ERNDIM
Quantitative Schemes
Organic Acids (urine)

ANNUAL REPORT 2019

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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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*

A total of 128 datasets have been submitted, for 3 of them an annual report could not be generated due to insufficient data submission.
4 laboratories did not submit results at all.

3. *Design*

The Scheme has been designed, planned and coordinated by Mme Clothilde Roux 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. As a subcontractor of ERNDIM, SKML prepare and dispatch EQA samples to the scheme participants and provide a website for on-line submission of results and access to scheme reports.

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 2-ketoglutaric acid, methylmalonic acid, 3-hydroxy-butyric acid and glycolic acid. Samples have been tested for stability and homogeneity according to ISO 13528.

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

Analyte	Source	Added to Pair 2019. 01 - 05	Added to Pair 2019. 02 - 08	Added to Pair 2019. 03 - 07	Added to Pair 2019. 04 - 06
2 methylcitric acid	CDN Isotopes X-4176	30,2	50,3	0,0	10,1
2-OH-glutarate	Sigma H8378	450,0	0,0	100,0	30,1
3 methylglutaconic acid	Sigma 06689	149,9	60,0	10,0	0,0
3-Methylglutarate	Aldrich M47604	0,0	79,9	20,1	150,0
3-OH-3 methylglutarate	Aldrich H4392	0,0	260,0	150,0	29,9
3-OH-Butyric acid	Aldrich 298360	500,0	230,0	0,0	50,1
3-OH-Glutaric acid	BioConnect SC-209609	3,0	0,0	20,0	5,0
3-OH-Isovalerate	Vumc*	150,3	15,9	41,0	0,0
4-OH-Butyrate	Brunet	0,0	15,0	250,2	100,1
Adipate	Sigma A26357	230,0	0,0	29,9	90,0
Ethylmalonate	TRC E922020	74,9	150,0	10,1	0,0
Fumarate	TRC F500380	160,0	50,0	20,1	0,0
Glutarate	Sigma G3407	50,0	0,0	100,0	300,0
Glycolate	Sigma G8284	0,0	99,8	299,8	499,8
Hexanoylglycine	Vumc*	20,1	0,0	9,9	5,0
Isovalerylglycine	Vumc*	20,0	5,0	0,0	15,0
2-Ketoglutarate	Sigma K2000	0,0	400,0	600,1	30,0
Methylmalonate	Aldrich M54058	100,3	10,0	0,0	500,0
Mevalonate	Sigma M4667	100,1	10,1	50,0	0,0
N-acetylaspartic acid	Sigma A5625	50,0	0,0	299,9	10,0
Pyroglutamate	Aldrich 83160	320,1	159,9	80,1	0,0
Sebacate	Aldrich 84809	5,0	40,2	0,0	150,0
Suberate	Aldrich S5200	150,0	0,0	40,0	10,1
Tiglylglycine	Vumc*	10,0	300,0	0,0	125,0
Vanillacetate	TCI H0538	0,0	4,0	60,0	10,0

***** *Supplied by University of Amsterdam*

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 (200 such

Analyte-in-Detail-reports could be consulted in the 2019 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 2019). 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 224 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 2019

Subsequently we present accuracy, recovery, precision, linearity, interlab CV and cross sectional relations. Creatinine has been excluded from the annual report because this analyte is not spiked (thus same concentration in all 8 samples); without spiking it is not possible to calculate recovery and linearity. 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 154 micromol/L.

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 was acceptable (80% < recovery < 120%)) for 19/25 analytes. Six analytes showing low median recoveries in the 2019 scheme: 3-OH-glutaric acid (59%), 3-OH-isovaleric acid (77%), Hexanoylglycine (68%), Isovalerylglycine (79%), Mevalonic acid (71%) and N-acetylaspartic acid (78%).
With the exception of for 3-OH-glutaric acid, the recovery of the others 5 compounds has decreased compared to previous years.

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. We strongly recommend that you revise the calibrations of analytes that show a clearly lower recovery in your lab as compared to the median of all labs. One pragmatic option for improved harmonization across diagnostic labs, is to use the residual samples of the previous ERNDIM EQA Scheme for Quantitative Organic Acids as calibrators, taking either added amounts (Table 1) or the median value reported by all labs (Annual Report, www.erndimqa.nl) as indicator of trueness/accuracy. The difficulties we face are certainly a challenge for developing improved methods.

4.3 Precision (intra-lab CV)

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”.

High imprecisions 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. 2-methylcitric acid, 3-OH-glutaric acid, 3-OH-isovaleric acid, Hexanoylglycine isovalerylglycine, Keto-glutaric acid, Mevalonic acid, N-acetylaspartic acid, Tiglylglycine and Vanillactic acid. Rigorous standardization of the extraction parameters, i.e. pH of the sample, exact volumes of extraction solvents and carefully controlled timings of various steps (evaporation of solvents, oximations,…) may be a way to improve this aspect of performance.

4.4 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 (128) whereas only 52 participated for vanillactic acid.

4.5 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 0.645 for 3-OH Glutaric acid to 0.997 for methylmalonic acid. Overall reported linearity is excellent for all compounds, suggesting that the major source of inter-laboratory variations reside at the level of sample extraction/derivatisation rather than at the level of instrument calibration of mass spectrometers.

4.6 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.

4.7 Your performance: red and green flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags (different colours starting from last year) in case of poor performance for accuracy, precision, linearity and recovery. Organic acids with satisfactory performance for at least three of the four parameters (thus no or only one flag) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular organic acid. Criteria for flags can be found in the general information on the website (on this website under general information; interactive website, explanation annual report).

4.8 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 39% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 4% of laboratories with more than 25% 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%	4%	4%
25%	2%	6%
20 – 25%	6%	12%
15 – 20%	6%	18%
10 – 15%	5%	23%
5 – 10%	13%	36%
0 – 5%	25%	61%
0%	39%	100%

4.9 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.

4.10 Conclusions & Summary

The high overall inter-lab CV (46.7%) demonstrates clearly the major problem in the analysis of organic acids: lack of standardization. Precision with a mean CV of 21.2% 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.

4.11 Additional Specific Remarks of the Scientific Advisor

This year, 3-OH Butyric acid was added. 83 labs submitted results for this compound. The median CV for all labs for 3-OH Butyric acid was 21.8% (poor precision) with a median recovery for all labs of 93%. In addition, the interlab CV% was high (57.0%).

5 Preview Scheme 2020

Each year, the composition of the scheme is reviewed, and adapted, based on the feedback of the scheme participants, collected during our Users' survey, and technical feasibility. For the 2020 scheme we removed Glycolic acid and we added Suberylglycine and 3-OH-propionic acid.

6 Questions, Comments and Suggestions

If you have any questions, comments or suggestions, please address to the scientific advisor of the scheme Mme Clothilde Roux-Petronelli (clothilde.roux@chuv.ch) 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.

Lausanne,
20 January 2020

Mme Clothilde Roux-Petronelli
Scientific Advisor