ERNDIM - Quantitative Schemes
Quantitative Organic Acids

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Brussels/Winterswijk, 31 January 2014

Annual Report ERNDIM-EQAS 2013

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*

106 Datasets were submitted by laboratories from 33 countries. 5 labs did not submit enough results to allow calculation of the annual report and 11 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-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 Prof. Geert Martens as scientific advisor and Dr. Cas Weykamp as scheme organiser (subcontractor on behalf of SKML), both appointed by and according to the procedures of the ERNDIM Trust 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 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, and pyroglutamic acid. Samples have been tested for stability an homogeneity according to ISO 13528.

As compared to the 2012 scheme, the 2013 scheme included novel compounds (3-hydroxy-3-methylglutaric acid, 3-hydroxyisobutyric acid and vanillactic acid). Others e.g. 3-hydroxybutyric acid were omitted from the panel.

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

Analyte	Source	Added to Pair 173 -179	Added to Pair 176-178	Added to Pair 175 -180	Added to Pair 174 -177
D-2-OH-glutarate	Sigma H8378	0	470	188	94
3-Methylglutarate	Sigma M47604	0	143	86	43
3-OH-3 methylglutarate	Aldrich H4392	0	144	72	481
3-OH-Isobutyrate	Sigma 36105	0	367	147	73
3-OH-Isovalerate	Brunet	0	378	151	75
4-OH-Butyrate	Sigma H3635	0	40	403	81
Adipate	Sigma A26357	0	144	72	481
D,L-Glycerate	BioConnect Lip0000373 / TCI G0232	0	559	280	1862
Ethylmalonate	Aldrich 102687	0	17	175	35
Fumarate	Sigma F2752	0	55	28	183
Glutarate	Sigma G3407	0	89	45	298
Glycolate	Sigma G8284	0	391	157	78
Hexanoylglycine	Ten Brink	0	72	43	22
2-Ketoglutarate	Sigma K2000	0	72	726	145
Malic acid	Sigma M9138	0	45	447	89
Methylmalonate	Aldrich M5,405.8	0	248	2484	498
Mevalonate	Sigma M4667	0	1239	496	247
N-acetylaspartate	Sigma A5625	0	78	157	2601
Pyroglutamate	Aldrich 83160	0	60	122	2021
Sebacate	Aldrich 84809	0	144	72	481
Suberate	Aldrich S5200	0	120	60	400
Tiglylglycine	Ten Brink	0	265	106	53
Vanillactate	TCI H0538	0	81	49	24

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 results of your laboratory are confidential and only accessible to you (with your name and password). The anonymised mean results of all labs are accessible to all participants. Statistics of the respective reports are explained in the general information section of the website.

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 (192 such Analyte-in-Detail-reports could be consulted in the 2013 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 2013). 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 inspect only the annual report (e.g. laboratory managers) or study all 192 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 2013

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". E.g. it can be seen that the mean of all labs for 2-OH-glutaric Acid is 185.

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 63% (4-OH-butyric acid) to 110% (3-OH-isovaleric acid). The low recovery 4-OH-butyric acids 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. Your Intra-Laboratory coefficient of variation (CV) is calculated from the 4 pairs of identical samples in the scheme design which can be regarded as technical duplicates, and compared to the median CV on all duplicate results for a given analyte, submitted by the total group of participating laboratories. These calculated precisions thus provide a rough indication of the reproducibility of your laboratory as compared to the total group of participating laboratories, and are shown in column “Precision”.
Median precision was excellent for many compounds with inter-lab CV ≤ 15% e.g. for the simple dicarboxylic acids (adipic/sebacic/suberic acids, glutaric acid, methylmalonic and ethylmalonic acids). Higher imprecision for several hydroxyacids may have been the consequence of non-optimal extraction efficacies. In line with the results of previous years, a number of problematic compounds show poor precision with intra-laboratory CV of > 25% e.g. 3-hydroxyisobutyric acid, 4-hydroxybutyric acid, hexanoylglycine and vanillactic acid. 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 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 Inter-laboratory 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 (101) whereas only 29 participated for vanillactic acid.

The Inter-lab CV ranges from 17.9 % for 3-methylglutaric acid to 96.4% for 3-OH-3-methylglutaric acid. As expected, the Inter-lab CV is typically 2 to 3-fold higher than the corresponding Intra-lab CV but for a number of organic acids this discrepancy is more than 3-fold. For a number of compounds, this discrepancy was persistently observed also in previous schemes from 2011 on. Key examples, with relevance for disease monitoring and/or diagnosis are glyceric acid, pyroglutamic acid and N-acetylaspartic acid. This is another reason to emphasize the need for harmonization of methods between the different laboratories.

4.2.3 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 0.969 for hexanoylglycine to 0.999 for adipic 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. 36% 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 6% 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%	6%	6%
20 – 25%	5%	11%
15 – 20%	6%	17%
10 – 15%	6%	23%
5 – 10%	18%	41%
0 – 5%	23%	64%
0%	36%	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 17.4% 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 2014

Except for N-acetylaspartic acid (removed from the list) the analytes in the 2014 scheme will be the same as in 2013. Furthermore the concentration ranges of some analytes have been updated and 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.

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.