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REVIEW

# Quality of analytical performance in inherited metabolic disorders: the role of ERNDIM

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**Summary** External quality assurance (EQA) schemes are essential for improvement of accuracy, reliability and comparability of results of biochemical genetic tests. ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism), established in 1994, operates nine EQA schemes for biochemical genetic testing according to international norms and recommendations. These comprise qualitative schemes for amino acids, organic acids, purines and pyrimidines, special assays in serum and urine and white cell cystine, qualitative organic acid and acylcarnitine schemes, as well as diagnostic proficiency testing. The total number of participants has increased from 123 in 1994 to 268 in 2007. Additional activities include participation in the Eurogentest project, a laboratory directory, training, education and development of guidelines. Results from the quantitative amino acid scheme with 170 participants reveal good variation within and between laboratories of below 10% for 10 amino acids; good within-laboratory variation but intermediate inter-laboratory variation of 10–22% for 11 amino acids; and higher variation within and between laboratories for 8 amino acids. Results on samples from 51 inherited metabolic disorders from two of five centres organizing diagnostic proficiency testing indicate overall diagnostic efficiency above 80% and improved performance of individual laboratories. Comparison of results for 10 and 12 compounds in the serum and urine special assay schemes respectively for 2000 and 2007 reveal clear improvement of precision within laboratories and in inter-laboratory variation. There is considerable evidence that performance in biochemical genetic testing has improved since the introduction of ERNDIM schemes.

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## Abbreviations

| | |
|---|---|
| DPT | diagnostic proficiency testing |
| EQA | external quality assurance |
| ERNDIM | European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism |

## Introduction

Following advances in screening and treatment of phenylketonuria, technological advances such as quantitative ion-exchange chromatography, gas chromatography cou-

pled with mass spectrometry, high-performance liquid chromatography and tandem mass spectrometry led to a dramatic rise in the number of detected diseases involving several different classes of compounds. Not only diagnosis but also monitoring of treatment demands precise methods that need to be organized in order to serve all potential patients at the national and international level and to be performed by suitably trained staff. It is necessary to raise the levels of accuracy, precision, reproducibility and harmonization of laboratory testing in this field, benefiting from experience of internal and external quality control gathered in clinical chemistry during the 1970s and 1980s. At the same time we need to recognize that reliable diagnosis and treatment often require biochemical analyses using highly specialized techniques and equipment and require interpretation of the results by experienced personnel. Further, the reliability and validity of methods and comparability of results is essential. This is especially important with the evolution of agreed treatment thresholds of metabolite levels in treated patients, the special need for consensus cut-off values in newborn screening by tandem MS, and also for meaningful comparison of results in multi-centre studies. As mobility of families between countries increases, it is essential that biochemical results can be used for treatment of patients in another country independently of where the analyses are performed. EQA for biochemical genetic testing needs to be provided on an international basis since the number of provider laboratories in any individual country is far too small for statistically meaningful evaluation of results within an EQA scheme.

The ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) foundation was established in 1994 in order to meet the challenges raised above. ERNDIM initially focused on the provision of external quality assurance (EQA) schemes, recognizing its importance in relation to accreditation of laboratories with the support of two EU Biomed grants. Latterly, ERNDIM has become engaged in wider issues relevant to provision of biochemical genetic testing.

ERNDIM organizes EQA schemes according to accepted norms on a mainly European-wide scale, although several laboratories from outside Europe participate. All schemes are operated according to guidelines summarized by Sciacovelli and colleagues (2001) and are harmonized as much as possible with respect to numbers and frequency of samples and submission of results and receipt of reports by internet. Schemes are provided by SKML (Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek, Dutch Foundation for Quality Assessment in Clinical Laboratories) or academic centres. The scheme providers work closely together with the Scientific Advisory Board and schemes are administered by the ERNDIM executive committee, which represents the ERNDIM Foundation Board (see http://www.erndim.unibas.ch/pdf/ssiem_structure.pdf for details of these bodies and the organization of ERNDIM).

In wider terms, ERNDIM aims to promote agreement between European biochemical genetic testing laboratories on reliable and standardized procedures for diagnosis, treatment and monitoring of inherited metabolic diseases. It also advances education through meetings and by providing relevant documentation such as guidance documents for the analysis of groups of metabolites and annual reports of EQA schemes on its web site.

ERNDIM aims to be financially self-sufficient through minimal administration costs and efficient subscription collection.

## ERNDIM EQA schemes

The different ERNDIM schemes, centres operating the schemes, year of establishment of the scheme and initial and present participant numbers are summarized in Table 1. Full details of the compounds included in the schemes and numbers of samples tested per year can be found at the ERNDIM website (http://www.erndimqa.nl/InfoFrame.php).

As well as an increase in schemes, the total number of participants has increased greatly from 123 in 1994 to 268 in 2007. Today ERNDIM offers nine different schemes of three types as follows.

*Quantitative* schemes use samples in which variable quantities of a range of metabolites are added to a physiological matrix. For plasma, dialysed pooled control samples are prepared; and for urine, pooled samples from institutionalized subjects receiving a very poor diet are used. Participant's results are computed to provide consensus values and to test accuracy, recovery, precision and linearity for each laboratory as well as to compare performance between laboratories. In selecting the sample content, emphasis is given to concentrations of clinical relevance including low as well as high levels.

*Qualitative proficiency* schemes (organic acids, acylcarnitines) use natural samples from control or IEM subjects and participants are required to analyse and interpret the overall pattern of metabolites to make or exclude a diagnosis. Attention is paid to the style of reports, which should be made in the same way they would be given to a nonspecialist paediatrician in a

**Table 1** Summary of ERNDIM external quality assurance schemes

| ERNDIM QC scheme | Sample type | Year established | Initial no. participants | 2007 no. participants |
|---|---|---|---|---|
| Quantitative amino acids | Physiological matrix plus standards | 1994 | 88 | 200 |
| Special assays in urine | Physiological matrix plus standards | 1994 | Total 66 | 134 |
| Special assays in serum | Physiological matrix plus standards | 1994 | | 176 |
| Quantitative organic acids urine | Physiological matrix plus standards | 1994 | 51 | 76 |
| Purines and pyrimidines in urine | Physiological matrix plus standards | 2002 | 34 | 51 |
| Cystine in white blood cells | Cystine and protein in a white blood cell extract matrix. | 2004 | 23 | 27 |
| Qualitative organic acids | Authentic urine from patients | 1994 | 38 | 159 |
| Proficiency test divided between 5 centres | Authentic urine from patients | 1994 (Nijmegen)<br>1998 (Sheffield)<br>1998 (Lyon)<br>2001 (Prague)<br>2005 (Basel) | 20 | 101 |
| Acylcarnitines | Blood from patients on filter paper | 2003 | 47 | 75 |

general hospital. The qualitative organic acid scheme has been reported in detail in this issue of the Journal (Peters et al 2008).

*Diagnostic proficiency testing* (DPT) schemes also use patient urine samples. The DPT schemes are limited to a maximum of 25 participants owing to the difficulty of obtaining sufficient urine from patients and also the need to create an intimate forum in which results, including mistakes, can be discussed at the annual meeting of participants.

This scheme is designed to assess test selection based on clinical details provided, analytical performance and interpretation of results and recommendations for further tests.

## Results and experience for particular schemes

### Quantitative amino acids

This scheme was briefly described by Fowler and colleagues (2003a). It is the longest established scheme and its main features also apply to other quantitative schemes. Eight samples per year are prepared by adding four different concentrations of each of 25 standard amino acids to lyophilized plasma in duplicate. These amino acids are present every year, allowing long-term comparison of performance. In addition, four unusual amino acids are added each year. Concentrations obtained for each amino acid are submitted online through our web site and reports are automatically produced for each sample for each participant, showing the participant's own values compared with the median value for all laboratories. Also the relationship of each value to all values is shown graphically as the centile. Full details of all results for each amino acid can be called up as shown in Fig. 1. Comments can be added to the report by the scientific advisor for the scheme. Results for all eight samples are summarized in the annual report, which shows: (1) accuracy as the average of the eight values; (2) precision expressed as the coefficient of variation for the four duplicate samples; (3) linearity and (4) recovery, both calculated from the measured values compared with the added quantities; and (5) the coefficient of variation between all laboratories. It must be borne in mind that reliable comparisons of variation between laboratories in different years requires that median values are similar in different years, that any zero values are excluded, and that median and mean values do not differ importantly. Table 2 shows the median values for all laboratories and coefficient of variation for the lowest, intermediate and highest levels. Those obtained in 2002, the earliest year in which low levels were provided, and in 2007 are shown and results for each of the samples met the above requirements for reliable comparisons. It is clearly evident that variability between laboratories is greater with lower than with high concentrations. Except for some of the low levels there is maintenance of good variability or some minor improvement over these years. An example of the results in detail for an individual amino acid, in this case phenylalanine, is shown in Fig. 1. This example shows the values divided according to gradations of 0.5 of the standard deviation for each participant and indicates the methods

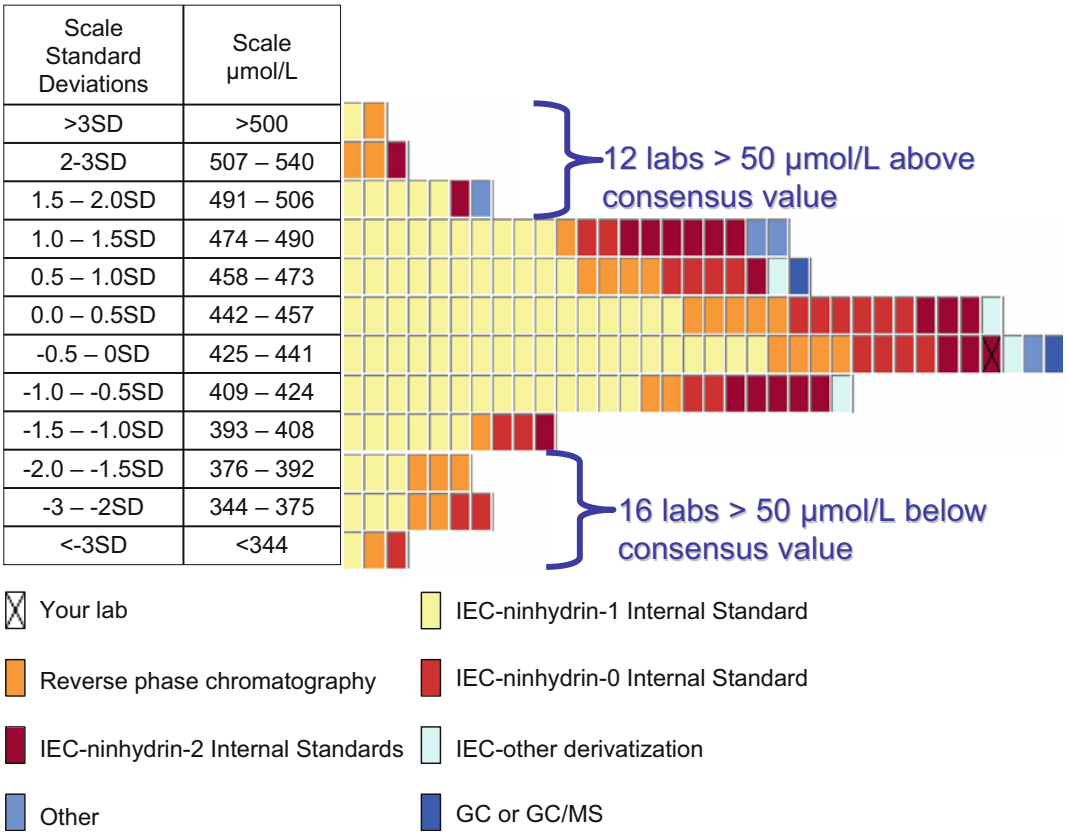


**Fig. 1** Results in detail for phenylalanine with a consensus value of 442 µmol/L. IEC, ion-exchange chromatography; GC/MS, gas chromatography–mass-spectrometry

**Table 2** Performance in the amino acid scheme in 2002 and 2007

| Amino acid | Low level (µmol/L) | | | | Intermediate level (µmol/L) | | | | High level (µmol/L) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2002 | | 2007 | | 2002 | | 2007 | | 2002 | | 2007 | |
| | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % |
| α-Aminobutyric acid | 6.0 | 40 | 6.0 | 23 | 9.7 | 21.4 | 9.9 | 10.4 | 25 | 14 | 29 | 11 |
| Alanine | 117 | 6.6 | 85 | 10 | 331 | 6.5 | 185 | 8.3 | 804 | 6.8 | 873 | 7.6 |
| Arginine | 19 | 16 | 9.7 | 23 | 219 | 8.4 | 220 | 8.0 | 301 | 8.5 | 362 | 7.6 |
| Citrulline | 12 | 19 | 10 | 17 | 110 | 10 | 99 | 8.8 | 151 | 9.1 | 494 | 8.0 |
| Cystathionine | 5.0 | 24 | 4.0 | 46 | 14.5 | 12.7 | 8 | 25 | 20 | 12 | 84 | 13 |
| Cystine | 9.5 | 22 | 27 | 15 | 37 | 12.2 | 53 | 10.2 | 49 | 8.8 | 72 | 10 |
| Glycine | 103 | 8.7 | 65 | 8.7 | 352 | 6.5 | 392 | 6.8 | 502 | 7.2 | 961 | 8.4 |
| Histidine | 33 | 14 | 46 | 9.4 | 104 | 10.4 | 102 | 9.9 | 146 | 9.2 | 427 | 9.5 |
| Isoleucine | 37 | 11 | 19 | 16 | 77 | 7.7 | 70 | 6.1 | 195 | 7.5 | 416 | 7.6 |
| Leucine | 75 | 9.9 | 52 | 9.8 | 302 | 7.2 | 355 | 6.7 | 421 | 6.9 | 1016 | 8.7 |
| Lysine | 87 | 8.6 | 60 | 8.9 | 158 | 8.5 | 145 | 8.7 | 346 | 7.4 | 531 | 6.7 |
| Methionine | 13 | 19 | 8.0 | 18 | 41 | 8.7 | 32 | 9.5 | 55 | 8.4 | 742 | 7.3 |
| Ornithine | 11 | 16 | 13 | 9.8 | 81 | 8.8 | 82 | 8.3 | 197 | 7.5 | 191 | 8.2 |
| Phenylalanine | 34 | 9.5 | 14 | 10 | 274 | 6.5 | 362 | 5.6 | 380 | 6.7 | 726 | 7.8 |
| Sarcosine | 33 | 27 | 46 | 22 | 195 | 12.3 | 237 | 10.9 | 485 | 12 | 714 | 14 |
| Serine | 26 | 12 | 9.0 | 25 | 77 | 7.3 | 54 | 9.4 | 195 | 7.1 | 166 | 7.5 |
| Taurine | 18 | 14 | 16 | 13 | 99 | 9.0 | 98 | 8.9 | 243 | 9.6 | 244 | 8.9 |
| Threonine | 33 | 9.4 | 33 | 8.9 | 87 | 7.2 | 85 | 8.3 | 207 | 6.2 | 297 | 6.7 |
| Tyrosine | 29 | 12 | 29 | 12 | 265 | 7.4 | 222 | 6.2 | 372 | 7.9 | 551 | 8.8 |
| Valine | 82 | 8.3 | 78 | 7.0 | 168 | 7.1 | 164 | 7.0 | 400 | 7.2 | 680 | 5.9 |

used. Presently 138 out of the 170 laboratories that submitted results used methods based on ion exchange chromatography. The practical importance of analytical performance is clearly shown in this example. This sample contained a phenylalanine concentration that is similar to a value that might be used as a cut-off for making decisions on changing the diet. With this sample values at least 50 μmol/L higher or lower than the consensus value were found by 12 and 16 laboratories respectively, with clear implications for patient management and comparability of outcome in relation to dietary control.

As a further indication of performance, the median coefficient of variation within laboratories is compared with variation between laboratories in Fig. 2. Data for 2006 are shown to allow illustration of performance with some particular special amino acids. It must be emphasized that these results have to be interpreted with caution since they are based on comparison of average median values for samples of low and high concentrations that differ considerably. Nevertheless, such limitations should apply equally to samples for the years compared here. Generally, three groups of amino acids are apparent: those with good precision and inter-laboratory variation below 10%; those with intermediate performance with good precision within laboratories but inter-laboratory variation of 10–22%; and those with poor precision above 16% and inter-laboratory variation above 26%. Prior to this there were few systematic data available on performance in amino acid analysis. However, in 1990 amino acid analysis performance within 27 UK laboratories was reported (Rattenbury and Townsend 1990). Examples of the coefficient of variation between laboratories for individual amino acids in plasma compared with the most recent ERNDIM values in brackets were as follows: glycine 13% (8.1%); isoleucine 14% (10.5%); phenylalanine 14% (8.1%); threonine 20% (8.2%); arginine 22% (14.2%); and histidine 41% (9.5%). This suggests a clear improvement of performance since the introduction of the ERNDIM scheme.

## Special assays for serum and urine

To illustrate performance achieved in the two special assay schemes, results are shown for analytes consistently included in the schemes for both serum and urine from 2001 to 2007. The logistics of the schemes are the same as those for the amino acid scheme. Tables 3 and 4 show the median values for all laboratories and coefficient of variation for the lowest, intermediate and highest levels for results of 10 and 12 important diagnostic metabolites in the serum and urine schemes respectively. Those obtained in 2001, when the scheme took on its present format, and in 2007 are shown. There has been a substantial increase in participation in both schemes. Returns for individual analytes ranged from 31 for 7-dehydrocholesterol

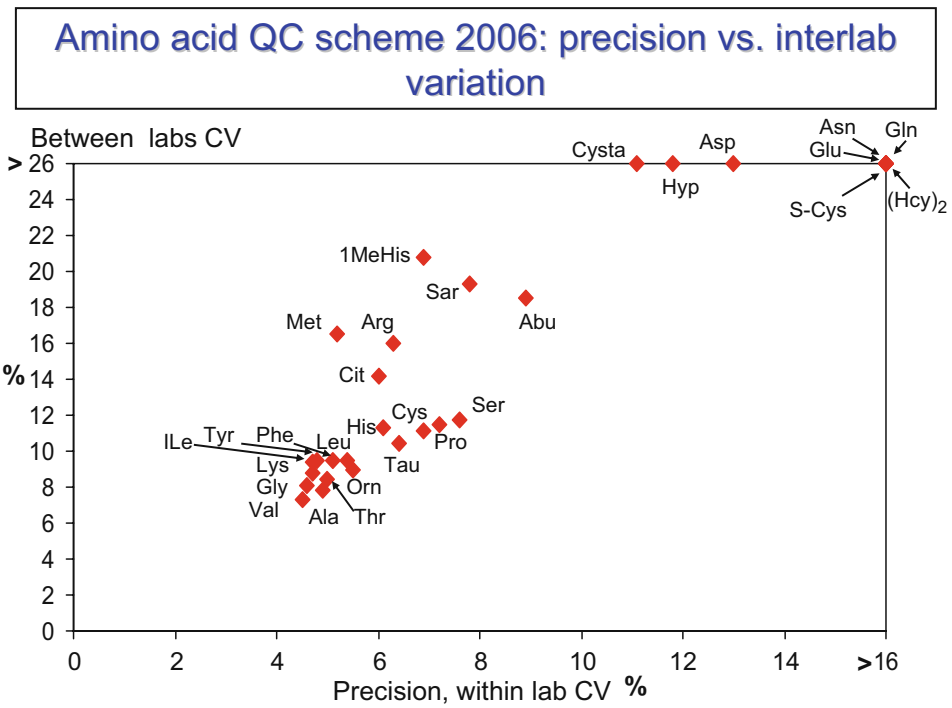


**Fig. 2** Comparison of precision within laboratories with inter-laboratory variation for all amino acids included in the amino acid scheme in 2006. CV, coefficient of variation; Ala, alanine; Abu, α-aminobutyric acid; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cit, citrulline; Cys, cystine; Cysta, cystathionine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; $(Hcy)_2$, homocystine; His, histidine; 1MeHis, 1-Me-histidine; Hyp, hydroxyproline; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Sar, sarcosine; Ser, serine; S-Cys, sulfocysteine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine

**Table 3** Comparison of overall performance in the special assays in serum scheme, 2001 and 2007

| Compound | Low level (μmol/L) | | | | Intermediate level (μmol/L) | | | | High level (μmol/L) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2007 | | 2001 | | 2007 | | 2001 | | 2007 | |
| | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % |
| 3-OH-Butyric acid | 80.5 | 37 | 46 | 50 | 1600 | 11 | 1635 | 11 | 4335 | 15 | 4560 | 13 |
| 7-Dehydrocholesterol | 38 | 63 | 77 | 25 | 74 | 32 | 77 | 25 | 119 | 41 | 241 | 31 |
| $C_{22:0}$, Behemic acid | 54 | 22 | 56 | 14 | 63 | 23 | 77 | 12 | 94 | 19 | 102 | 12 |
| $C_{24:0}$, Lignoceric acid | 41 | 23 | 46 | 20 | 58 | 19 | 65 | 17 | 86 | 19 | 92 | 13 |
| $C_{26:0}$, Cerotic acid | 0.475 | 55 | 0.52 | 32 | 3.3 | 24 | 3.7 | 16 | 9.0 | 28 | 9.8 | 15 |
| Carnitine | 32 | 11 | 36 | 14 | 66 | 11 | 67 | 12 | 128 | 11 | 132 | 9.2 |
| Homocysteine (total) | 11 | 8.5 | 12 | 10 | 43 | 11 | 48 | 8.2 | 106 | 11 | 117 | 8.7 |
| Lactic acid | 2310 | 11 | 2220 | 6.8 | 5725 | 7.1 | 4590 | 6.0 | 12700 | 9.0 | 9250 | 5.0 |
| Phytanic acid | 3.6 | 25 | 3.1 | 36 | 14 | 27 | 12 | 20 | 34 | 23 | 29 | 21 |
| Pyruvic acid | 49 | 32 | 66 | 20 | 540 | 32 | 365 | 16 | 1385 | 58 | 495 | 17 |

to 99 for homocysteine in 2001 and increased to 41 and 102 for these metabolites in 2007 for the serum scheme. For the urine scheme, returns ranged from 8 for guanidino acetate to 73 for orotic acid in 2000 and from 21 for sialic acid to 93 for orotic acid in 2007. As for amino acids, variability is clearly greater at lower concentrations. For both the serum and urine schemes there is improvement of variability over these years for a number of metabolites. The urine scheme has illuminated the importance of precision of creatinine measurements, since even moderate inaccuracies can markedly effect the final results of metabolites that have to be expressed in relation to creatinine, such as organic acids.

In general this experience with the special assay schemes provides clear evidence of improved performance, although further improvements are clearly needed for some compounds.

## Diagnostic proficiency testing

In this scheme six samples per year from patients with a specific inborn error of metabolism are circulated by each of the five centres to participants in their own scheme. One of these is a common sample sent to all laboratories. Samples from subjects without an inherited metabolic disorder (IMD) may also be included. Participants receive some information on

**Table 4** Performance in the special assays in urine scheme in 2001 and 2007

| Compound | Low level (μmol/L) | | | | Intermediate level (μmol/L) | | | | High level (μmol/L) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2007 | | 2001 | | 2007 | | 2001 | | 2007 | |
| | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % | Median | CV % |
| 5-OH-Indoleacetic acid | 4.3 | 22 | 2.2 | 58 | 38 | 23 | 37 | 14 | 102 | 29 | 105 | 18 |
| Carnitine | 20 | 13 | 16 | 23 | 54 | 11 | 17 | 10 | 67 | 13 | 462 | 12 |
| Creatinine | 2975 | 5.6 | 2740 | 5.5 | 6275 | 6.7 | 6180 | 5.8 | 12750 | 6.4 | 12800 | 5.7 |
| Guanidino acetic acid | 74 | 23 | 64 | 16 | 85 | 45 | 100 | 15 | 158 | 19 | 168 | 14 |
| Homovanillic acid | 5.8 | 34 | 2.9 | 47 | 38 | 19 | 35 | 21 | 110 | 14 | 102 | 23 |
| Hydroxyproline | 345 | 24 | 331 | 7.8 | 675 | 9.5 | 673 | 9.7 | 991 | 16 | 986 | 11 |
| Lactic acid | 3175 | 20 | 3200 | 12 | 6645 | 18 | 6470 | 15 | 9585 | 28 | 9515 | 14 |
| Mucopolysaccharides | 37 | 19 | 36 | 12 | 73 | 17 | 61 | 18 | 101 | 22 | 95 | 15 |
| Orotic acid | 7.5 | 46 | 30 | 12 | 13 | 32 | 57 | 13 | 20 | 30 | 81 | 16 |
| Sialic acid | 57 | 33 | 24 | 83 | 189 | 12 | 140 | 28 | 389 | 15 | 315 | 30 |
| Succinylacetone | 10 | 33 | 8.4 | 58 | 17 | 72 | 20 | 57 | 34 | 53 | 28 | 64 |
| Uric acid | 231 | 15 | 407 | 17 | 568 | 8.9 | 599 | 14 | 748 | 11 | 954 | 12 |

the clinical presentation and treatment details if applicable. They are required to perform any relevant tests in order to reach a diagnosis and it is expected that laboratories are able to perform tests for detection of amino acid, organic acid, mucopolysaccharide (MPS) and oligosaccharide disorders, and in some schemes also for purine and pyrimidine disorders. During recent years a harmonized scoring system has been adopted by all five DPT centres. For each sample, three criteria—analytical performance, interpretative proficiency and recommendations for further investigations—are evaluated and scored to give a maximum of 5 points per sample. In the absence of any results, the sample is scored as 0 points.

Satisfactory *analytical performance* (2 points) depends on clearly described and correct analytical results, which may be semi-quantitative evaluation of an abnormal level of metabolite(s) or quantitative value(s) or a description of a profile of analytes suggestive of or excluding a specific diagnosis. Analytical results may be considered only partially correct (1 point) if incomplete procedures have been performed (e.g. quantitative mucopolysaccharide measurement without performing profile analysis of MPS), or if the results are insufficiently specific. Zero points are given if the appropriate test has not been carried out, or an elevated metabolite has not been detected, or a decreased or absent metabolite has been reported.

Satisfactory *interpretative proficiency* (2 points) is judged as a correct diagnostic conclusion that is achievable by analysing urine and may be a single possible diagnosis or several diagnoses (e.g. methylmalonic aciduria due to mutase deficiency or cobalamin defects or secondary to vitamin $B_{12}$ deficiency). Helpful but incomplete diagnostic conclusions (1 point) should eventually lead to establishing the diagnosis, usually pointing to a group of diseases (e.g. diagnosis of mucopolysaccharidosis in the case of elevated MPS but without a clearly described profile or with assignment of a wrong type of MPS disorder). Wrong/misleading diagnosis (0 points) can be 'over-diagnosis' in the case of no known IMD, completely inappropriate diagnosis, or missed diagnosis in a patient with a known IMD.

**Table 5** Numbers of poor performers related to numbers of participants in the Prague and Lyon DPT schemes

| | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 |
|---|---|---|---|---|---|---|---|
| Prague | 2/21 | 1/20 | 3/20 | 1/20 | 0/24 | 3/18 | 0/17 |
| Lyon | | 6/17 | 2/20 | 2/20 | 0/22 | 0/19 | 0/22 |
| Total | | 7/37 | 5/40 | 3/40 | 0/46 | 3/37 | 0/39 |

*Recommendations for further investigations* should be unambiguous and avoid redundancy of recommendations, detachment from practice (tests that will not be ordered in a real patient), non-specificity of recommendations, or over-invasive testing in cases of samples from patients with no IMDM.

To illustrate the level of performance within these schemes, results obtained since 2001 for the Lyon and Prague schemes, which were the first centres to introduce a harmonized scoring system can be considered (Table 5).

Samples from 51 different inherited metabolic disorders were included, those from 12 different disorders on more than one occasion (2–4 times). Also, one sample is distributed by both centres each year. Full details of these samples can be found in Supplementary Table S1 accessible on-line. Efficiency of performance as the percentage of correct results for all participants for the sum of analytical and interpretative performance and recommended investigations is just over 80% on average for all samples. Relatively poor performance of between 28% and 69% efficiency was found for samples from patients with a range of disorders, in order of increasing efficiency as follows: adenylosuccinate lyase deficiency, mucopolysaccharidosis type III, sialidosis, a patient with both adenine phosphoribosyltransferase deficiency and MPS type IV, molybdenum cofactor deficiency, aspartylglucosaminuria, isolated sulfite oxidase deficiency, biotinidase deficiency, aromatic amino acid decarboxylase deficiency, septic shock, peroxisomal disorder, fucosidosis, and MPS type VII. All samples for which good performance of over 90% efficiency was found came from patients with an amino acid or organic acid disorder, although performance was not satisfactory for all samples for these types of disorder. For 11 samples that were distributed in different years, efficiency of performance remained over 90% for four, showed no important change in four and improved in three of them. Overall trends in performance over the years are difficult to evaluate owing to the use of widely differing samples, but there has been a clear drop in the number of laboratories judged to have performed poorly, as shown in Table 5. The experience from the Sheffield scheme was briefly reported by Bonham (2003).

## Future developments of EQA schemes

Much progress has already been made in improving performance in biochemical genetic testing, but further improvement is still necessary. Steps to improve performance include educational activities (see below),

provision of expert guidance papers on quality issues and specific methods and development of reference materials and calibrators. The recently published comprehensive book on methods for biochemical genetic testing (Blau et al 2008) could be a valuable aid in standardizing procedures.

Scoring and assessment of performance are an essential part of an EQA scheme and needs to be harmonized in order to define good performance. Steps are currently under way for our schemes, and from 2007 certificates of participation for the individual laboratories will include indicators of performance achieved. Laboratories that fail to reach a satisfactory level of performance are issued with a 'warning' letter, which is in fact a 'helping' letter that is meant to initiate dialogue on identification of problems and their remedies.

An essential development is to allow web-site submission of results and on-line reporting, presently available only for quantitative schemes, for all schemes. Moves are now underway to develop web-site submissions for the DPT and other qualitative schemes.

As well as encouragement of accreditation of the participating laboratories, the EQA schemes themselves will need to be accredited and this demands close cooperation with professionally operated EQA providers such as SKML and possibly others. Beyond this, moves have begun to towards accreditation of ERNDIM itself. Thus, the way is clear to bring our scheme into line with international requirements for EQA schemes within the context of accreditation of individual laboratories themselves.

### Pilot schemes

To meet expanding needs, ERNDIM supports the development of new schemes. First, potential scheme organizers and scientific advisors need to ascertain the potential need; 25 participants is the very lowest figure possible, since the involvement of fewer does not allow reliable statistical ascertainment of results. Second, feasibility of the scheme needs to be proved during a pilot phase, which usually lasts for two years. Sample suitability, stability, robustness of result presentation and reporting can all be tested. Then a new scheme can be incorporated as an official ERNDIM scheme. Presently two pilot schemes are underway. A scheme for lysosomal enzymes, organized by SKML with Dr O. van Diggelen as scientific advisor, was begun in 2007. A new scheme for disorders of glycosylation has been operated by SKML together with Professor R. Wevers in 2008.

## Other ERNDIM activities

### Training and education

Since its inception, ERNDIM has been active in this area and from an early stage workshops on diagnostic issues have been organized at the SSIEM annual symposium. Since 2001 these have been held following the parallel meetings for the DPT participants and are open to all persons attending the SSIEM symposium. They address clinical pointers and analytical procedures and performance in relevant ERNDIM schemes for a particular group of disorders. Topics since 2000 have been amino acids, mucopolysaccharides, organic acids, general aspects of schemes (Fowler et al 2003b), acylcarnitines, purines and pyrimidines, lysosomal enzymes (held at a separate ERNDIM meeting in 2006), peroxisomal disorders and creatine synthesis disorders. These workshops provide the basis for guidance documents on general issues of quality as well as analysis of specific groups of metabolites. These are mounted on the ERNDIM web site (http://www.erndim.unibas.ch/ 'Training and Education' and currently include: Theoretical Aspects of QC in IEM and Method Validation; Control of Accuracy and Precision; Amino Acid Analysis Recommendations; Biomed 2 Recommendations; Polymorphonuclear Leukocyte Preparation; Mixed Leukocyte Preparation; White Cell Cystine Determination and the role of EQA; and Role of EQA in Special Assays for IEM. Further planned documents include recommendations for analysis of organic acids, mucopolysaccharides, purines/pyrimidines, lysosomal enzymes, acylcarnitines, and performance assessment in ERNDIM diagnostic proficiency schemes.

ERNDIM endeavours to support the training of biochemists in biochemical genetic testing, similar to that existing for paediatricians in metabolic medicine. It is clear that training programmes will inevitably vary between countries. This is a natural consequence of the differences in professional and institutional environments. It will also reflect genetic variation and the differing geographic incidence of individual diseases. However, there are many principles of good practice and analytical techniques in common that we can learn from each other. There is also much that countries that plan to develop these services can learn from those where they are well established. Accordingly, ERNDIM has developed an area of its web site as a resource where information can be sought about existing training programmes and web-based resources can be shared. The web site provides a place for infor-

mation on the approaches to training in each individual country. Also, some training resources, such as the training syllabus approved by SSIEM, the MetBioNet training log, information about the French training scheme, and a metabolic map, are directly available from this site.

A new training initiative is to provide training days for laboratory workers in collaboration with the SSIEM and under the aegis of Education and Training Advisory Committee. The first one-day course in parallel with one for clinicians preceded the annual SSIEM symposium at Lisbon in 2008.

## ERNDIM/Eurogentest directory of laboratories

This has been established with support of a previous EU project and currently within the Eurogentest project. The aim of the directory is to aid specialist workers in the field in selection of laboratories for sample referral. Provision of information on quality management such as accreditation status and participation in EQA schemes helps users to judge the suitability of laboratories to which samples are sent. Tests, referred to as analytes, for single or groups of metabolites, enzyme activity and mutation analysis are included. In using the directory, a specific analyte is chosen from the pull-down list and laboratories providing the service are listed, first from a specified country followed by other countries. Details of the listed laboratories are found under 'more info' according to country with details on the laboratory including contacts, general description, metabolic clinic, EQA schemes participated in, accreditation status and special interests.

Laboratories wishing to register their services within the directory apply by submitting their details directly to the web site. Subject to approval of the laboratory by ERNDIM, the laboratory details are automatically entered into the web site and a password is issued to the laboratory by e-mail allowing entry to the 'Existing Participants' area of the site. Existing participants can enter this area in order to add or delete analytes they perform as well as to update laboratory information. The directory presently contains about 130 laboratories for which information has been fully validated. Currently about 480 analytes or tests are listed. Approximately 200 additional European laboratories that perform biochemical genetic testing have been identified and encouraged to join the directory. Further developments will be to link this directory to the Eurogentest QAu database and to the disease-oriented Orphanet web site.

Our aim is to increase awareness of quality issues in the minds of users of biochemical genetic testing services when they select the laboratories to which they send samples. Ultimately we should all look at the ERNDIM laboratory directory and check EQA participation before sending out samples.

## The Eurogentest Project and the role of ERNDIM

The Eurogentest project aims to promote the proper utilization and management of genetic services; harmonization of accreditation and certification of genetic testing laboratories (http://www.eurogentest.org/); and establishment of procedures and guidelines for the validation of methods and technologies.

ERNDIM represents biochemical genetic testing within the project (http://www.eurogentest.org/web/info/unit1/biochemical.xhtml) and is currently engaged in the following activities within the work packages 1.5 and 1.9. Specific aims are to expand opportunities for biochemical genetic testing laboratories in the EU to participate in EQA and to link this through agreed best practice in internal and external quality control to improve the quality of biochemical genetic testing. Examples of achievements so far are as follows:

- Production of an interim report on biochemical genetic testing in Europe: deficits and needs and EQA. This report is based on two meetings with national representatives from each EU country, held in Basel, 2 December 2005 and Prague, 5–6 October 2006 (see http://www.erndim.unibas.ch/Meeting_Rep/06_dec_basel/Best%20practice%20EUGT%20UPDATED%20report%203Sept2007.pdf).
- In order to expand the capacity and scope of our schemes, a fifth diagnostic proficiency testing EQA scheme has been established in Basel and two new schemes for lysosomal enzymes and congenital disorders of glycosylation are under development.
- We have developed criteria for performance assessment of both proficiency and quantitative schemes within best-practice meetings of the ERNDIM Scientific Advisory and Executive Committees.
- Several educational activities have been consolidated as mentioned above.
- The new ERNDIM web site has been developed, including the directory of laboratories and training and methodological guidance documents.

Meetings have been held together with our partners responsible for EQA in cytogenetics and molecular

genetics and accreditation experts in order to promote accreditation of individual genetic EQA schemes as well as EQA umbrella organizations. Resulting documentation includes checklists and quality manuals to aid us all in the accreditation process.

Future plans include further best-practice meetings with all EU national representatives to establish guidelines for organization of biochemical genetic testing services at the national level.

## Conclusion

There is ample evidence that ERNDIM has fulfilled its early aims of improvement of patient services and is now synonymous with EQA for biochemical genetic testing in Europe. Nevertheless, challenges remain to continue to improve performance of laboratories and to increase awareness by users of biochemical genetic testing services of the importance of EQA. Introduction of measures highlighted above and increasing cooperation with other genetic disciplines and the SSIEM should help to sustain our important activities in the future.

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