QUALITY CONTROL IN INHERITED DISORDERS OF METABOLISM (IDM) LABORATORIES ERNDIM SCHEMES AND THEORETICAL ASPECTS

This document has been developed within the framework of the EU project BMH4-98-3404 led by Baudouin François MD, Belgium. It was undertaken by a working group with the following members: Dr. P. Divry, Lyon; Prof. B. François, Chairman, Diepenbeek; Dr. A.C. Sewell, Frankfurt; Dr. L. Spaapen, Maastricht. C. Vandervorst, Diepenbeek.

Reproduced and edited from ERNDIM BIOMED 2 Newsletters of October 198 and June 1999 by B. Fowler (May 2002)

INTRODUCTION TO QUALITY MANAGEMENT IN IDM LABORATORIES

The Project 'Development of a European Quality Assurance Program of Analytical Methods used in the Detection of and Monitoring of Treatment of Inherited Disorders of Metabolism. An Inter-Laboratory Reproducibility Study, Phase II' has been accepted recently by the European Commission. The aims of the phase II proposal are:

a) To improve and standardise the quality of accuracy and precision in laboratories performing analyses to optimise diagnosis at lowest costs by:

1. Improvement of the analytical capabilities of individual IDM laboratories.
2. Improvement of the inter-laboratory reproducibility in the E.U. by standardisation of analytical methods and reference material
3. Improvement of the efficacy of the individual IDM laboratories by introduction of an efficiency proficiency testing system on a European-wide scale
4. Alignment of reliable reference values among IDM laboratories as a guideline for therapeutic decisions (monitoring of treatable IDM)

b) To promote co-operation and exchange of information between E.U. laboratories by:

1. Establishment of age-related and diet-related reference ranges for metabolite concentrations based on quality assured results.
2. Updating of the European Directory with QS scheme recognition of laboratories
3. Information exchange between laboratories through meetings and an ERNDIM newsletter

c) Defining conditions for accreditation of IDM laboratories in all E.U. countries.

The general aim of the project is to add insight into the clinical research of orphan diseases (Area 4. 6). Due to the low prevalence of these rare diseases no country can afford the implementation on a national basis of diagnostic criteria or quality assessment of the biochemical methods used in the diagnostic or therapeutic procedures of IDM. The set- up of an educational system for exchange information on clinical expression of rare diseases and exchange samples of patients with IDM throughout a harmonisation of the biochemical methods, should offer unique opportunities to link the experience of each individual IDM centre at the E.U. level.

The content of this BIOMED II edition of the ERNDIM newsletter is devoted to various topics concerning quality control in order to support the participants in the ERNDIM Quality Assurance Program in their efforts to improve quality care in their laboratories.

Why should we control the quality of our analytical data ?

Quality control of analytical procedures employed for specific tests has received considerable attention in the recent years from industrial committees and regulatory agencies. There are however no official guidelines referring to biological fluids. In the medical field one should establish that an analytical method is suitable for its purpose and that the obtained data are close to the reality. Therapeutic action based on erroneous data may be harmful. Therefore, controlling the quality of the data is essential.

Need for Quality assurance in Inherited Disorders of Metabolism.

Decisions on diagnosis, follow-up, treatment, and prognosis depend largely on biochemical analyses using highly specialised techniques and equipment and on interpretation of the data by experienced personnel. The capabilities of academic personnel involved in the analytical process vary among the different European countries. In contrast to more commonly requested medical laboratory investigations, investigation of these rare disorders is not (yet) legally subject to external quality control. Because of existing fear that bad QC results will become public, some people may decide not to participate in external QC programmes. Implementation of **quality control on a national basis is unrealistic since the number of participating laboratories in any individual country is too small** for statistically meaningful evaluation of results.
Results of analytical measurements made in one location should be consistent with those obtained elsewhere. Laboratories must participate in multinational quality assurance programs so that biochemical results can be used for treatment of patients in any other country and are independent of the laboratory or in any country the analyses are performed. In a previous study, sponsored by the E.U. BIOMED I program, we were able to compile an anonymous inventory of the quality of analytical performance by means of quality assurance schemes and to assess diagnostic capabilities of IDM laboratories by operating a proficiency testing system.
Results revealed a lack of reproducibility among the participating IDM laboratories. It must be emphasised that both accuracy and precision needed to be improved. The tendency towards large variations in the analytical data from the quality control samples of the participating laboratories is obvious.
One of the aims of the ERNDIM-QAP Phase II project sponsored by the E.U. is to improve the quality of the analytical performance in IDM laboratories. Two general approaches have been used and quality control schemes were developed as follows:

1. Addressing quantitative data (technical quality assurance program):
   Accuracy[1], what is the range of deviations from the true value between different laboratories for the same samples?
   Day to day precision[2], its impact on long-term monitoring of treatment in patients with certain metabolic disorders is obvious.

2. Addressing qualitative analysis (proficiency testing program):
   Is a participating laboratory able to identify the pathognomonic metabolites in a urine sample from a patient with a confirmed IDM and correctly interpret the significance of these finding; in other words can the diagnosis of a disorder be made?

[1] accuracy= ability of a measuring instrument to give responses close to a true value. Inaccuracy deviation from the true value (Eurachem A1.1)

[2] imprecision= day to day dispersion of the measures of an analyte of the same specimen in a given laboratory.

We present the issues related to method validation and to increase the reader's understanding of what is involved, why it is important, and give some idea of how it can be achieved in IDM laboratories.

Systems designed to evaluate effectiveness of a method have to be designed with care. Too simple a system can result in crude distortions, too complicated a system can be unworkable due to the increasing bureaucracy.

Ce qui se conçoit bien s'énonce simplement (Nicolas Boileau). What is well conceived, states itself simply. These newsletters will provide reviews and simple strategies for the validation of analytical methods used in IDM laboratories.
One of the problems faced by the laboratory worker in the understanding of method validation[3] is the fact that they are not familiar with the expressions used in quality assurance[4]. In addition most of the participants at one or another QAP scheme of the ERNDIM foundation do not use English as mother language. For whom those are familiar with Quality assessment we hope that this simple approach will stimulate further discussion to improve the quality assurance of the methods used in IDM laboratories. In order to avoid any misinterpretation definitions provided by the Eurachem guide will be provided in footnote.

[3] Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. (Eurachem A32.2)

[4] Quality assurance = all those planned and systematic actions necessary to provide adequate confidence that an analytical measurements will statistically given requirement for quality (Eurachem A16.1).

A SMALL GUIDE TO HELP US IN THE MAZES OF TERMINOLOGY ISO 9000 & 445001

Every laboratory establishes its own quality requirements as a function of the scientific relevance of it in that particular analytical field. The high and adequate quality level is assured by the competence of the laboratory personnel. The importance of quality assurance is that it guarantees the maintenance of this high quality within the laboratory.
One of the most important tools for quality assurance is to take part in an external quality assessment scheme. The European Accreditation organisation EA firmly encourages this policy towards its members, the accreditation bodies of the individual countries.

It is clear that external quality assessment schemes and quality assurance are part of a total quality system.
In the field of quality control and management in the laboratory, international accepted guides and standards exist : EN 45001, part of the ISO9000 series of standards and the principles of Good Laboratory practice. (GLP).
These three systems each focus in different fields of activity :

- testing, inspection, and calibration (EN 45001)
- the production of goods or services in an industrial environment (ISO 9001-9003), and the
- execution of safety studies required by law (GLP)

Experiments in the laboratory can differ from unique research like projects (R&D) to routine tests. Depending on the nature and application of the experiments the demonstration of the technical competence and the quality management system can be accomplished by accreditation (EN45001), certification (ISO 9000), or a Statement of Compliance (GLP). The first two are voluntary; the latter is based on legislation.

R&D :
Research *is a scientific investigation aimed at discovering and applying new facts, techniques and natural laws.*
Development in an industrial context is the work done to finalise the specification of a new project or new manufacturing process. It uses many of the methods of scientific inquiry, and may generate much new knowledge, but its aim is to create practicable economic solutions.
The combined term **Research and Development** can be seen as the work in an industrial or governmental context concentrating on finding new or improved processes, products, etc., and also on ways of introducing such innovations.
GLP : Good Laboratory Practice (GLP) is concerned with the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported. These principles are applicable to the testing of chemicals to obtain data on their properties and/or their safety with respect to human health or the environment.

EN 45001 : General criteria for the operation of testing laboratories (1989)
ISO 9001 : Quality Systems – Model for quality assurance in design, development, production, installation and servicing.
ISO9002 : Quality systems – Model for quality assurance in production, installation and servicing.
ISO 9003 : Quality systems : Model for quality assurance in final inspection and test.
Proficiency testing[5] : the organisation of an external measure of performance.

[5] A periodic assessment of the performance of individual laboratories and groups of laboratories that is achieved by the distribution by an independent testing body of typical materials for unsupervised analysis by the participants (Eurachem A15)

THEORETICAL ASPECTS OF METHOD VALIDATION

The professional duty of the analytical chemist

The operator carrying out the studies must be competent in the field of work and have sufficient knowledge related to the work to be able to make appropriate comments or decisions. By entrusting a sample to a IDM laboratory for analysis, it is assumed that the laboratory has a degree of expert knowledge that the clinician lacks. The laboratory and its staff have a clear responsibility to provide reliable data from the required analyses. Implicit in this is that the tests carried out are appropriate for the analytical part of the problem put forward by the clinician. The priority given to the various laboratory procedures is completely dependent on the suspected IDM which is at least, in part, determined by the clinical presentation. Therefore, a **close collaboration between the biochemical geneticist and the clinician remains essential** for a fast course of the diagnostic procedure. The final report has to be presented in such a way that the clinician can readily understand it and draw appropriate conclusions.

When should methods be validated?

Methods need to be validated:

- Before their introduction into routine use
- Whenever the condition for which the method has been validated, changes, for example, a change to an instrument with different characteristics

Strategy for validation of methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards with a matrix similar to those of the unknown samples analysed in the routine. The preparation and execution should follow a validation protocol, written in a step-by-step instruction format. The scope of the method and its validation criteria should be defined. These include:

- Compounds
- Type of information: qualitative or quantitative
- Detection and quantitation limits
- Linear range
- Precision and accuracy
- Type of equipment

In IDM laboratories, the samples are generally blood, urine, CSF, or others (skin biopsies, liquid of the eye, etc…). The composition as well as the concentration of the metabolites varies with the matrix of the samples. The characteristics of the method's performance should be clearly defined based on the intended use of the method. For example, if the amino acid analyser will be used for quantitative analysis of amino acids in urine and in serum, there is a need to test the method's linearity in both conditions. The same procedure should be followed when assaying one or more metabolites in a normal or pathological sample. For example, there exists a difference in 1 or 2 degrees of magnitude for phenylalanine in a normal compared to a PKU sample.

Performance parameters

The possible parameters which should be considered for method validation are listed on table 1

Table 1: Parameters for method validation

- ♦ Specificity
- ♦ Selectivity
- ♦ Precision
  - → Repeatability
  - → Reproducibility
- ♦ Accuracy
- ♦ Robustness
- ♦ Bias
- ♦ Recovery
- ♦ Range
- ♦ Linearity
  - → Limit of detection
  - → Limit of quantitation

Selectivity[6] / Specificity[7]

The terms selectivity and specificity are often used each for the other. The term specific generally refers to a method that produces a response for a single analyte only. In IDM laboratories where methods are used to provide data on different metabolites, the term selectivity appears to be more appropriate.
Selectivity in the amino acid analysers is obtained by setting the column temperature at the optimal value or by modifying the pH of the running buffer.
It is a difficult task in gas chromatography to ascertain whether the peaks within a sample chromatogram are pure or consist of more than one compound. While, in the past, chromatographic parameters such as the composition of the stationary phase, its thickness, the nature and velocity of the carrier gas, the temperature programming of the oven etc. have been modified to optimise the method, the application of mass spectrometry or tandem MS-MS spectrometry coupled on line to the gas-chromatograph has improved the selectivity of the analyses dramatically. Mass spectrometers acquire spectra on-line throughout the entire chromatogram. These spectra are further analysed and identified by computer. In this method the selectivity of the gas chromatography is now far less important.

Precision and Reproducibility[8]

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. The repeatability is the precision obtained with the same method, by the same operator within A short interval of time. Intermediate precision has been defined as the long term variability of the measurement process (several weeks).
The primary aim of an *internal quality control* [9] system is the reduction of the analytical variation by assaying the same sample repeatedly. An International survey done within the participants in the ERNDIM QAP system revealed that 34% clearly stated the use of internal QC in the amino acids analysis against 26.4% who did not; and for the organic acids analysis, it is 15.6% against 49.4%.
Guidelines for introduction of internal quality control in IDM laboratories are given below.

[6] Selectivity: the ability of a method to determine accurately the analyte(s) of interest in a sample matrix under the stated conditions of the analyse.(Eurachem A24.2)
[7] Specificity: the ability of a method to measure only what it is intended to measure. (Eurachem A27)
[8] Reproducibility = Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment
[9] Internal quality control = Set of procedures undertaken by laboratory staff for the continuous monitoring of operations and the results of measurements in order to decide whether results are reliable enough to be released.

Internal Quality Control

Material to be used (the matrix must be similar to the samples analysed daily):
Pooled plasmas and/or urines possibly spiked with metabolites; either home made or commercial available

Frequency of internal quality controls

- in case of batch processing: analyse a 'high-concentration' and a 'low-concentration' pool sample each run
- in case of continuous processing: analyse a pool sample each 24 hours
- analyse a pool sample after: * change of chromatographic parameters
  * new batches of buffers
  * new batches of derivatization agent (e.g. ninhydrin)

From the pool analyses relative standard deviations in the concentrations of the compounds to be quantified are calculated. The ERNDIM National experts are prepared to provide additional information on commercially available control samples.

External Quality Control

This has to be used to check our internal quality controls.
Never use the samples of the external quality control for your internal QC.

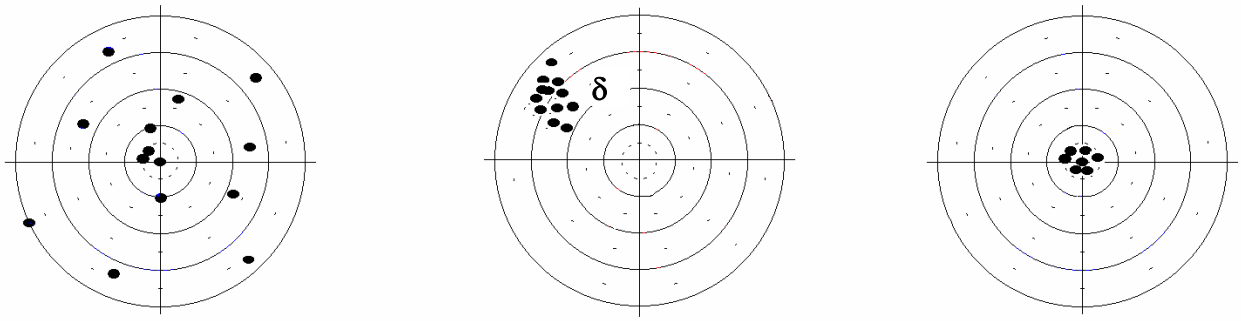
Reproducibility

This represents the precision obtained between laboratories. The objective is to verify that the analyses will provide comparable results in different laboratories. The aim of an *external quality control system* (e.g. the ERNDIM Quality Assurance Program) is to achieve inter-laboratory comparability, and eventually to approach the accuracy of the analytical data when the number of participants are high. The implementation of quality control on a national basis is unrealistic since the number of participating laboratories in any one country is too small for statistically meaningful evaluation of results. The ERNDIM foundation started in 1993 to provide aliquots from homogeneous lots of metabolites within 3 different schemes

The QC scheme for quantitative amino acids analysis.
The QC scheme for the quantitative analysis of organic acids
The QC scheme for special assays in serum and urine as in 1999 (see table).

In Urine	**in Serum**
Lactic acid	Lactic acid
Pyruvate	pyruvate
3-OH Butyrate	3-OH Butyrate
Carnitine total	Acetoacetate
Carnitine Free	Phytanic acid
Creatinine	Very long chain fatty acids
Orotidine	Carnitine total
Orotic acid	Carnitine free
Uracil	Creatinine
Uric acid	hydroxyproline
Sialic acid	Total homocysteine
Mucopolysaccharides	phenylalanine
Hydroxyproline	Uric acid
Total homocysteine	7-dehydrocholesterol

The execution of these schemes has been entrusted to two highly experienced laboratories, Laboratoire de biochimie génétique, Pr P. Kamoun, Paris and SKZL Foundation, Dr J. Willems, Nijmegen. Early evaluation reveals wide discrepancies between the results obtained in the different laboratories



(1) **(2)** **(3)**

Figure 1: Difference between Precision and Accuracy

	(#1)	(#2)	(#3)
Precision	very bad	excellent	excellent
Accuracy	Random error or systematic error not recognised due to the bad precision	Systematic error (δ= bias)	excellent
Conclusion :	Unusable system	Useful system	This is the reference method

Accuracy

The accuracy of a method is the extent to which test results generated by the method and the true value agree. The true value for accuracy assessment can be obtained in several ways. The best approach is to compare results of the method with these from a well established reference method. In most of the methods used in investigations for IDM, no such reference method exists. Accuracy can be assessed by analysing a blank sample matrix spiked with known concentration of the analyte from interest. Because this last accuracy assessment measures the effectiveness of sample preparation, a high number of data from laboratories using the same technique is needed for statistical evaluation of the results.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. One must identify variables in the method that may be expected to influence the results.

Range

The range of an analytical procedure is the interval between the upper and lower concentration of analytes in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Bias[10]

The bias could be considered as the difference between the mean value obtained during the precision step and the true value. It reflects the interference of one or more systematic errors during the analytical procedure (Fig. 1, example (2) with δ in contrast to random errors as in example (1)

[10] Bias: The difference between the expectation of the test results and an accepted reference value.

Recovery[11]

recovery should be determined by comparing the response at different concentrations. The expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration.

Linearity [12] and Calibration Curve[13]

Determination of the linearity of an analytical method (figure 2) is the procedure correlating directly the test results with the concentration of the metabolite in the sample. A calibration curve is obtained by injecting a succession of 3 to 5 injections of 5 or more standards whose concentration span the expected concentration range. A linear equation applied to the results should have an intercept not significantly different from zero. The non zero intercept represents the *Bias* as mentioned above.

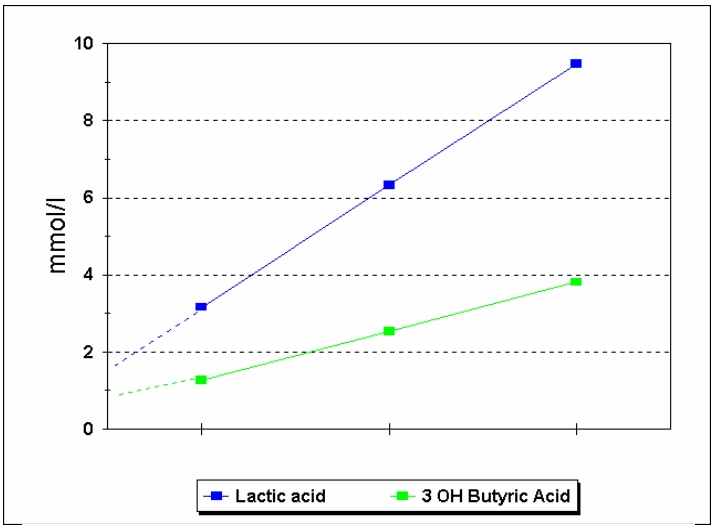


Figure 2: linearity curve for two organic acids

Limits of detection[14] and quantitation[15].

The limit of detection is the point at which the method used gives a reasonable response. In amino acid analysis the detection limit is a peak found in a chromatogram with a height at least 2-3 times above the baseline noise level. The limit of quantitation is different and represents the minimum amount which gives precise measurement. Going back to amino acid analysis the correct integration of a peak requires that the peak height is 5 to 10 times greater than the base noise level.

Conclusion

The purpose of method validation is to ensure the production of reliable high quality data from diagnostic analyses. The time invested in validating your methods is essential and will pay rich rewards in the long term. This is also very important with regard to inter-laboratory comparison of data, probably leading to less variation. The proficiency testing[16] program, in addition to the ERNDIM external quantitative QC schemes, is set-up in order to assure the attained quality in the laboratory.

[11] Recovery : the fraction of analyte added to the test sample prior to analysis
[12] Linearity: Defines the ability of the method to obtain test results proportional to the concentration of the analyte.
[13] Calibration curve: Graphical representation of measuring signal as a function of quantity of analyte.
[14] The lowest concentration of an analyte that can be detected but not necessarily quantitated.
[15] The lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test.
[16] Proficiency testing is a type of external quality control. The practice of testing unknown specimens from an outside source provides an essential means to assure quality laboratory testing results. The purpose of proficiency testing is to verify the performance of each step from clinical data towards the diagnosis. (adapted from American Proficiency Institute, 1999)