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9 Discussion and Conclusions

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9.1 Discussion of the Protein Projects

The first aim of The Nordic Protein Project was to improve analytical quality of specific protein measurements in the Nordic countries. The main problems were a) the variability of commercial protein calibrators and b) the specificity problem of turbid samples.

a) The problem of calibration was solved by introdution of The Nordic Protein Calibrator to all the participating laboratories for daily use. This reduced the variability of calibration considerably (except for α_1-Antitrypsin). Here, the IFCC-initiative of producing an international protein reference preparation (IFCC/CAP/BCR 470) was a lucky co-incidence (even though it delayed the project for nearly two years) as the traceability of the concentration values was established. Further, the IFCC-preparation was a success, and all Nordic countries have decided to use only protein calibrators with concentration values traceable to this, and probably US and the most of Europe will do the same. This, makes the Nordic calibrator a valuable link in the hierarchical structure of traceable calibrators and concentration values. The success of the project with the introduction of The Nordic Protein Calibrator was therefore, to a large extent supported by the IFCC-reference preparation. On the other hand, the Nordic Protein Project may make the introduction of the IFCC reference-preparation easier, due to the advantages from the project and from the establishment of common reference intervals for the Nordic countries.

b) The problems with measurement of turbid samples were evaluated in the project and it was found that the turbidimetric analytical principles in current use are robust, whereas, the nephelometric principles are sensible to turbidity, and therefore need clearing - best by ultracentrifugation, e.g. by a top-desk ultracentrifuge - before measurements.

The combined introduction in the project of analytical quality specifications related to the use of common reference intervals - and the co-ordination with projects which established these common reference intervals - was an important improvement of the total concept of the projects. Thereby, another traceability - between the Nordic Protein Calibrator (to BCR 470) and the common reference intervals - was established.

Further, the control system was related to

a) the two analytical problems (calibrator and specificity) and

b) the analytical quality specifications, as derived for the establishment of common reference intervals.

These two conditions, were combined in the design of control systems with control samples produced for purpose a) and with the measure of analytical quality according to b).

The general evaluations revealed that the calibration with the Nordic Protein Calibrator made the analytical quality for immunoglobulins, Orosomucoid and Haptoglobin acceptable for the use of common reference intervals in the majority of laboratories. Evaluations of the individual laboratories were divided into

a) specificity (capability to handle turbid samples)

b) calibration function
   (i) proportionality
   (ii) reproducibility of the function

c) general reproducibility
   (i) within-run imprecision
   (ii) between-run imprecision

and this was a guideline for internal trouble-shooting.

For the two proteins S-Albumin and S-α_1-Antitrypsin the aim was only fulfilled to a partial extent.

The analytical quality specifications used for S-Albumin are very demanding and it looks like the immunological measurement procedures are less reproducible, probably due to the immense dilutions which are necessary for getting the measurement signals down to a level where the instruments are able to measure the signals. For the dye-binding methods other problems may be actual, but only the validity of calibration functions was investigated and found insufficient in most laboratories.

For S-α_1-Antitrypsin the nature of problems was different. The transfer of concentration values from the IFCC reference preparation to the Nordic Calibrator revealed a difference related to analytical principles of about 20 %, which was related to changes in the structure of the protein in the calibrator as disclosed by different antisera. Further, the control project disclosed a variation, which was not consequent but might be explained by a variable denaturation of the protein - not disclosed by the comparison of different calibrator-pools produced over eight years. One reason could be the mailing and storage conditions in the participating laboratories in the project compared to the stable -80 °C conditions for the calibrator pools. These facts, reduce the validity of the calibrator and the applicability of the reference intervals for this protein.

The communication was mainly one-way, but telephone calls and letters from some of the participants disclosed general problems like the use of molar units in Denmark, without using updated molar weights. The problems specific for the laboratories could often be solved by telephone.

Whether the populations really are homogeneous for the plasma proteins investigated - except from S-Haptoglobin and S-IgA - and thereby gives the basis for common reference intervals, seems to be confirmed for the non-immunoglobulin proteins, whereas, the immunoglobulins may turn up more deviating in an extended evaluation. This is impossible to answer now, but the reference intervals estimated in these projects are - for the time being - the best basis for common reference intervals, and much better than if each laboratory established its own intervals.

In conclusion, the aim of improvement in protein measurement in the Nordic countries was succesful and the aspects of traceability to CRM 470 and the introduction of common reference intervals further improved the total quality for the patients.

9.2 Discussion of General Applications

The second aim was to develop a more general model for analytical quality management, which could be applied to other laboratory analytes as well.

The principles as well as the practical accomplishment show that it is possible for plasma proteins, and the model should be transferable to other laboratory quantities. But a number of specific problems for these, must be considered separately for each analyte.

a) The general strategy of establishing common reference intervals is applicable to all naturally occurring components, but it might be more relevant to apply a clinical strategy, which should be confirmed by clinical investigations, and the analytical quality specifications should be formulated according to this strategy.

b) The use of a serum pool calibrator may be rather special for plasma proteins and for other quantities, other types of calibrators may be more relevant. The establishment of a common reference basis (a reference method or a stable reference preparation) is essential and should be created on an international level when possible.

c) Problems with specification of the structure of the analyte (to be quantitated) are general, but must be solved according to the component under consideration.

d) Specificity and interference problems must be identified and the most important should be challenged first. The principle, however, of using pairs (or more) of identical preparations, except from the content of suspected component, is valid to all analytes.

e) The design of the control system and the frequency of surveys should be reasonable also for other quantities, as there is no need for frequent control of the permanent factors, and the variable factor cannot be monitored by external systems (unless there is a daily electronic communication) and this should be handled by internal control. Our design with few specially produced control samples and the evaluations with analysis of variance as well as different types of difference plots (measured minus target, and measurements of pairs) should be applicable to any other analyte.

The workload, however, by establishing a comparable project is considerable, and a lot of experts need to be associated, as well as assistance from a lot of colleagues is needed for the completion of such a project. And it is expensive, the investments by NORDKEM for meeting activities and publication of the book were 69 500 Finnish Mark and for LABQUALITY the expenses for the calibrator and control samples were about 200 000 Finnish Mark (which was paid back). Further, The Nordic Societies of Clinical Chemistry supported the projects with 10 to 20 000 Danish Kroner each. Moreover, all the laboratories and their staffs have invested money and a lot of time, both in the control project proper, but also in the extra work of additional analyses and blood sampling for the reference interval projects. Further, a lot of experts are needed for data handling and for computations as well as for advises needed throughout the whole project.

To our knowledge, the only project where all the same elements have been integrated to a comparable level, is the US National Cholesterol Education Program. This programme, however, is performed professionally and accomplished on a large scale. Here, the overall strategy is clinical, but except from this, the elements are the same as in the Nordic protein projects.

In conclusion, this type of integration of all the elements of analytical quality with the creation of reference intervals based on a common traceability should be applied to all naturally occurring analytes in order to ensure patients reliable results over time and geography.