ERNDIM - Quantitative Schemes
Purines & Pyrimidines

J. Bierau, Ph.D.
Maastricht University Medical Centre
Department Clinical Genetics
P.O. Box 5800
NL – 6202 AZ Maastricht
e-mail: jorgen.bierau@mumc.nl

Dr. C.W. Weykamp
Queen Beatrix Hospital
MCA Laboratory
P.O. Box 9005
NL – 7100 GG Winterswijk
e-mail : c.w.weykamp@skbwinterswijk.nl

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Annual Report ERNDIM-EQAS 2015

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 purines and pyrimidines in urine in laboratories involved in the screening and diagnosis of patients with inherited metabolic disorders. For details see www.erndim.org / www.ERNDIMQA.nl

2. Participants

53 Datasets from 22 countries have been submitted, for 3 of them an annual report could not be generated due to insufficient data submission.

3. Design

The Scheme has been designed, planned and co-ordinated by Dr. Jörgen Bierau as scientific advisor and Dr. Cas Weykamp as scheme organizer (subcontractor on behalf of SKML), both appointed by and according to the procedures of the ERNDIM 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.

Samples

The scheme consisted of 8 lyophilised samples, all prepared from the same basic urine but with various amounts of added analyte. The samples were identical two by two: the pairs, analytes and their source as well as the added amounts are in the table below. Samples have been tested for stability and homogeneity according to ISO 13528.

Analyte	Source	Added Quantities in micromol/liter			
		Sample Pair 2015. 01 - 06	Sample Pair 2015. 02 - 05	Sample Pair 2015. 03 - 08	Sample Pair 2015. 04 - 07
5-OH methyluracil	Aldrich 852589	50,6	100,2	0,0	75,7
Adenine	Sigma A8751	0,0	49,9	75,4	10,6
Adenosine	Sigma A9251	9,9	75,4	0,0	49,6
AICAR	RI. Chemicals A1300	50,0	0,0	10,1	150,1
Deoxy-adenosine	Sigma D7400	75,3	25,0	50,0	0,0
Deoxy-guanosine	Sigma D7145	0,0	49,6	75,1	24,9
Deoxy-inosine	Sigma D5287	24,9	75,3	0,0	49,8
Deoxy-uridine	Sigma D5412	49,8	0,0	25,2	100,2
Dihydro-thymine	Ikemi L01996	0,0	99,9	150,1	50,2
Dihydro-uracil	Sigma D7628	150,0	49,8	100,9	0,0
Guanosine	Sigma G6752	74,8	0,0	50,0	100,2
Hypoxanthine	Sigma H9377	249,8	50,1	149,6	0,0
Inosine	Sigma I4125	0,0	49,7	124,8	25,1
Orotic Acid	Sigma O2875	249,9	25,3	49,9	0,0
Orotidine	Sigma O9505	21,0	0,0	7,1	28,1
Pseudo-uridine	Berry &Ass PYA 11080	0,0	49,6	150,0	25,4
Thymidine	Sigma T9250	9,7	50,6	0,0	25,0
Thymine	Sigma T0376	50,4	0,0	24,6	100,9
Uracil	Sigma U0750	150,6	26,3	74,3	0,0
Xanthine	Sigma X4002	50,3	250,4	0,0	99,5

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.

An important characteristic of the website is that it supplies short-term and long-term reports. Short-term reports are associated with the eight individual specimens, for each of which there has been a specific deadline in the year 2015. Two weeks after the respective deadlines participants could request their reports and as such had eight times up-to-date information on their analytical performance. Although technically not required (the website can work with a delay time zero) a delay time of 14 days has been chosen to enable the scientific advisor to inspect the results and add his comment to the report. Contrary to the fast short-term report is the annual long-term report. The annual report is based on the design-anchored connection between samples which enables to report a range of analytical parameters (accuracy, precision, linearity, recovery and interlab dispersion) once an annual cycle has been completed. The annual report is discussed below.

A second important characteristic of the website is the wide range in aggregation of results which permits labs 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 (176 such Analyte-in-Detail-reports can be requested in the year 2015 cycle). A more

condensed report is the “Current Report” which summarizes the performance of all analytes in a specific sample (8 such Current Reports can be requested in 2015). The highest degree of aggregation has the Annual Report which summarizes the performance of all analytes of all 8 samples (1 such Annual-Report can be requested in 2015). Depending on their position in the laboratory one can choose to have a glance at only the annual report (managers) or at all 176 detailed reports (technicians).

4. Discussion of Results in the Annual Report 2015

In this part the results as seen in the annual report 2015 will be discussed. Subsequently we will regard accuracy, recovery, precision, linearity, interlab CV and crosssectional relations. Please print 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 interprete the specific results of your laboratory.

4.1 Accuracy

A first approach to describe the accuracy is comparison of your mean outcome in the eight samples with the mean of all labs. This is shown in the columns "your lab" and "all labs" under the heading "Accuracy", respectively. For Adenine the mean of all labs is 28.0 micromol/Liter with which you can compare the mean of your lab.

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 your measured quantities (on the y-axis) has been calculated. The slope of the correlation multiplied with 100% is your recovery of the added amounts. Outcome for your lab in comparison to median outcome of all labs is shown in the column “Recovery” in the annual report. For all labs the recovery ranges from 86% for 5-OH methyluracil to 111% for Thymidine. The overall recovery is 97%.

4.3 Precision

Reproducibility is an important parameter for quality in the laboratory and is encountered in the schemes’ design. Samples come in pairs which can be regarded as duplicates from which CV’s can be calculated (Intra Laboratory CV as indicator for reproducibility). Outcome for your lab in comparison to the median of all labs is shown in the column “Precision” of the Annual Report. Precision ranges from 4.9% for Uric acid to 13.7% for Orotidine. The overall intralab CV is 8.7%.

4.4 Linearity

Linearity over the whole relevant 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 0.936 for Thymidine to 0.999 for Orotic acid.

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 harmonization between results of various laboratories. Part of the schemes' design is to monitor this by calculating the Interlaboratory CV. This, along with the number of laboratories who submitted results, is shown in the column "Data All labs" in the Annual Report. It can be seen that most laboratories submitted results for xanthine (49) whereas only 17 labs assayed dihydro-thymine. The Interlab CV ranges from 8.21% for uric acid to 26.8% for Orotidine. The mean Interlab CV for all analytes is 16.7%.

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, clearly seen in the other ERNDIM schemes is less prominent in the Purines and Pyrimidines.

4.8 Your performance: red and green flags

After some years of discussion and planning a system to judge performance of individual laboratories is implemented starting from January 2009. In the annual report of an individual laboratory red flags indicate 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 red flag or no result) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular analyte while a red flag indicates that your laboratory has failed to attain satisfactory performance. Criteria for red flags can be found in the general information on the website (general information; interactive website, explanation annual report).

4.9 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 22% of the laboratories have no red 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% red flags. Following intensive discussion within the ERNDIM 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 Diagnostic Proficiency schemes and qualitative schemes. We have also tested a scoring system for 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 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.

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	4%	4%
20 – 25%	2%	6%
15 – 20%	8%	14%
10 – 15%	12%	26%
5 – 10%	22%	48%
0 – 5%	30%	78%
0%	22%	100%

4.10 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 purines and pyrimidines 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.

5. *Summary*

The purpose of the ERNDIM scheme for Purines and Pyrimidines is the monitoring of the analytical quality of the quantitative assay of these compounds in urine**.** The interlaboratory CV has gradually decreased throughout the years. It used to be 30 to 40% in the beginning but dropped below 20% in recent years. We see especially improvement for the dihydropyrimidines. The results confirm the educational relevance of the scheme: participation in the scheme is associated with harmonisation between laboratories.
Nevertheless, each participant should re-validate the analytical method for those compounds for which the various parameters are not acceptable (e.g. acceptable means: precision CV<10%, linearity r>0.99 and recovery 90 < rec % <110. In case these goals cannot be achieved with the present method another method should be considered.

6. *Preview Scheme 2016*

The design of the 2016 scheme is essentially the same as in 2015

7. Questions, Remarks, Suggestions

If you have any questions, remarks or suggestions please address to the scientific advisor Dr. Jörgen Bierau (jorgen.bierau@mumc.nl) or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).