ERNDIM
Quantitative Schemes
Purines and Pyrimidines (urine)

ANNUAL REPORT 2023

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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1. *Purpose*

The ERNDIM External Quality Assurance Scheme for Quantitative Purines and Pyrimidines in Urine monitors the analytical performance of laboratories providing screening and diagnosis of patients with inherited metabolic disorders. For details see www.erndim.org / www.ERNDIMQA.nl

2. *Participants*

Fifty-three laboratories ordered 53 sets of samples and submitted 51 sets of data. For two laboratories it was not possible to prepare an annual report due to the insufficient number of data submitted.

3. *Design*

The ERNDIM Board has appointed Dr Jörgen Bierau as Scientific Advisor and Dr C. W. Weykamp as Scheme Organiser (on behalf of MCA Laboratory) to design, plan and co-ordinate the scheme in accordance with ERNDIM procedures.
The scheme provides both short and long term information on analytical performance and detailed reports with overall performance assessments. Reports are available shortly after each term and an annual report is issued at the completion of the scheme. The MCA laboratory is subcontracted by ERNDIM to prepare and dispatch EQA samples and to host a website for online 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

The scheme works with four pairs of lyophilised samples. The compounds included in the scheme are added at 3 levels and at basal levels, resulting in 4 concentration levels. All samples are prepared from the same native urine sample. The compounds, their source and the amounts added are listed in the table below. The compounds reported are referred to as analytes. The samples were tested for stability and homogeneity according to ISO 13528.

Analyte	Source	Added quantities in µmol/liter			
		Sample pair 2023. 01 - 06	Sample pair 2023. 02 - 08	Sample pair 2023. 03 - 05	Sample pair 2023. 04 - 07
3-Ureidoisobutyric acid	Sigma 74005	11,2	49,3	36,1	0,0
3-Ureidopropionic acid	Sigma 94295	0,0	500,6	149,3	749,1
5-OH methyluracil	Aldrich 852589	49,6	0,0	76,8	26,5
Adenine	Sigma A8751	25,5	75,1	51,0	0,0
Adenosine	Sigma A9251	74,3	0,0	150,7	24,6
AICAR	Sigma A9978	49,9	11,3	0,0	26,4
Cytidine	Sigma C122106	101,0	26,0	0,0	51,0
Deoxy-adenosine	Sigma D7400	0,0	25,1	16,1	74,2
Deoxy-guanosine	Sigma D7145	11,1	50,3	24,7	0,0
Deoxy-inosine	Sigma D5287	25,1	0,0	51,1	10,6
Deoxy-uridine	Sigma D5412	50,6	10,1	0,0	24,6
Dihydro-thymine	TRC D449440	51,2	126,0	75,7	0,0
Dihydro-uracil	Sigma D7628	0,0	76,7	49,0	149,2
Guanosine	Sigma G6752	49,8	10,3	0,0	24,5
Hypoxanthine	Sigma H9377	0,0	150,1	75,0	251,9
Inosine	Sigma I4125	26,3	100,6	50,8	0,0
Orotic Acid	Sigma O2875	0,0	50,3	15,8	76,1
Orotidine	SC-222103	39,7	10,1	0,0	20,2
Pseudo-uridine	Berry &Ass PYA 11080	24,2	150,6	74,5	0,0
Succinyladenosine	Carbosynth NS16562	20,3	40,0	0,0	0,0
Thymidine	Sigma T9250	76,3	0,0	100,4	25,1
Thymine	Sigma T0376	124,6	25,6	0,0	75,4
Uracil	Sigma U0750	0,0	75,7	49,5	175,7
Uridine	Sigma U3750	49,8	0,0	100,6	24,9
Xanthine	Sigma X4002	102,3	0,0	150,3	51,2

Reports

All data submissions, report consultations, and requests are made via the the interactive website www.erndimqa.nl, which is also accessible through the ERNDIM website (www.erndim.org).

Your laboratory's results are confidential and can only be accessed in a password-protected area. The anonymised mean results for all laboratories are available to all participants. An explanation of the statistics behind the reports can be found in the general information section of the website.

An important feature of the website is that it provides both short-term and long-term reports. Short-term reports accompany the 8 individual samples, each with its own deadline in 2023. Two weeks after the respective deadlines, sample reports were available to participants, providing up-to-date information on analytical performance

on 8 occasions. A delay of 14 days was chosen to allow the scientific advisor to review the results and add optional comments.

The annual report is the long-term counterpart to the sample reports. It is only after the completion of an annual cycle that the analytical parameters (accuracy, precision, linearity, recovery and interlaboratory variation) monitored by the scheme can be analysed and reported. The annual report is discussed below.

The second important feature of the website is the aggregation of results, allowing the participant to choose between detailed reports or an overview of overall performance. "Analyte in Detail" is the most detailed report available as it shows the result of a specific analyte in a sample. There are 216 such reports available in the 2023 scheme. "Current Report" is a more condensed report summarising the performance of all analytes in a particular sample. Eight such reports are available. The 'Annual Report' is a single report summarising the performance of all eight samples. Depending on the level of detail you require, you can choose to review only the Annual Report or delve into the detailed reports.

4. Discussion of Results in the 2023 Annual Report

In this part, we discuss information that the 2023 annual report provides, and regard accuracy, precision, linearity, recovery, inter-laboratory CV and cross-sectional relations. Creatinine and Uric Acid are not included in the annual report because these analytes have not been added. Please keep your annual report at hand when you go through the “guided tour” below. Do remember we only discuss the results of “all labs”; it is up to you to inspect and interpret the results of your own laboratory.

4.1 Accuracy

A first approach to assessing accuracy is to compare your average result over 8 samples with the mean of all laboratories. This is shown in the 'Your lab' and 'All labs' columns under the 'Accuracy' heading. For example, the mean of all laboratories for adenine is 36.0 µmol/litre. You can compare your laboratory's mean to this collective mean.

4.2 Precision

Reproducibility is an important quality parameter and the scheme is designed to assess the intra-laboratory coefficient of variation (CV) as an indicator. The sample pairs can be considered as duplicates and used to calculate intra-laboratory CVs. The precision column shows how your results compare to the mean of all participants. The precision ranges from 4.7% for cytidine to 16.6% for 3-ureidoisobutyric acid. The overall intra-laboratory CV is 8.9%.

4.3 Linearity

Linearity over the relevant analytical range is another important parameter for analytical quality. This is also included in the scheme design. With the weighted quantities on the x-axis and your measured quantities on the y-axis, the coefficient of regression (r) is calculated. In the linearity column you can see how your results compare with the mean of all participants. The mean r ranges from 0.978 for orotidine to 0.997 for adenosine, cytidine, deoxy-guanosine, guanosine and uracil.

4.4 Recovery

A second way of assessing accuracy is to determine recovery. Recovery is the amount of analyte measured relative to the amount of the same analyte added. This approach assumes that the weighted amount is the target amount. The correlation

between the weighted amounts added (on the x-axis) and your measured amounts (on the y-axis) has been calculated. The slope of the correlation curve multiplied by 100% is your recovery. In the Recovery column you can see how your results compare to the average of all participants. The mean recovery ranges from 85% for 3-ureidopropionic acid and adenine to 108% for deoxyguanosine. The overall recovery is 97%.

4.5 Inter-lab CV

A high degree of harmonisation between analytical results from different laboratories is very important for patient care and the use of common reference values. It should be irrelevant in which laboratory the analytical results were obtained. The scheme is also designed to monitor inter-laboratory CV. The column "Data All Labs" shows the number of participants who submitted results per analyte. Most laboratories submitted results for xanthine (52), whereas only 9 laboratories measured 3-ureidoisobutyric acid. The inter-laboratory CV ranges from 10.1% for hypoxanthine to 28.3% for orotidine. The mean inter-laboratory CV for all analytes is 15.3%.

4.6 Cross Sectional Relations

The various parameters discussed above are often interrelated. Often several parameters point to good or poor analytical control.
This pattern, which is clearly visible in the other ERNDIM schemes, is less pronounced in the Quantitative Purines and Pyrimidines scheme.

4.8 Your performance: Flags

The Annual Report includes flags to help you easily assess your performance. The flags have different colours to indicate poor performance for accuracy, precision, linearity and recovery. Compounds with satisfactory performance in at least 3 out of 4 parameters (no or one flag) are marked with a green flag. Thus, a green flag indicates satisfactory analytical performance. The criteria for flagging can be found in the General Information section of the website.

4.9 Poor Performance Policy

There is a wide variation in the overall performance of individual laboratories. Table 2 shows the percentage of flags scored. 18% of the laboratories have no flags at all and have achieved excellent overall performance. At the other extreme, 2% of the participants scored more than 25% of flags.

After careful consideration, the ERNDIM Board and the Scientific Advisory Board (SAB) agreed on a harmonised scoring system for the various diagnostic proficiency and quality schemes. We tested a scoring system for the quantitative schemes as described in our Spring 2009 newsletter. In line with this, the SAB has agreed levels of adequate performance for all schemes which will be assessed annually. After careful consideration by the members of the SAB, we have applied scoring systems to our schemes since 2007. As decided by the ERNDIM Board, the Scientific Advisor assesses the performance of each participant against the agreed level of satisfactory performance. The laboratories that do not meet the criteria are sent a letter of advice. The purpose of this letter is to initiate a dialogue between the Scientific Advisor and the participant to resolve any analytical problems and improve performance. It is offered in the spirit of ERNDIM's objective to harmonise and improve the quality of diagnostic services for inborn errors of metabolism.

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%	2%	2%
25%	2%	4%
20 – 25%	6%	10%
15 – 20%	14%	24%
10 – 15%	12%	36%
5 – 10%	21%	57%
0 – 5%	25%	82%
0%	18%	100%

4.10 Certificates

Your annual ERNDIM certificate of participation will show the programmes to which you have subscribed. For this scheme, it shows the number of purines and pyrimidines included in the scheme, the number for which you have submitted results and the number for which you have achieved satisfactory performance. Remember that the certificate of participation must be accompanied by your annual report for the scheme in the event of an audit.

4.11 Additional Specific Remarks of the Scientific Advisor

We aim to include as many purines and pyrimidines as possible, but this is limited by availability, cost and solubility. For this reason, some analytes are included at two levels or not included at all. The inclusion of 2,8-dihydroxyadenine is not feasible. This compound has not been found to be soluble in its solid state.

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 been reduced to 15.3%. The educational relevance of the scheme is demonstrated 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.

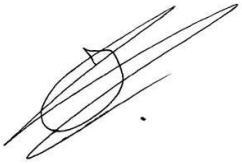
6. *Preview 2024 Scheme*

The design of the scheme in 2024 will be essentially the same as the 2023 scheme.

7. Questions. Remarks. Suggestions

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

Rotterdam, 10 January 2024



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	11 January 2024	• 2023 annual report published

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