ERNDIM
Quantitative Schemes
Purines and Pyrimidines (urine)

ANNUAL REPORT 2024

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1. Purpose

The purpose of the ERNDIM External Quality Assurance Scheme for Quantitative Purines and Pyrimidines in Urine is the monitoring of the analytical quality of the quantitative assay of a range of analytes in urine in laboratories providing screening and diagnosis of patients with inherited metabolic disorders. For details see www.erndim.org / www.ERNDIMQA.nl

2. Participants

A total of 52 datasets have been submitted. No annual report could be generated for two laboratories because no results were submitted

3. Design

The scheme has been designed, planned and coordinated by the scientific advisor, Dr. Jörgen Bierau, and Dr. C.W. Weykamp as scheme organiser (on behalf of the MCA Laboratory), both appointed by and according to the procedure of the ERNDIM 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 sub-contractor of ERNDIM, the MCA Laboratory prepares and distributes the EQA samples and provides a website for on-line submission of results and access to scheme reports.

[1] 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.

Samples

Each year, eight lyophilised urine samples, comprising four identical pairs, are distributed to participants. All samples are prepared from the same native urine sample and were enriched with varying amounts of the analytes. Thus, the final concentration of each sample is the physiological concentration (for many purines and pyrimidines practically zero) plus the spiked amount. The compounds, their source and the amounts added are listed in the table below. Thus, the final concentration of each sample is the remaining physiological concentration plus the spiked amount.

Analyte	Source	Added quantities in µmol/liter			
		Sample pair 2024. 01 & 08	Sample pair 2024. 02 & 05	Sample pair 2024. 03 & 07	Sample pair 2024. 04 & 06
3-Ureidoisobutyric acid	Sigma 74005	51.4	10.8	0.0	35.1
3-Ureidopropionic acid	Sigma 94295	501.6	0.0	748.9	149.1
5-OH methyluracil	Sigma 852589	16.3	50.8	35.9	0.0
Adenine	Sigma A8751	0.0	59.5	19.8	79.8
Adenosine	Sigma A9251	20.2	74.9	50.1	0.0
AICAR	Sigma A9978	34.9	0.0	50.1	10.4
Cytidine	Sigma C122106	0.0	50.0	25.0	99.9
Deoxy-adenosine	Sigma D7400	49.7	15.5	0.0	25.7
Deoxy-guanosine	Sigma D7145	0.0	24.7	10.1	50.2
Deoxy-inosine	Sigma D5287	25.1	75.2	50.1	0.0
Deoxy-uridine	Sigma D5412	24.4	0.0	50.6	10.2
Dihydro-thymine	TRC D449440	0.0	50.1	25.6	100.6
Dihydro-uracil	Sigma D7628	149.2	49.0	0.0	74.8
Guanosine	Sigma G6752	25.4	0.0	49.8	10.5
Hypoxanthine	Sigma H9377	300.3	50.6	0.0	150.4
Inosine	Sigma I4125	0.0	49.9	15.4	75.3
Orotic Acid	Sigma O2875	150.6	50.6	0.0	100.2
Orotidine	Bio Connect SC-222103	25.3	0.0	0.0	10.1
Pseudo-uridine	Berry &Ass.11080	0.0	50.0	15.2	125.4
Succinyladenosine	Bio Connect S688825	0.0	20.3	10.1	29.8
Thymidine	Sigma T9250	50.1	249.8	100.7	0.0
Thymine	Sigma T0376	74.8	0.0	100.5	24.9
Uracil	Sigma U0750	199.8	26.7	0.0	75.9
Uridine	Sigma U3750	49.6	250.0	99.6	0.0
Xanthine	Sigma X4002	75.3	249.5	150.1	0.0

Reports

All data-transfer, the submission of data and the request and viewing of reports, proceed 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.

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.

A second important characteristic of the website is the different levels of detail of results which allows individual laboratories the choice of fully detailed and/or summarised 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 can be requested in the 2024 cycle for the added analytes, as well as an additional 16 for creatinine and uric acid). A more condensed report is the "Cycle Review" which summarises the performance of all analytes in a specific sample (16 such Cycle-Review-Reports can be requested in 2024). The most comprehensive report is the Annual Report which summarises the performance of all analytes in each of the four pairs of samples. One such Annual-Report can be requested in 2024.

4. Discussion of Results in the 2024 Annual Report

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. Please keep at hand your annual report from the Interactive Website when you read the "guided tour" below and keep in mind that we only discuss the results of "all labs": it is up to you to inspect and interpret the specific results of your laboratory.

4.1 Accuracy

A first approach to describe accuracy is to compare the mean outcome of the eight samples from your lab with the mean outcome from all labs. This is done in the first column of the annual report. For example, it can be seen that the mean of all laboratories for adenine is 37.8 µmol/litre.

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

Reproducibility is an important parameter for quality in the laboratory and is encountered in the schemes' design. Samples are prepared in pairs and each pair can be regarded as duplicates from which CV's can be calculated (intra-laboratory CV as an indicator of reproducibility). Outcome for your lab in comparison to the median of all labs is shown in column "Precision" of the Annual Report. The all laboratory mean imprecision ranges from 3.4% for cytidine to 19.5% for orotidine. The overall intra-laboratory CV is 7.9%.

4.3 Linearity

Linearity over the analytical range is another important parameter for analytical quality. Again, this is encountered in the Schemes' design. With weighed quantities on the x-axis and your measured quantities on the y-axis the coefficient of regression (r) has been calculated. Outcome for your lab in comparison to the median of all labs is in the column "Linearity" of the annual report. It can be seen that the coefficient of regression ranges from 0.986 for orotidine to 0.998 for AICAr and deoxy-uridine.

4.4 Recovery

A second approach to describe accuracy is the percentage recovery of added analyte. In this approach, recovery is measured relative to the weighed amount of analyte used

to enrich the sample. The correlation between the weighed quantities added to the samples (on the x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the correlation multiplied by 100% is your recovery of the added amounts. Outcome for your laboratory in comparison to median outcome of all laboratories is shown in the column “Recovery” in the Annual Report. The all laboratory mean recovery ranges from 54% for orotidine to 106% for deoxy-adenosine and succinyl adenosine. The overall recovery is 96%.

4.5 Inter-lab CV

For comparison of outcome for one patient in different hospitals and for use of shared reference intervals and/or the use of consensus target treatment ranges (less relevant for purines and pyrimidines) it is important to have a high degree of inter-laboratory harmonisation. Part of the schemes’ design is to monitor this by calculating the inter-laboratory CV. This, along with the number of laboratories who submitted results, is shown in the column “Data all Labs” in the Annual Report.. Most laboratories submitted results for xanthine (47), whereas only 12 laboratories measured 3-ureidoisobutyric acid. The inter-laboratory CV ranges from 9.06% for xanthine to 44.1% for orotidine. The mean inter-laboratory CV for all analytes is 14.8%.

4.6 Cross Sectional Relations

The various parameters as described above often have an inter-relation: more than one parameter directs towards good or poor analytical performance.

For example, for xanthine all parameters indicate good performance: precision (CV = 4.5%), linearity (r = 0.997), recovery (96%) and inter-lab variation (inter-lab CV 9.06%) with the majority of laboratories (n=47 datasets) submitting results.
Conversely, for orotidine most parameters indicate that good performance is challenging: precision (CV = 19.5%), linearity (r = 0.986), recovery (54 %) and inter-lab variation (inter-lab CV 44.1%) with the half of laboratories (n=26 datasets) submitting results.

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 case of poor performance for accuracy, precision, linearity and recovery. Analytes 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 analyte. 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

It is evident that there is considerable variation in the overall performance of individual laboratories. Table 2 shows the percentage of flags observed. 18% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme, 6% of laboratories have more than 25% red 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 advice of failure 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 and the participating laboratory in order to solve any

particular analytical problems in order to improve quality of performance of labs in the pursuit of our overall aim to improve quality of diagnostic services in this field.

If your laboratory is assigned poor performance and you wish to appeal against this classification, please email the ERNDIM Administration Office (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 flags

% Red flags seen in annual report	Percentage labs in this category	Cumulative percentage of labs
>25%	6%	6%
25%	2%	8%
20 – 25%	4%	12%
15 – 20%	4%	16%
10 – 15%	20%	36%
5 – 10%	12%	48%
0 – 5%	34%	82%
0%	18%	100%

4.9 Certificates

Overall performance (as is indicated by red/green flags in each laboratory's annual report) is summarised in the annual participation certificate. The certificate lists the total number of special assays 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 Additional Specific Remarks of the Scientific Advisor

We aim to include as many purines and pyrimidines as possible. However, this is limited by availability, cost and solubility. This is why certain analytes are included at low concentration levels or not included at all.
The inclusion of 2,8-dihydroxyadenine proved to be impossible as was not possible to make prepare solution from the solid compound.

5. Summary

The ERNDIM External Quality Assurance Scheme for Quantitative Purines and Pyrimidines in Urine monitors the analytical performance of laboratories involved in the screening and diagnosis of patients with inherited metabolic disorders. During the first 10 years of the scheme, the inter-laboratory CV decreased significantly. In recent years, it has gradually stabilised at 20%, and this year it has even gone down to 14.8%. This demonstrates the educational relevance of the scheme.
Notwithstanding the success of the scheme, each participant should carefully evaluate, adjust and revalidate any analytical method that is not performing at a satisfactory standard. Satisfactory performance is defined as precision CV <10%, linearity r>0.99. If this cannot be achieved, an alternative method should be considered.

The Annual Report deals with analytical performance in terms of accuracy, precision, linearity, recovery and inter-lab CV. All parameters (intra-laboratory CV, linearity, recovery, inter-laboratory CV and the number of participating laboratories) are broadly comparable to that seen in 2023. Intra-laboratory variation was acceptable for all analytes; mean variance 7.9%, range 4.5 – 19.5%. Inter-laboratory imprecision highlights the ongoing challenges associated with the measurement of some of these

analytes; mean inter-laboratory variation 14.8%, ranges from 9.06% for xanthine, to 44.1 for orotidine.

6. *Preview 2025 Scheme*

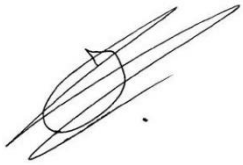
The design of the scheme in 2025 will be the same as the 2024 scheme.

As an alternative to the above mentioned 'problematic compounds', we would like to distribute educational samples of patients with 2,8-dihydroxyadeninuria (Adenine Phosphoribosyl Transferase (APRT) deficiency), Adenylosuccinate lyase (ADSL) deficiency and AICAribosiduria (ATIC deficiency).
To prepare, test and distribute lyophilised samples, we need at least 300 mL with a creatinine concentration of 1 mM or higher. Please contact the scientific advisor of you are willing to contribute a sample. In case of APRT deficiency it is important that the sample has been collected prior to treatment with allopurinol, as this, of course, effectively inhibits xanthine oxidase and prevents the formation of 2,8-dihydroxyadenine. We also do not know for certain yet if solubility of 2,8-dihydroxyadenine in lyophilised urine differs from the pure solid compound. It may be that for these special samples, we may have to distribute liquid samples. We welcome any samples, tips and advice.

7. Questions. Remarks. Suggestions

If you have any questions, comments or suggestions please address to the scientific advisor of the scheme Dr Jörgen Bierau (jorgen.bierau@mumc.nl) and/or to the scheme organiser D. C.W. Weykamp (mca.office@skbwinterswijk.nl).

Rotterdam, 14 January 2025



Dr. J. Bierau
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Purines & Pyrimidines in Urine scheme. The content may not be used for any publication without permission of the scheme advisor.

The fact that your laboratory takes part in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will be shared only within ERNDIM to evaluate your laboratory's 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.

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

Version Number	Published	Amendments
1	20th January 2025	• 2024 annual report published

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