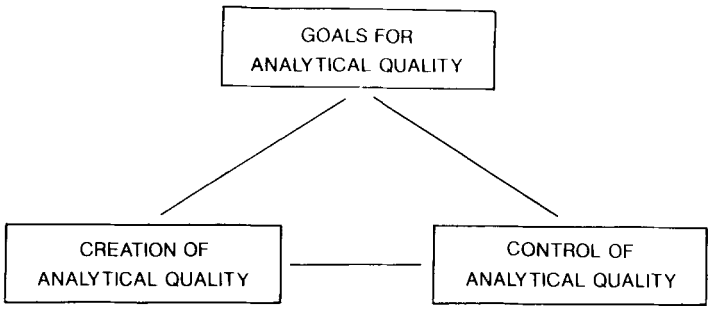

Figure 8. The indispensable parts of analytical quality. From Blaabjerg et al. (2) with permission.

CONCLUSIONS

(i) The clinical specifications of quality are considered equal to the analytical quality specifications as the preanalytical variations (and errors) in practice are included in the estimated biological variation.

(ii) Analytical specifications

The analytical quality specifications can be read off from the diagram in Figure 2.

(iii) Guidelines for control system

The control system must always be 'problem related' and control materials and theory for evaluation of control data must be designed according to these objectives.

This has been done in The Nordic Protein Project where the control is directed against external as well as internal problems (combined and separately) with four selected control materials and problem related data evaluation related to calibration and correction for turbidity.

ACKNOWLEDGEMENT

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