ERNDIM Quantitative Schemes Pterins in Urine

ANNUAL REPORT 2018

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
Dr. C. Weykamp Queen Beatrix Hospital MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail: c.w.weykamp@skbwinterswijk.nl	Prof. Dr. N. Blau University Children's Hospital Dietmar-Hopp Metabolic Center Im Neuenheimer Feld 669 D-69120 Heidelberg Germany e-mail: nenad.blau@med.uni-heidelberg.de	Mrs. Irene de Graaf Queen Beatrix Hospital MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail : i.degraaf@skbwinterswijk.nl	ERNDIM Administration Office Manchester Centre for Genomic Medicine 6th Floor, St Mary's Hospital, Oxford Road, Manchester M13 9WL, United Kingdom. e-mail: admin@erndim.org

Heidelberg-Winterswijk, 27.12.2018
Amended report issued 25 June 2019[1]

1. *Purpose*

The purpose of the ERNDIM External Quality Assurance Scheme for Pterins in Urine is the monitoring of the analytical quality of the assay of pterins in laboratories involved in the screening and diagnosis of patients with inherited metabolic disorders. The scheme consists of a quantitative assay of pterins in urine and will be discussed in this report. For details: www.erndim.org / www.ERNDIMQA.nl

2. *Participants*

A total of 31 datasets have been submitted, for 1 of them an annual report could not be generated due to insufficient data submission. One laboratory did not submit results at all.

3. *Design*

The Scheme has been designed, planned and coordinated by Prof. Dr. Nenad Blau as scientific advisor and Dr. Cas Weykamp as scheme organizer (subcontractor on behalf of SKML), both appointed by and according and in line with 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 lyophilized samples, all prepared from the same basic urine, but with various amounts of added analytes. The analytes included are biopterin, neopterin, and primapterin (7-biopterin) and results are expressed in both micromol/L and mmol/mol creatinine. The samples were identical two by two: the pairs, the biochemical and (mimicked) clinical characteristics are in the table below. Samples have been tested for stability and homogeneity according to ISO 13528.

Version Number (& Date)	Amendments
[1] version 2 (25th June 2019)	• Page 5: Preview of 2019 scheme updated.

Table 1. Samples

Sample Pair	Biochemical Characteristics	Clinical Characteristics
1 and 7		GTP cyclohydrolase (GTPCH) deficiency
2 and 8		Pterin-4a-carbinolamine dehydratase (PCD) deficiency
3 and 5		6-pyruvoyl-tetrahydropterin synthase (PTSP) deficiency
4 and 6	Normal levels	Normal pattern

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 2018. 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 early 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 (56 such Analyte-in-Detail-reports can be requested in the year 2018 cycle). A more condensed report is the “Current Report” (Called “Cycle Review” on the website), which summarizes the performance of all analytes in a specific sample (8 such Current Reports can be requested in 2018). 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 2018). Depending on their position in the laboratory, one can choose to have a glance at only the annual report (managers) or at all 56 detailed reports (technicians).

4. Discussion of Results in the Annual Report 2018

In this part, the results as seen in the annual report 2018 will be discussed. Subsequently we will focus on accuracy, recovery, precision, linearity, interlab CV and cross-sectional 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 interpret 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. E.g. for biopterin the mean of all labs is 1.64 micromol/L 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) have 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 98% for biopterin (micromol/L) and neopterin (mmol/mol creatinine) to 102% for primapterin (micromol/L). The overall recovery is 100%.

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 11.2% for neopterin (mmol/mol creatinine) to 44.5% for primapterin (micromol/L). The overall intralab CV is 20.0%.

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. The coefficient of regression ranges from 0.900 for biopterin (micromol/L) to 0.999 for neopterin (micromol/L).

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. Most laboratories submitted results for neopterin and biopterin in mmol/mol creatinine (31) whereas only 16 labs assayed primapterin. The Interlab CV ranges from 26.8% for biopterin to 122% for primapterin. The mean Interlab CV for all analytes is 46.9%.

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 pterins scheme.

4.7 Your laboratory performance: Flags

Since January 2009 a flagging system to judge performance of the individual laboratories has been implemented. In the annual report an individual laboratory 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 flag or no result) receive a green flag. Thus, a green flag indicates satisfactory performance for analysis of that particular analyte while a 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).

4.8 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of flags observed. 46% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 10% of laboratories with more than 25% flags. Following intensive discussion within the ERNDIM board and Scientific Advisory Board (SAB) and feedback from participants we could 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 to solve any analytical problems 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 Flags

% Red Flags seen in Annual Report	Percentage Labs in this Category	Cumulative Percentage of Labs
>25%	10%	10%
20 – 25%	17%	27%
15 – 20%	7%	34%
10 – 15%	0%	34%
5 – 10%	10%	44%
0 – 5%	10%	54%
0%	46%	100%

4.9 Interpretation

In this scheme, we also requested the interpretation. Table 3 shows the interpretation frequency for the respective sample pairs. The correct interpretation is marked with a green box. For sample #1 25 laboratories reported the correct interpretation “GTP cyclohydrolase (GTPCH) deficiency”; 1 lab reported the incorrect normal pterins pattern. In the paired sample (sample #7) 29 laboratories reported the correct interpretation. In general, the reported interpretation of the laboratories is nearly always correct

Table 3. Interpretation

Description	Pair 1-7	Pair 2-8	Pair 3-5	Pair 4-6
Normal pterins pattern	1 – 0	9 – 7	0 – 0	29 – 29
Pterin-4a-carbinolamine dehydratase (PCD) def.	0 – 0	17 – 21	1 – 0	0 – 0
GPT cyclohydrolase (GTPCH) def.	24 – 27	0 – 0	1 – 0	1 – 0
6-pyruvoyl-tetrahydropterin synthase (PTPS) def.	0 – 0	1 – 2	26 – 28	0 – 0

4.10 Additional Specific Remarks of the Scientific Advisor

Interestingly, 15 laboratories did not report primapterin, but 4 of them hit the correct diagnosis of PCD deficiency in the first round and 6 of them in the second round, respectively. These laboratories may miss the diagnosis of a rather benign hyperphenylalaninemia, which however present with a late-onset diabetes type 2 (MODY).

5. Certificates

Starting from 2017 the pterins are included on the 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 pterins 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.

6. *Preview Scheme 2019*

The ERNDIM Scientific Advisory Board have agreed that the inclusion of scoring of interpretation in addition to scoring of quantitative results may improve the utility of this scheme for participants. Therefore during 2019 pilot scoring of interpretation will be performed by the Scientific Advisor based on the interpretations selected by participants when submitting their quantitative results. The planned pilot scoring will assign 1 point for a correct interpretation and 0 points for incorrect or missing interpretations. No negative scores will be assigned and where a laboratory does not perform the necessary testing required to identify an abnormality an interpretation of 'normal' will be assigned a score of 1.
As scoring of interpretation will be in the pilot phase for the 2019 scheme, it will not affect the performance assessment for participants and will not be included in the 2019 certificates of participation. Further information about these changes will be included in the ERNDIM annual newsletter later in 2019.

7. Questions, Remarks, Suggestions

If you have any questions, remarks or suggestions please address to the scientific advisor Prof. Dr. Nenad Blau (nenad.blau@med.uni-heidelberg.de), his deputy Dr. Glynis Klinke (Glynis.Klinke@med.uni-heidelberg.de) or the scheme organizer Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).

Heidelberg, 27.12.2018

Prof. Dr. N. Blau
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Pterins in urine scheme. The contents should not be used for any publication without permission of the scheme advisor.