ERNDIM - Quantitative Schemes
Purines & Pyrimidines

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Annual Report ERNDIM-EQAS 2009

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*

51 Datasets have been submitted by Laboratories from 14 countries.

3. *Design*

The Scheme has been designed, planned and co-ordinated by Dr. Jörgen Bierau as scientific advisor and Dr. Cas Weykamp as scheme organiser, both appointed by 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.

Analyte	Source	Added Quantities in micromol/liter			
		Sample Pair 141-146	Sample Pair 143-148	Sample Pair 144-147	Sample Pair 142-145
5-OH methyluracil	Aldrich 852589	72	24	0	120
Adenine	Sigma A8751	0	110	66	22
Adenosine	Sigma A9251	30	0	150	90
AICAR	RI. Chemicals A1300	100	60	20	0
Deoxy-adenosine	Sigma D7400	72	24	0	120
Deoxy-guanosine	Sigma D7145	0	380	228	76
Deoxy-inosine	Sigma D5287	0	163	98	33
Deoxy-uridine	Sigma D5412	90	30	0	150

Dihydro-thymine	Ikemi L01996	30	0	149	89
Dihydro-uracil	Sigma D7628	200	120	40	0
Guanosine	Sigma G6752	72	24	0	120
Hypoxanthine	Sigma H9377	400	240	80	0
Inosine	Sigma I4125	0	165	99	33
Orotic Acid	Sigma O2875	144	48	0	240
Orotidine	Sigma O9505	0	47	28	9
Pseudo-uridine	Berry &Ass 11080	30	0	150	90
Thymidine	Sigma T9250	400	240	80	0
Thymine	Sigma T0376	30	0	150	90
Uracil	Sigma U0750	50	0	250	150
Xanthine	Sigma X4002	300	180	60	0

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).

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 2009. 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 (168 such Analyte-in-Detail-reports can be requested in the year 2009 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 2009). 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 2009). Depending on their position in the laboratory one can choose to have a glance at only the annual report (managers) or at all 168 detailed reports (technicians).

4. Discussion of Results in the Annual Report 2009

In this part the results as seen in the annual report 2009 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 46.2 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 Dihydro-thymine to 102% for xanthine. The overall recovery is 96%.

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 3.0% for creatinine to 23.4% for dihydro-thymine. The overall intralab CV is 8.9%.

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 0.902 for dihydro-thymine to 0.999 for creatinine.

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 hypoxanthine (42) whereas only 9 labs assayed dihydro-thymine. The Interlab CV ranges from 5.89% for creatinine to 156% for dihydro-uracil. The mean Interlab CV for all analytes is 30.4%.

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. 17% 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 5% 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%	5%	5%
20 – 25%	5%	10%
15 – 20%	5%	15%
10 – 15%	7%	22%
5 – 10%	41%	63%
0 – 5%	20%	83%
0%	17%	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 amino 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 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 was the monitoring of the analytical quality of the quantitative assay of these compounds in urine**.** The

most dominating in the outcome is the huge Interlab Variation for all analytes except creatinine whereas precision, linearity and mean recovery are quite acceptable. 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.
The results seem to confirm the relevance of the scheme and an indication that improvement of standardization to achieve harmonisation between laboratories seems a major task associated with the organisation of this scheme.

6. *Preview Scheme 2010*

The design of the 2010 scheme is essentially the same as in 2009.

7. Questions, Remarks, Suggestions

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