ERNDIM - Pilot Schemes
Pterins

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Annual Report ERNDIM-EQAS 2015

Purpose

The purpose of the ERNDIM External Quality Assurance Scheme for Pterins in Urine and Dry Blood Spots (DBS) 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 two parts: a) quantitative assay of pterins in urine and b) assay of pterins and DHPR activity on dry blood spots and will be evaluated in parts 1 and 2 of this report, respectively. For details: www.erndim.org / www.ERNDIMQA.nl

1. Pterins in Urine

1.1 Participants

25 Datasets from 16 countries 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.

1.2 Design

The Scheme has been designed, planned and co-ordinated 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 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 analytes included are biopterin, neopterin, and primapterin 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.

Table 1. Samples

Sample Pair	Biochemical Characteristics	Clinical Characteristics
1 and 6	Normal levels	Normal pattern
2 and 5	Normal levels	Normal pattern
3 and 7	Elevated neopterin and primapterin	Pterin-4a-carbinolamine dehydratase (PCD) deficiency
4 and 8	Elevated neopterin and low biopterin	6-pyruvoyl-tetrahydropterin synthase (PTSP) 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 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 (56 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 56 detailed reports (technicians).

1.3 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 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.

1.3.1 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 4.32 micromol/Liter with which you can compare the mean of your lab.

1.3.2 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 94% for biopterin to 107% for primapterin. The overall recovery is 94%.

1.3.3 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 11.7% for primapterin to 21.4% for biopterin in mmol/mol Creatinine. The overall intralab CV is 13.4%.

1.3.4 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 0.980 for biopterin to 0.999 for primapterin .

1.3.5 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 neopterin (26) whereas only 12 labs assayed primapterin. The Interlab CV ranges from 21.2% for neopterin to 34.7% for primapterinl. The mean Interlab CV for all analytes is 27.1%.

1.3.6 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.

1.3.7 Your laboratory 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 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).

1.3.8 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 30% 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%	12%	16%
15 – 20%	4%	20%
10 – 15%	23%	43%
5 – 10%	23%	66%
0 – 5%	4%	70%
0%	30%	100%

1.3.9 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. It can be seen that interpretation is nearly always correct.

Table 3. Interpretation

Description	Pair 1-6	Pair 2-5	Pair 3-7	Pair 4-8
6-pyruvoyl-tetrahydropterin synthase def.			4 - 2	22 - 22
Pterin-4a-carbinolamine dehydratase def.		1 – 0	14 - 18	
Normal pterins pattern	22 - 22	21 - 22	4 - 2	0 - 1

1.3.10 Certificates

As the pterins scheme is a pilot scheme, pterins are not included on the certificates.

1.3.11 *Preview Scheme 2016*

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

1.3.12 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) or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).

2. Pterins and DHPR activity in Dry Blood Spots

2.1 Participants

17 Datasets from 16 countries have been submitted, 2 for pterins only, 10 for DHPR only and 5 for pterins and DHPR. 15 laboratories did not submit results at all.

2.2 Design

The Scheme has been designed, planned and co-ordinated 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 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 4 dried blood samples, all prepared from the same basic blood, but two incubated with methotrexate (MTX) to inactivate DHPR. Incubation with MTX resulted in additional pterin-like peaks in HPLC that need to be separated from pterins. Pterin results are expressed in both nmol/L and nmol/g Hb. Samples have been tested for stability and homogeneity according to ISO 13528.

Table 1. Samples

Samples	Biochemical Characteristics	Clinical Characteristics
1	Normal levels for pterins and DHPR	Normal pattern
2	Normal levels for pterins and very low DHPR activity	DHPR deficiency
3	Normal levels for pterins and DHPR	Normal pattern
4	Normal levels for pterins and very low DHPR activity	DHPR deficiency

Reports

Mean in nmol/L and range pilot pterines and DHPR in dried blood spots

Table 2.

Analyte	n	Sample 1	Sample 2	Sample 3	Sample 4
Neopterin	6	7.2 (4.3-12.1)	6.7 (5.3-7.6)	6.5 (1.1-11.0)	7.7 (6.1-11.2)
Biopterin	6	8.0 (4.3-13.2)	9.5 (1.9-17.0)	8.0 (1.4-13.0)	10.9 (8.3-16.0)
DHPR*	7	2.6 (0.8 – 5.1)	0 (0 – 0.6)	3.1 (0.1 – 5.7)	0 (0 – 0.2)

*mU/mg Hb

2.3 Discussion of Results in the Annual Report 2015

A very good separation was essential for quantitative analysis of neopterin and biopterin with HPLC. Two labs with very high results for neopterin and biopterin (due to MTX) were not included. One lab reported very low DHPR activity in sample #1. DHPR activity was reported with 5 different units, making statistical evaluation impossible for most samples.

Conclusion is that there is a complete lack of standardisation, which makes reporting impossible. For this reason we suggest to ask participants to use only one standardized procedure with a well defined amount of DBS circles

2.4 *Preview Scheme 2016*

The design of the 2016 scheme is essentially the same as in 2015, but with additional interpretation (similar to the urine scheme).

2.5 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) or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).