ERNDIM - Quantitative Schemes
Quantitative Organic Acids

Dr. M. Duran
Academic Medical Center Amsterdam
Lab. Genetic Metabolic Diseases
P.O. Box 22700
NL – 1100 DE Amsterdam
e-mail :
m.duran@amc.uva.nl

Dr. C.W. Weykamp
Queen Beatrix Hospital
MCA Laboratory
P.O. Box 9005
NL – 7100 GG Winterswijk
e-mail :
c.w.weykamp@skbwinterswijk.nl

Annual Report ERNDIM-EQAS 2006

1. *Purpose*

The purpose of the ERNDIM External Quality Assurance Scheme for Quantitative Organic Acids is the monitoring of the analytical performance of the quantitative assay of organic acids in urine in laboratories involved in the diagnosis and follow-up of patients with inherited metabolic disorders. For detailed information see www.ERNDIMQA.nl

2. *Participants*

66 Laboratories from 20 countries participated in the Scheme. As there are more labs which take part in the qualitative OA Schemes, apparently not all diagnostic laboratories feel the need for quantitative analysis of organic acids.
Nevertheless the Scientific Advisory Board recommends to implement quantitative organic acid assays. These can be most informative in detecting subtle increases of significant organic acids such as ethylmalonic acid in SCAD-deficiency and 3-hydroxyisovaleric acid in Biotinidase deficiency.

3. *Design*

The Scheme has been designed, planned and coordinated by Dr. Cas Weykamp as scheme organiser and Dr. Ries Duran as scientific advisor, appointed by the ERNDIM Board. The design includes samples and reports which are connected to provide information with a balance between short-term and long term-reports and between detailed and aggregated information.

Samples

The scheme of the year 2006 consisted of 8 lyophilised samples, all prepared from the same basic human urine but with various amounts of added analyte. The samples were identical two by two: the pairs, along with the added amounts of analyte and their source are in the table below. The type and level of the analytes were discussed in the Scientific Advisory Board and agreed by the Trust Board. As before, the concentrations varied between the physiological range and the typical pathological range. The latter may be quite high, e.g. in methylmalonic aciduria and ketosis. One

drawback of this system is the relative weight of the sample with the highest concentration with respect to the linearity and recovery calculations. These aspects will have to be reconsidered.

Pairs, added amounts (in micromol/L) of organic acids and their source

Analyte	Source	Added to Pair 117-122	Added to Pair 120-124	Added to Pair 118-121	Added to Pair 119-123
4-OH Butyrate	Sigma H3635	0	40	404	81
Glutarate	Sigma G4126	0	89	45	298
Suberate	Sigma S5200	0	24	49	807
Hexanoylglycine	VU A'dam	0	72	43	22
3OH Butyrate	Sigma H3145	0	149	299	4968
3OH Propionate	Brunet	0	32	317	63
3 Methylglutarate	Sigma M1512	0	144	86	43
Methylmalonate	Sigma M5,405.8	0	496	4965	992
Tiglylglycine	VU A'dam	0	265	106	53
3OH Isovalerate	Brunet	0	420	168	84
Fumarate	Sigma F2752	0	56	27	185
Glycolate	Sigma G8284	0	492	197	98
2-Keto Glutarate	Sigma K2000	0	121	1209	243
Ethylmalonate	Sigma E8758	0	29	291	58
2OH Glutarate	Sigma H8378	0	471	188	94
Malonate	Sigma M1875	0	196	98	652
N-acetylaspartate acid	Sigma A5625	0	391	782	13000
D,L-Glyceric acid	Sigma G7274	0	489	244	1629

Reports

All data-transfer, the introduction of data by the subscribers (methods, results) as well as their request of reports was done via the interactive website www.erndimqa.nl.

The website supplies short-term and long-term reports. Short-term reports are associated with the eight individual specimens, for which a specific deadline has previously been established for each . Two weeks after the respective deadlines participants can request their reports and thus can update the information on their analytical performance. Although technically not required, a delay time of 14 days has been arbitrarily chosen to enable the scientific advisor to inspect the results and add his comment to the report. In contrast to the rapidly available short-term reports the annual long-term report is based on the designed connection between samples – as described above - which enables to report a range of analytical parameters (accuracy, precision, linearity, recovery and inter-laboratory dispersion) once an annual cycle has been completed.

Another characteristic of the website is the variety of result presentations which allows laboratories to make an individual choice for detailed and/or aggregated reports. The most detailed report which can be requested from the website is the "Analyte in Detail" which shows results of a specific analyte in a specific sample (136 such Analyte-in-Detail-reports could be consulted in the 2006 cycle). A more condensed report is the "Cycle Review" which summarizes the performance of all analytes in a specific sample (8 such Cycle-Review-Reports were available in 2006). The highest

degree of aggregation is the Annual Report which summarizes the performance of all analytes of all 8 samples. Depending on the information one wants to obtain one can choose to have a glance at only the annual report (e.g. laboratory managers) or study all 136 detailed reports (person in charge of the workplace, technicians).

4. Discussion of Results in the Annual Report 2006

Subsequently we present accuracy, recovery, precision, linearity, interlab CV and cross sectional relations. It may be helpful to print your results of the annual report from the Interactive Website before reading the following comments and keep in mind that we only discuss the results of all labs in general: it is up to you to inspect and interpret the specific results of your laboratory and - where needed – to investigate the cause of unsatisfactory results and to correct the procedure.
Whenever serious problems are encountered, contact may be made with your National Representative or eventually with the Scientific Advisor.

4.1 Accuracy

A first approach to describe accuracy is to compare the mean outcome in the eight samples in your lab with the mean in all labs. This is shown in the column "Your Lab" and "All labs" under the heading "Accuracy".

4.2 *Recovery*

A second approach to describe accuracy is the percentage recovery of added analyte. In this approach it is assumed that the recovery of the weighed quantities is the target value. The correlation between weighed quantities as added to the samples (on the x-axis) and the measured quantities (on the y-axis) have been calculated. The slope of the correlation multiplied with 100% is the recovery of the added amounts. The column “Recovery” shows your recovery in the respective organic acids in comparison to the median recovery of all laboratories. The median recovery ranges from 39% 3-OH-propionic acid (we have doubt on the purity of the spiking material used as the recovery was 95% in 2005) to 109% (2-OH Glutaric acid). The recovery of 4-OH-butyric acid was quite low (64%), possibly as a result of lactone formation, either during the production of the samples of during the extraction / derivatisation.The overall mean recovery is 86%. Conclusions from aggregated data are generalisations which should render the participants to the QC-programs (and even more the end- users of the data) cautious about utilizing data from other labs without asking about proof of reliability. The difficulties we face are certainly a challenge for developing improved methods.

4.2.1 *Precision*

Reproducibility is an important parameter for quality in the laboratory. The CV is calculated from the pairs of the scheme which can be regarded as duplicates (Intra Laboratory CV as indicator of reproducibility). Since there are only four pairs, the calculated precision can only give an indication about the reproducibility of the individual laboratory. It allows, however, comparison total group of the individual performance with that of the participants. The results in comparison to the median of all labs is shown in the column “Precision” of the Annual Report. Precision ranges from 2.9 % for creatinine to a poor 37.4% for 3-OH-propionic acid with an overall intra-lab CV of 21.5%.

In general the best precision was observed for the simple dicarboxylic acids such as ethylmalonate and glutarate; lower scores of the hydroxyacids may have been the consequence of non-optimal extraction efficacies. Rigorous standardization of the extraction parameters, i.e. pH of the sample and exact volume of extraction solvent may be a way to improve this aspect.

4.2.2 *Linearity*

Linearity over the whole relevant analytical range is another important parameter for analytical quality. The regression has been calculated taking the final measured concentration of the addition as independent (x) variable and the measured concentrations as the dependent (=y). The regression coefficient r of the individual and the median of all labs are shown in the columns "Linearity"of the annual report. It can be seen that the coefficients of regression range from 0.9532 for hexanoylglycine to 0.9991 for suberic acid.

4.2.3 *Interlab CV*

For comparison of outcome for one patient in different hospitals and for use of shared reference values it is relevant to have a high degree of harmonization between results of various laboratories. Part of the scheme design is to monitor this by calculating the Interlaboratory CV. This, along with the number of laboratories who submitted results, is shown in the column "Data All labs" in the Annual report. It can be seen that most laboratories submitted results for methylmalonic acid (66) whereas only 43 participated for tiglylglycine. The Inter-lab CV ranges from 5.6 % for creatinine to 413.8% for D,L-glyceric acid.

4.2.4 *Cross Sectional Relations*

The various parameters as described above often have an interrelation: often more than one parameter directs towards good or bad analytical control. This pattern is not clearly seen in the organic acids scheme.

5 *Conclusions & Summary*

The high interlab CV demonstrates clearly the major problem in the analysis of organic acids: lack of standardization. Precision with a mean CV of 21.5% is much better indicating that reproducibility within the labs is not too bad. Linearity is also no major problem and recovery is also quite acceptable. In this respect it should be noted that steps are being taken to prepare extra samples which may be used as calibrators, given that the weighed additions and the median calculated values are known. More information will be distributed through the regular ERNDIM-channels.
We invite you to review your data carefully and especially study your recoveries. These may give an indication of deviant calibration.

6 *Preview Scheme 2007*

The 2007 scheme will be similar to 2006, except that malonic acid has been removed.

7 *Questions, Comments and Suggestions*

If you have any questions, comments or suggestions, please address to the scientific advisor of the scheme Dr. Ries Duran (m.duran@amc.uva.nl) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).
Alternatively you may approach your local National Representative, a list of which is available from ERNDIM.