ERNDIM - Quantitative Schemes
Quantitative Organic Acids

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Annual Report ERNDIM-EQAS 2012

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 analysis of organic acids in urine. For detailed information see www.erndim.org / www.ERNDIMQA.nl

2. Participants

107 Datasets were submitted by laboratories from 33 countries. 6 labs did not submit enough results to allow calculation of the annual report and 8 labs did not submit any results. Although the number of participants in this scheme is steadily increasing, the number of labs which take part in the qualitative OA Schemes is larger than that in the quantitative scheme. 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, 3-methylglutaconic acid and 3-methylglutaric acid in the 3-methylglutaconic acidurias, and 3-hydroxyisovaleric acid in biotinidase deficiency. Another important area of quantitative analysis is that of treatment monitoring.

3. Design

The Scheme has been designed, planned and coordinated by Dr. Ries Duran as scientific advisor and Dr. Cas Weykamp as scheme organiser, both appointed by the ERNDIM Trust Board. In 2012 Ries Duran has retired as scientific advisor from the Scientific Advisory Board. Subsequently, the Trust Board appointed dr. Geert Martens as his successor.
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 consisted of 8 lyophilised urine 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 Table 1 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. for methylmalonic acid, pyroglutamic acid, and 3-hydroxybutyric acid.

Table 1: Pairs, added amounts (in micromol/L) of organic acids and their source

Analyte	Source	Added to Pair 165 -169	Added to Pair 167-171	Added to Pair 168 -170	Added to Pair 166 -172
D-2-OH-glutarate	Sigma H8378	0	470	188	94
3-Methylglutarate	Sigma M47604	0	144	86	43
3-OH-Butyrate	Aldrich 29,836-0	0	148	299	4966
3-OH-Isovalerate	Brunet	0	458	183	92
4-OH-Butyrate	Sigma H3635	0	40	404	81
Adipate	Sigma A26357	0	240	121	801
D,L-Glycerate	BioConnect Lip0000373	0	559	280	1861
Ethylmalonate	Sigma E8758	0	29	291	58
Fumarate	Sigma F2752	0	55	28	183
Glutarate	Sigma G3407	0	89	45	298
Glycolate	Sigma G8284	0	489	196	98
Hexanoylglycine	Ten Brink	0	72	43	22
2-Ketoglutarate	Sigma K2000	0	121	1210	242
Malic acid	Sigma M9138	0	240	121	801
Methylmalonate	Aldrich M5,405.8	0	496	4966	995
Mevalonate	Sigma M4667	0	620	248	124
N-acetylaspartate	Sigma A5625	0	388	782	12997
Pyroglutamate	Aldrich 83160	0	303	610	10136
Sebacate	Sigma S2625	0	240	121	801
Suberate	Sigma S5200	0	240	120	799
Tiglylglycine	Ten Brink	0	265	106	53

Reports

All data-transfer, the submission of data as well as request and viewing of reports proceeded via the interactive website www.erndimqa.nl which can also be reached through the ERNDIM website (www.erndim.org).

The website supplies short-term and long-term reports. Short-term reports are associated with the eight individual specimens, for which a deadline has previously been established . 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 (176 such Analyte-in-Detail-reports could be consulted in the 2012 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 2012). 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 176 detailed reports (person in charge of the workplace, technicians).
Inevitably, every sign of inadequate performance arising from the Annual Report will be followed up by inspecting the relevant Analyte-in-detail reports.

4. Discussion of Results in the Annual Report 2012

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 results of your laboratory and - where needed – to investigate the cause of unsatisfactory results and to make plans for improving your performance..
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". It can be seen that the mean of all labs for 2-OH-glutaric Acid is 145.

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 of the respective organic acids in comparison to the median recovery of all laboratories. The median recovery ranges from 66% (4-OH-butyric acid) to 106% (hexanoylglycine). The low recovery of 4-OH-butyric acid is possibly due to lactone formation, either during the production of the samples or during the extraction / derivatisation. Also 2-OH-glutaric acid and mevalonic acid are prone to lactone formation which should always be kept in mind when interpreting the recovery data. Conclusions from aggregated data are generalisations which should render the participants of 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 coefficient of variation (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 of the

individual performance with that of the toal group of participants. Your results in comparison to the median of all labs is shown in the column “Precision” of the Annual Report. Precision ranges from 9.4 % for 3-methylglutaric acid to a poor 24.3% for 4-OH-Butyric acid with an overall intra-lab CV of 16.4%. Pyroglutamic acid suffers from a low extraction yield, therefore subtle changes of the extraction conditions may lead to deviant values.
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 of performance.

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 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 column “Linearity” of the annual report. It can be seen that the coefficients of regression range from 0.971 for 3-OH-Isovaleric acid to 1.000 for 3-OH-Butyric 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 which 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 (100) whereas only 44 participated for malic acid. The Inter-lab CV ranges from 20.5 % for 3-methylglutaric acid to 77.8% for N-acetylaspartic 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.

4.3 Your performance: red and green flags

ERNDIM has implemented a system to judge performance of individual laboratories. Red flags in the annual report of an individual laboratory indicate poor performance for accuracy, precision, linearity, and/or recovery. Organic acids with satisfactory performance for at least three of the four parameters (thus no or only one red flag or no result) are marked in green.
Thus a green mark indicates satisfactory performance for analysis of that particular organic acid while a grey mark together with two or more red flags indicates that your laboratory has failed to attain satisfactory performance for this analyte.
Criteria for red flags can be found in the general information on the website (general information; interactive website, explanation annual report).

4.4 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 33% of the laboratories have no red flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 5% of laboratories with more than 25% red flags. Following intensive discussion within the ERNDIM Trust Board and Scientific Advisory

Board (SAB) and taking into account feedback from participants we have been able to agree on a harmonised scoring system for the various branches of the quantitative schemes as described in our Newsletter of Spring 2009. In parallel to this the SAB has agreed levels of adequate performance for all the schemes and these will be re-evaluated annually. The scoring systems have been carefully evaluated by members of the SAB and have been applied to assess performance in our schemes from 2007 onwards. The ERNDIM Board has decided that the Scientific Advisor will judge the performance of the individual laboratories based on these levels of satisfactory performance and issue a letter of failure with advice to achieve satisfactory performance to those laboratories which do not achieve satisfactory performance. The letter is intended to instigate dialogue between the EQA scheme organiser/advisor and the participating laboratory in order to solve any particular analytical problems, eventually resulting in an improved quality of performance of labs

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	5%	5%
20 – 25%	6%	11%
15 – 20%	7%	18%
10 – 15%	12%	30%
5 – 10%	11%	41%
0 – 5%	26%	67%
0%	33%	100%

4.5 Certificates

As for other schemes the performance as it is indicated by the red/green flags in the individual laboratories annual report is summarised in the new style of annual participation certificate. The certificate lists the total number of organic acids in the scheme, the number for which results have been submitted and the number for which satisfactory performance has been achieved. It is important to bear in mind that the certificate has to be backed up by the individual annual report in the case of internal or external auditing.

5 Conclusions & Summary

The high overall interlab CV demonstrates clearly the major problem in the analysis of organic acids: lack of standardization. Precision with a mean CV of 16.4% is much better indicating that reproducibility within the labs is acceptable. Linearity is no major problem and recovery is also quite acceptable. In this respect it should be noted that extra samples can be purchased from the scheme organizer, which may be used as calibrators, given that the weighed additions and the median calculated values are known. These samples are prepared by mixing equal amounts of the four levels of one of the previous years. Over the years it has become clear that these 'mixed' samples are ideally suited to serve as internal quality assurance samples.

We invite you to review your data carefully and especially study your recoveries. These may give an indication of deviant calibration.

6 Preview Scheme 2013

The 2013 scheme will be expanded by the addtions of 3-hydroxyisobutyrate, 3-hydroxy-3-methylglutarate, and vanillactate to the scheme. Furthermore the concentration ranges will be brought more in line with the other \ERNDIM quantitative schemes, i.e. less exceptional concentration values in order to allow for comparable

calculation of the performance parameters. The SAB has expressed the wish to include methylcitrate in the present scheme; however, no accessible source of this analyte could be found so far. Suggestions for supplying methylcitrate will be welcomed by the scheme organizer.

7 Questions, Comments and Suggestions

If you have any questions, comments or suggestions, please address to the scientific advisor of the scheme Dr. Geert Martens (Geert.Martens@uzbrussel.be) 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.