

**ANNUAL REPORT 2024**

<b>Scheme Organiser</b>	<b>Scientific Advisor</b>	<b>Website for reporting results</b>	<b>Administration office</b>
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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](http://www.erndim.org) / [www.ERNDIMQA.nl](http://www.ERNDIMQA.nl)

**2. Participants**

A total of 140 datasets have been submitted, for 6 of them an annual report could not be generated due to insufficient data submission. 1 Laboratory 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. C.W. Weykamp as scheme organiser (on behalf of the MCA Laboratory), both appointed by and according to the procedures of the ERNDIM Trust Board. The design includes samples and reports to provide information with a balance between short-term and long term-reports and between detailed and aggregated information. As a subcontractor of ERNDIM, the MCA Laboratory prepares and distributes the 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-methylcitric acid, glutaric acid, 4-hydroxybutyric acid and pyroglutamic acid. Samples have been tested for stability and homogeneity according to ISO 13528.

<sup>1</sup> If this Annual Report is not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document

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

Analyte	Source	Added to Pair 2024. 01 - 07	Added to Pair 2024. 02 - 05	Added to Pair 2024. 03 - 06	Added to Pair 2024. 04 - 08
2 methylcitric acid	Bioconnect M265080	30.5	300.7	99.8	0.0
2-OH Glutaric acid	Sigma H8378	50.0	199.9	25.0	0.0
3 methylglutaconic acid	Sigma 06689	0.0	49.6	225.2	550.0
3 Methylglutaric acid	Sigma M47604	30.5	60.2	0.0	150.2
3-OH-3 methylglutaric acid	Sigma H4392	300.3	100.5	0.0	20.1
3-OH-Butyric acid	Sigma 298360	400.1	299.5	100.6	0.0
3-OH-Glutaric acid	Sigma 04725	150.1	0.0	99.9	50.2
3-OH-Isovaleric acid	Merck 55453	0.0	40.2	300.0	120.0
3-OH-Propionic acid	Sigma 792659	200.2	24.8	49.6	0.0
4-OH-Butyric acid	Brunet	124.6	0.0	249.5	20.1
Adipic acid	Sigma A26357	0.0	199.9	100.1	15.3
Ethylmalonic acid	TRC E922020	15.1	0.0	200.3	51.0
Fumaric acid	Sigma 47910	0.0	20.2	160.0	39.4
Glutaric acid	Sigma G3407	50.6	199.8	25.1	0.0
Hexanoylglycine	VUMC*	0.0	4.6	14.5	69.3
Isovalerylglycine	VUMC*	149.3	500.0	0.0	19.7
Keto-glutaric acid	Sigma K2000	299.7	50.3	0.0	49.6
Malic acid	Sigma M9138	199.9	0.0	120.4	10.6
Methylmalonic acid	Sigma M54058	24.7	0.0	400.1	999.6
Mevalonic acid	Sigma M4667	79.9	0.0	14.9	4.8
N-acetylaspartic acid	Sigma A5625	0.0	400.1	40.1	25.1
Pyroglutamic acid	Sigma 83160	50.5	99.6	0.0	404.6
Sebacic acid	Sigma 84809	40.1	20.1	59.9	0.0
Suberic acid	Sigma S5200	40.0	20.0	59.7	0.0
Suberylglycine	Sigma 52888	119.8	4.9	0.0	29.9
Tiglylglycine	Bioconnect T440100	0.0	30.2	299.9	10.2
Vanillactic acid	TCI H0538	30.0	0.0	20.3	4.9

\* Supplied by University of Amsterdam

## Reports

All data-transfer, the submission of data and the request and viewing of reports proceed via the interactive website [www.erndimqa.nl](http://www.erndimqa.nl) which can also be reached through the ERNDIM website ([www.erndim.org](http://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** on the individual specimens are available two weeks after the submission deadline and provide up-to-date information on analytical performance. Although it is technically possible to produce reports immediately there is a delay of 14 days to enable the scientific advisor to inspect the results and add comments to the report when appropriate. The **annual long-term report** is based on the design-anchored connection between samples which enables a range of analytical parameters (accuracy, precision, linearity, recovery and inter-lab dispersion) to be reported once the 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 (208 such Analyte-in-

Detail-reports could be consulted in the 2024 cycle). A more condensed report is the "Cycle Review" which summarises the performance of all analytes in a specific sample (8 such Cycle-Review-Reports were available in 2024). The highest degree of aggregation is the Annual Report which summarises 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 208 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 2024**

In this section the results of the annual report 2024 are summarised in terms of accuracy, precision, linearity, recovery, inter-laboratory co-efficient of variation (CV), and relations between these parameters. 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 keep at hand 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, you can contact your National Representative or 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 71.5 µmol/L.

It is important to recognise that using ERNDIM Quantitative EQA material to establish bias is potentially a limitation. The bias of the method has been determined by comparing results to a derivation of the ERNDIM all laboratory trimmed mean, not a true target value. As the materials produced by the scheme are not reference materials, the bias determined is not a measure of absolute accuracy and is simply a measure of performance relative to other laboratories.

##### **4.2 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. 3-OH-3-methylglutaric acid, 3-OH-isovaleric acid, and suberylglycine. Rigorous standardisation 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, etc) may be a way to improve this aspect of performance.

##### **4.3 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 0.970 for 3-OH-Glutaric acid to 0.996 for glutaric 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.

#### **4.4 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 ( $80\% < \text{recovery} < 120\%$ ) for 25/27 analytes. Two analytes shows a low median recovery in the 2024 scheme: 2-methylcitric acid (76%) and 3-OH-isovaleric acid (62%).

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 utilising 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 harmonisation 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.erndimga.nl](http://www.erndimga.nl)) as indicator of trueness/accuracy. The difficulties we face are certainly a challenge for developing improved methods.

#### **4.5 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 harmonisation 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 (135) whereas only 57 participated for vanillic acid.

#### **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: Flags**

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags (in different colours) 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. 40% of the laboratories have no 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% flags. However, it should be noted that not all laboratories return results for all analytes. Intensive discussion within the Scientific Advisory Board (SAB) resulted in a harmonised scoring scheme that has been in place for the quantitative schemes for more than ten years. Likewise, there has been agreement as to what constitutes satisfactory performance. Both parameters are checked annually and if necessary re-evaluated. 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.

If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office ([admin@erndim.org](mailto:admin@erndim.org)), with full details of the reason for your appeal, within one month receiving your Performance Support Letter. Details of how to appeal poor performance are included in the Performance Support Letter sent to poor performing laboratories.

Table 2. Percentage Red Flags

<b>% Red Flags seen in Annual Report</b>	<b>Percentage Labs In this Category</b>	<b>Cumulative Percentage Of Labs</b>
>25%	6%	6%
25%	2%	8%
20 – 25%	3%	11%
15 – 20%	5%	16%
10 – 15%	9%	25%
5 – 10%	13%	38%
0 – 5%	22%	60%
0%	40%	100%

#### 4.9 **Certificates**

Overall performance (as indicated by the red/green flags in each laboratories annual report) is summarised in the 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 should be viewed in conjunction with the individual annual report in the case of internal or external auditing.

#### 4.10 **Conclusions & Summary**

The high overall inter-lab CV (41.9%) demonstrates clearly the major problem in the analysis of organic acids: lack of standardisation. Precision with a mean CV of 18.5% 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 organiser, which may be used as calibrators, given that the weighed additions and the median calculated values are known.

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**

Malic acid was added for the first time in 2024. 58 labs submitted results for this compound. The median CV for all labs for Malic acid was 16.6% with a median recovery for all labs of 85% and an interlab CV% of 39.7%. These results are in line with the last results we obtained 9 years ago.

A notable decrease of the recovery has been observed this year for the 3-Hydroxyisovaleric acid (86% in 2023 and 62% in 2024) accompanied by an increase in the interlab CV% from 42.3% in 2023 to 55.10% in 2024.

High decrease for interlab CV% should be mentioned for 3-Hydroxybutyric acid, 4-Hydroxybutyric acid and 3-Hydroxyglutaric acid: from 51.8% in 2023 to 31.4% in 2024, from 51.5% in 2023 to 34.7% in 2024 and from 69.3% in 2023 to 52% in 2024, respectively. On the contrary, we observed an high increase for interlab CV% for the suberylglycine (from 49.7% to 81.2%).

#### 5 **Preview Scheme 2025**

Each year, the composition of the scheme is reviewed, and adapted, based on the feedback of the scheme participants collected during our participant survey, and technical feasibility. For the 2025 scheme we have added malonic 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. C.W. Weykamp ([mca.office@skbwinterswijk.nl](mailto:mca.office@skbwinterswijk.nl)).

Alternatively you may approach your local National Representative, a list of which is available from ERNDIM.

Lausanne, 15<sup>th</sup> of January 2025



Mme Clothilde Roux-Petronelli  
Scientific Advisor

Please note:

This annual report is intended for participants of the Organic Acids(urine) scheme. The contents should not be used for any publication without permission of the scheme advisor.

The fact that your laboratory participates in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will be shared within ERNDIM for the purpose of evaluating your laboratory performance, unless ERNDIM is required to disclose performance data by a relevant government agency. For details, please see the terms and conditions in the ERNDIM Privacy Policy on [www.erndim.org](http://www.erndim.org).

#### **APPENDIX 1. Change log (changes since the last version)**

Version Number	Published	Amendments
1	20 <sup>th</sup> January 2025	<ul style="list-style-type: none"><li>2024 annual report published</li></ul>

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