ERNDiM
Quality Assurance in Laboratory Testing for IEM

ERNDIM Quantitative Schemes Pterins in Urine

ANNUAL REPORT 2021

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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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 36 datasets have been submitted, for 4 of them an annual report could not be generated due to insufficient data submission.

3. *Design*

The Scheme has been designed, planned and coordinated by Dr. Alessio Cremonesi and Prof. Dr. Nenad Blau as scientific advisors and Dr. Eline van der Hagen as scheme organizer (on behalf of the MCA Laboratory), both appointed by and according and in line with the procedures of the ERNDIM Board. The design includes samples and reports to provide information with a balance between short-term and long-term reports and between detailed and aggregated information. As a subcontractor of ERNDIM, the MCA Laboratory prepares and dispatches EQA samples to the scheme participants and provide a website for on-line submission of results and access to scheme reports.

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 µmol/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. Unfortunately, due to a manufacturing issue, the samples did not contain the desired amount of analytes to

[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

perfectly mimic the different disorders. In particular all samples contained moderate amounts of primapterin. Please see section 4.10 for further details

Table 1. Samples

Sample Pair	Biochemical Characteristics	Clinical Characteristics
1 and 6	Moderately high Primapterin Low/normal Neopterin Low/normal Biopterin	A pterin-4a-carbinolamine dehydratase (PCD) deficiency or A GTP cyclohydrolase (GTPCH) deficiency
2 and 8	Moderately high Primapterin High Neopterin Low Biopterin	A pterin-4a-carbinolamine dehydratase (PCD) deficiency or A 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency
3 and 7	High primapterin Normal/high Neopterin Normal/high Biopterin	A pterin-4a-carbinolamine dehydratase (PCD) deficiency or A 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency on treatment with BH4
4 and 5	Moderately high Primapterin High Neopterin Low Biopterin	A pterin-4a-carbinolamine dehydratase (PCD) deficiency or A 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency

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 2021. Two weeks after the respective deadline 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 without any delay time), 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 2021 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 2021). 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 2021). 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 2021

In this part, the results as seen in the annual report 2021 will be discussed. Subsequently we will focus on accuracy, recovery, precision, linearity, interlab CV and cross-sectional relations. Please keep at hand 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 neopterin the mean of all labs is 59.5 µmol/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 by 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 94% for neopterin (mmol/mol creatinine) to 107% for primapterin (mmol/mol creatinine). The overall recovery is 101%.

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 10.3% for neopterin (mmol/mol creatinine) to 21.4% for primapterin (mmol/mol creatinine). The overall intralab CV is 11.3%.

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 0.991 for neopterin (µmol/L and mmol/mol creatinine) to 0.999 for primapterin (µmol/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 (27) whereas only 19 labs assayed primapterin. The Interlab CV ranges from 21.2% for neopterin (mmol/mol creatinine) to 151% for primapterin (µmol/L). The mean Interlab CV for all analytes is 69.9%.

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 for 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. 38% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 3% of laboratories with ≥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%	0%	0%
25%	3%	3%
>20 – <25%	16%	13%
>15 – ≤20%	3%	22%
>10 – ≤15%	19%	31%
>5 – ≤10%	12%	50%
>0 – ≤5%	9%	63%
0%	38%	100%

4.9 Interpretation

In this scheme, we also requested the interpretation with respect to metabolic conditions. Table 3 shows the interpretation frequency for the respective sample pairs. The correct interpretation is marked with a green box. For sample #1 24 laboratories reported the correct interpretations “PCD deficiency” or “GTPCH deficiency”; 1 lab reported the “PTPS deficiency on treatment with BH4” and 8 laboratories a “normal pterin pattern”.

Table 3. Interpretation.

Description	Pair 1-6	Pair 2-8	Pair 3-7	Pair 4-5
a normal pterins pattern	8 – 4	0 – 0	1 – 1	0 – 0
Pterin-4a-carbinolamine dehydratase (PCD) deficiency	13– 11	10 – 7	13 – 14	6 – 8
6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency	0 – 0	20 – 20	1 – 1	26 – 23
a GTP cyclohydrolase (GTPCH) deficiency	11 – 17	0 – 1	1 – 0	0 – 0
a 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency on treatment with BH4	1 – 0	1 – 2	15 – 11	0 – 0

4.10 Additional Specific Remarks of the Scientific Advisor

Unfortunately, due to an issue with a standard used to prepare the EQA samples, all pairs of samples in the 2021 scheme mistakenly contained primapterin instead of biopterin, which caused the diagnostic patterns of the samples to be ambiguous. To ensure that no participants were disadvantaged by these issues, two diagnoses were accepted as correct for each sample and biopterin has been excluded from the individual laboratory online annual reports.

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 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 2022*

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.

The scoring of interpretation was in the pilot phase for the 2021 scheme, so it will not affect the performance assessment for participants and will not be included in the 2021 certificates of participation.

However, it has been agreed that **interpretations will be formally scored for the 2022 scheme and will be included in the 2022 certificates.** Laboratories will be expected to participate in 6 out of 8 distributions with a score of at least 10 points out of 16 (2 points for correct interpretation, 0 points for incorrect interpretation) with no critical errors in order to attain satisfactory performance.

7. Questions, Remarks, Suggestions

If you have any questions, remarks or suggestions please address to the Scientific Advisor Dr. Alessio Cremonesi (alessio.cremonesi@kispi.uzh.ch) or the scheme organizer Dr. Eline van der Hagen (E.vanderHagen@skbwinterswijk.nl).

Zürich, 22 February 2022

Dr. Alessio Cremonesi
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.

The fact that your laboratory participates in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will be shared within ERNDIM for the purpose of evaluating your laboratory 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 February 2022	• 2021 annual report published

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