ERNDIM
Quantitative Schemes
Purines and Pyrimidines (urine)

ANNUAL REPORT 2021

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 for laboratories providing screening and diagnosis of patients with inherited metabolic disorders.For details see www.erndim.org / www.ERNDIMQA.nl

2. Participants

Fifty laboratories ordered 50 sample sets and submitted 50 datasets. For one of them an annual report could not be generated due to insufficient data submission.

3. Design

The ERNDIM board has appointed Dr Jörgen Bierau as scientific advisor and Dr Eline van der Hagen as scheme organiser (on behalf of MCA Laboratory) to design, plan and co-ordinate the scheme adhering to ERNDIM procedures.
The scheme provides both short- and long-term information on analytical performance, and detailed reporting with overall performance assessments. Reports are available shortly after each term, and an annual report is issued after completion of the scheme. The MCA Laboratory is subcontracted by ERNDIM to prepare and dispatch EQA samples, and to host a website for online result submission and access to scheme reports.

[1] If these scheme instructions are 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 level, giving 4 concentration levels. All samples are prepared from the same native urine sample. The compounds, their source and added amounts are listed in the table below. The reported compounds are referred to as analytes. Samples have been tested for stability and homogeneity according to ISO 13528.

Analyte	Source	Added quantities in µmol/liter			
		Sample pair 2021. 01 - 08	Sample pair 2021. 02 - 07	Sample pair 2021. 03 - 05	Sample pair 2021. 04 - 06
5-OH methyluracil	Aldrich 852589	25,7	75,3	49,6	0,0
Adenine	Sigma A8751	0,0	49,6	25,5	100,6
Adenosine	Sigma A9251	24,6	100,1	50,0	0,0
AICAR	Sigma A9978	25,4	0,0	50,8	10,4
Cytidine	Sigma C122106	50,0	0,0	100,9	26,0
Deoxy-adenosine	Sigma D7400	49,6	9,9	0,0	25,3
Deoxy-guanosine	Sigma D7145	0,0	25,6	10,2	51,1
Deoxy-inosine	Sigma D5287	9,6	50,1	26,0	0,0
Deoxy-uridine	Sigma D5412	24,5	0,0	50,1	9,6
Dihydro-thymine	TRC D449440	0,0	75,9	24,7	100,6
Dihydro-uracil	Sigma D7628	102,2	27,7	0,0	76,7
Guanosine	Sigma G6752	24,9	0,0	49,7	10,3
Hypoxanthine	Sigma H9377	150,0	25,0	0,0	76,8
Inosine	Sigma I4125	0,0	75,2	25,4	150,4
Orotic Acid	Sigma O2875	150,2	25,0	0,0	75,1
Orotidine	SC-222103	20,2	0,0	39,6	10,1
Pseudo-uridine	Berry &Ass PYA 11080	0,0	49,8	24,9	99,5
Succinyladenosine	Carbosynth NS16562	0,0	0,0	20,3	39,0
Thymidine	Sigma T9250	10,0	50,2	26,1	0,0
Thymine	Sigma T0376	75,2	0,0	150,3	25,1
Uracil	Sigma U0750	149,6	49,9	0,0	99,7
Uridine	Sigma U3750	0,0	0,0	0,0	0,0
Xanthine	Sigma X4002	24,9	100,5	50,8	0,0

Reports

All data submission, report viewing, and requests proceed through the interactive website www.erndimqa.nl also accessible through the ERNDIM website (www.erndim.org).

Your laboratory's results are confidential and only accessible in a password-protected area. The anonymised mean results of all labs are accessible to all participants. An explanation of the statistics behind the reports is provided in the general information section of the website.

An important characteristic of the website is that it supplies short-term and long-term reports. Short-term reports accompany the 8 individual samples, each with their own deadline in 2020. Two weeks after the respective deadlines participants sample reports were available, providing up-to-date information on analytical performance on 8 occasions. A delay of 14 days has been chosen to allow the scientific advisor to inspect the results and add optional comments.

The annual report is the long-term counterpart of the sample reports. It is only after completion of an annual cycle that the analytical parameters (accuracy, precision, linearity, recovery, and inter-laboratory dispersion) the scheme monitors can be analysed and reported. We discuss the annual report below.

The second important feature of the website is the aggregation of results allowing the participant to choose to view detailed reports or rather an overview of overall performance. “Analyte-in-Detail” is the most detailed report available as it shows the result of one specific analyte in one sample. In the 2020 scheme, 168 such reports are available. “Current Report” is a more condensed report summarising performance for all analytes in a specific sample. There are eight such reports available. The “Annual Report” is a single report that summarises performance over all eight samples. Depending on the level of detail you require, you can choose to only check the annual report or delve into detailed reports.

4. Discussion of Results in the 2021 Annual Report

In this part, we discuss information that the 2021 annual report provides, and regard accuracy, recovery, precision, linearity, 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 assess accuracy is to compare your mean outcome over 8 samples with the mean of all labs. This is shown in the columns "your lab" and "all labs" under the heading "Accuracy", respectively. For example, the mean of all labs for adenine is 38.3 µmol/liter. You can compare the mean of your lab with this collective mean.

4.2 Recovery

A second way to assess accuracy is to determine recovery. Recovery is the amount of analyte measured relative to the amount of that same analyte added. In this approach, the assumption is made that the weighted quantity is the target value. The correlation between weighted quantities added (x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the correlation curve multiplied by 100% is your recovery. In the column “Recovery”, you can see how your results compare with the mean of all participants. Mean recovery ranges from 90% for 5-OH methyluracil and adenine to 101% for deoxy-inosine and succinyladenosine. The overall recovery is 97%.

4.3 Precision

Reproducibility is an important quality parameter, and the scheme is designed to assess intra laboratory coefficient of variation (CV) as an indicator. The sample pairs can be considered duplicates and used to calculate intra laboratory CVs. In the column “Precision”, you can see how your results compare with the mean of all participants. Precision ranges from 6.7% for xanthine to 17.4% for succinyladenosine. The overall intra-lab CV is 9.6%.

4.4 Linearity

Linearity over the entire relevant analytical range is another important parameter for analytical quality. Again, this is included in the scheme-design. With weighted quantities on the x-axis and your measured quantities on the y-axis, the coefficient of

regression (r) is calculated. In the column “Linearity”, you can see how your results compare with the mean of all participants. Mean-r ranges from 0.979 for orotidine to 0.997 for orotic acid, thymine and xanthine.

4.5 Inter-lab CV

A high degree of harmonisation between analytical results produced by multiple laboratories is very important for patient care and the use of shared reference values. It should be irrelevant in what laboratory analytical results were obtained. The scheme is also designed to monitor inter-laboratory CV. The column “Data All Labs” shows the number of participants that submitted results per analyte. Most laboratories submitted results for xanthine (47), whereas only 10 labs measured cytidine. The inter-lab CV ranges from 10.1% for pseudo-uridine to 36.2% for succinyladenosine. The mean inter-lab CV for all analytes is 16.3%.

4.6 Cross Sectional Relations

The various parameters discussed above often show an interrelation. Often multiple parameters direct towards good or poor analytical control.
This pattern, clearly seen in the other ERNDIM schemes is less prominent in the Quantitative Purines and Pyrimidines scheme.

4.8 Your performance: Flags

The annual report includes flags to help you easily assess your performance. Flags have different colours signifying poor performance for accuracy, precision, linearity, and recovery respectively. Compounds scoring a 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. You can find the criteria for flagging in the general information on the website.

4.9 Poor Performance Policy

There is a wide dispersion in the overall performance of individual laboratories. Table 2 shows the percentage of flags scored. 19% of the laboratories have no flags at all and have attained excellent overall performance. At the other extreme end, 6% of the participants scored over 25% flags.

After careful consideration, the ERNDIM board and Scientific Advisory Board (SAB) agreed on a harmonised scoring system for the various Diagnostic Proficiency and qualitative schemes. We have tested a scoring system for the quantitative schemes as described in our Newsletter of Spring 2009. In line with this, the SAB have agreed upon levels of adequate performance for all schemes and this is evaluated annually. After careful evaluation by members of the SAB, we have applied scoring systems to our schemes since 2007. As per the ERNDIM Board’s decision, the Scientific Advisor assesses the performance of the individual participants based on the agreed level of satisfactory performance. A letter of advice addressing the failure to achieve satisfactory performance is issued to the laboratories falling short of the criteria. This letter is intended to start a dialogue between the scientific advisor and the participant to help solve any analytical problems and to improve performance. It is offered in the spirit of ERNDIM’s goal to harmonise and improve the quality of diagnostic services of inborn errors of metabolism.

Table 2. Percentage flags

% Red flags seen in annual report	Percentage labs in this category	Cumulative percentage of labs
>25%	6%	6%
25%	4%	10%
20 – 25%	4%	14%
15 – 20%	10%	24%
10 – 15%	10%	34%
5 – 10%	14%	48%
0 – 5%	33%	81%
0%	19%	100%

4.10 Certificates

On your annual ERNDIM participation certificate, the schemes you subscribed to are stated. For this scheme, it states the number of purines and pyrimidines included in the scheme, the number for which you have submitted results and the number for which you obtained satisfactory performance. Remember that for an audit the participation certificate needs to be accompanied by your annual report for the scheme.

4.11 Additional Specific Remarks of the Scientific Advisor

This year, cytidine and uridine have been included in the scheme. Uridine and cytidine are excreted into the urine in elevated amounts in individuals with uridine-cytidine-uria (OMIM 618477) caused by homozygous or compound heterozygous mutations in the SLC28A1 gene (OMIM 606207). This gene encodes the concentrative nucleoside transporter-1 (CNT1). This inborn error of metabolism probably has a benign phenotype. It is important to recognize this condition and to discriminate it from other pyrimidinurias.

5. *Summary*

The ERNDIM External Quality Assurance Scheme for Quantitative Purines and Pyrimidines in Urine monitors the analytical performance for laboratories providing screening and diagnosis of patients with inherited metabolic disorders. Over the first 10 years of the scheme, the inter-laboratory CV has significantly decreased. It has been gradually levelling off towards 20% in recent years, and this year it is even below 20%. This success confirms the educational relevance of the scheme. Regardless of the success of the scheme, every participant should carefully evaluate, adjust and re-validate any analytical method falling short of satisfactory performance. Satisfactory performance means precision CV <10%, linearity r>0.99 and recovery 100% ± 10%. If this cannot be achieved, consider another method.

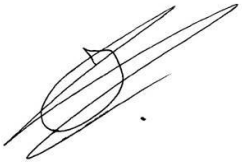
6. *Preview 2022 Scheme*

The design of the 2022 scheme will essentially remain the same as in 2021.

7. Questions. Remarks. Suggestions

If you have questions, remarks or suggestions please address them to the scientific advisor Dr Jörgen Bierau (jorgen.bierau@mumc.nl) or the scheme organiser D. Eline van der Hagen (E.vanderHagen@skbwinterswijk.nl).

Rotterdam, 22 December 2021



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	22 December 2021	• 2021 annual report published

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