ERNDIM - Quantitative Schemes
Purines & Pyrimidines

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Annual Report ERNDIM-EQAS 2007

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.erndimqa.nl

2. *Participants*

51 Laboratories subscribed and 45 laboratories from 14 countries submitted results.

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 (all Sigma)	Added Quantities in micromol/liter			
		Sample Pair 127-131	Sample Pair 125-130	Sample Pair 128-132	Sample Pair 126-129
Uracil	U0750	360	216	72	0
5-OH methyluracil	852589	81	49	16	0
Thymine	T0376	151	91	30	0
Dihydro-uracil	D7628	457	152	0	761
Uridine	U3750	47	16	0	78
Adenosine	A9251	55	18	0	92
Deoxy-adenosine	D7400	83	28	0	138

Orotidine	O9505	24	8	0	40
Hypoxanthine	H9377	44	0	220	132
Xanthine	X4002	53	0	267	160
Adenine	A8751	14	0	69	41
Thymidine	T9250	46	0	230	138
Orotic Acid	O2875	0	138	83	28
Inosine	I4125	0	92	55	18
Guanosine	G6752	0	74	44	15
Deoxy-inosine	D5287	0	138	83	28
Deoxy-guanosine	D7145	0	92	55	18
Deoxy-uridine	D5412	0	90	54	18

Reports

All data-transfer, the submission of data as well as the request of reports proceeded via the interactive website www.erndimqa.nl

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 2007. 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 2007 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 2007). 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 2007). 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 2007

In this part the results as seen in the annual report 2007 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 29.7 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 17% for dihydrouracil to 102% for deoxu-uridine. The overall recovery is 91% (95% without dihydrouracil). The quantification of dihydrouracil appears to be challenging. Only a few participants submitted results for this analyte. About half of the participants use GC techniques for the measurement of DHU and half of them use liquid chromatography using tandem mass spectrometry for detection and quantification. The reported values for DHU are judged against the added amount of DHU in the sample. It is striking that the results of LC-MS/MS users are almost invariably close to the target value, while the GC results are often disappointing. It appears that LC-MS/MS is a more suitable method for the quantification of DHU.

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 3.2% for creatinine to 13.7% for 5-OH-methyluracill. The overall intralab CV is 7.4%.

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 0.9733 for dihydro-uracil to 0.9986 for guanosine. The overall r is 0.9949.

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 (45) whereas only 14 labs assayed dihydro-uracil. The Interlab CV ranges from 5.8% for creatinine to 672.6% for orotidine. The mean Interlab CV for all analytes is 133.1%.

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.

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 2008*

The design of the scheme is essentially the same as in 2007.

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).