ERNDIM - Quantitative Schemes
Pilot Neurotransmitters

Simon Heales
Neurometabolic Unit
National Hospital for Neurology
Queen Square
London
WC1N 3BG
UK
e-mail:simon.heales@gosh.nhs.uk

Dr. C.W. Weykamp
Queen Beatrix Hospital
MCA Laboratory
P.O. Box 9005
NL – 7100 GG Winterswijk
e-mail : c.w.weykamp@skbwinterswijk.nl

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Annual Report ERNDIM-EQAS 2015

1. *Purpose*

The purpose of the ERNDIM External Quality Assurance Scheme for Neurotransmitters is the monitoring of the analytical quality and interpretation of the quantitative assay of neurotransmitters in CSF 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*

17 Datasets from laboratories in 15 countries were submitted. 4 Laboratories did not submit results at all.

3. *Design*

The Scheme has been designed, planned and co-ordinated by Dr. Simon Pope and Prof. Simon Heales as scientific advisors 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 samples (4 lyophilised pooled CSF and 4 lyophilised artificial CSF), all prepared from the same basic CSF/artifical matrix but with various amounts of added analyte either with or without diluting with distilled water. The samples were identical two by two: the pairs, analytes and their source as well as the added amounts are in the table below. Samples have been tested for stability and homogeneity according to ISO 13528.
The idea of comparing artificial vs. pooled CSF was to compare analyte stability in each matrix as there had been some concerns about analyte stability in pooled CSF in the previous year of the scheme.

Analyte	Source	Estimate Quantities in nmol/liter			
		Sample Pair 2015. 01-07	Sample Pair 2015. 02-05	Sample Pair 2015. 03-06	Sample Pair 2015. 04-08
3-methyl dopa	Sigma-Aldrich M4255	1027	2685	46.4	24.1
5HIAA	Sigma-Aldrich H9772	156	138	282	142
5-OH-Tryptophan	Sigma-Aldrich H1252	284	439	12.4	5
Homovanillic acid	Sigma-Aldrich H8876	355	328	542	288
HVA:5HIAA ratio	Not applicable	2.30	2.35	1.95	1.89

Samples 02, 05, 04 and 08 were made in artificial matrix and samples 01, 07, 03 and 06 were made in pooled CSF.
Unfortunately the exact spiking is not known for this set of CSFs as (1) the spike was added to pooled CSF that had been diluted to varying degrees (therefore the endogenous level of metabolites was variable) and (2) the sample was made into more aliquots than originally intended due to higher than expected participant numbers. The values above correspond to the median results.

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 (40 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 40 detailed reports (technicians).

4. *Discussion of Results in the Annual Report 2015*

In this part the results as seen in the annual report 2014 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.

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 3-methyl dopa the mean of all labs is 93.5 nmol/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) 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 95% for HVA to 105% for 5-OH-tryptophan. As spiked plus endogeneous amounts were not known exactly, median results were chosen as estimated weighed amounts. Therefore the mean recovery is (of course) nearly 100% and in fact meaningless.

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 6.0% for HVA to 14.6% for 3-methyl dopa. 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 0.978 for HVA to 0.998 for 5-OH-tryptophan**.** Also here the medians were used as estimated weighed amounts.

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 5HIAA (17) whereas only 14 labs assayed 5-HTP. The Interlab CV ranges from 11.0% for HVA to 23.9% for 3-methyl dopa.The mean Interlab CV for all analytes is 15.8%.

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 Neurotransmitter scheme.

4.7 *Cross Sectional Relations*

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).

4.8 *Poor Performance Policy*

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 40% 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 12% 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%	12%	12%
20 – 25%	6%	18%
15 – 20%	6%	24%
10 – 15%	12%	36%
5 – 10%	18%	54%
0 – 5%	6%	60%
0%	40%	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. It can be seen that interpretation is nearly always correct.

Table 3.

Description	Pair 2015. 01-07 (4y-2y)	Pair 2015. 02-05 (6y-7y)	Pair 2015. 03-06 (1y-2y)	Pair 2015. 04-08 (5y-6y)
patient with a pterin-related disorder receiving treatment	17 - 17	17 - 16	1 - 0	1 - 0
Tyrosine hydroxylase deficiency		0 - 1		1 - 0
An isolated disorder of serotonin metabolism				1 - 0
A dopamine transporter defect				
No obvious disorder of dopamine or serotonine metab			16 - 17	14 - 17

To prevent laboratories from deriving the duplicate samples from the age of the patients, ages of samples for a duplicate were not the same (Example: Samples 2 and 5 were identical but were given ages of 6 and 7 years)

4.10 Certificates

Neurotransmitters are not included yet in the certificates as this is a pilot scheme.

5. *Summary*

This was the second year of the neurotransmitter pilot scheme. In the first year, there was general agreement with regards to 5HIAA and HVA concentrations (CVs <12.5%) but there was quite a large discrepancy in 3-methyl dopa and 5-hydroxytryptophan values between laboratories (CVs >40%), which may have been due to variable analyte degradation in the pooled CSF matrix. This was investigated in this years scheme by comparing pooled CSF versus artificial CSF to see if this improved the consistency of results between laboratories. Overall there has been improvement in interlab CVs this year compared to the 2014 scheme (15.8% vs 26.0% - all analytes combined). This may be due to the (1) use of the artificial matrix, (2) a less degradative pooled CSF, (3) higher concentrations of the minor metabolites, further away from the limit of quantitation, or (4) general improvement in analysis and reporting, perhaps due to the implementation of this EQA scheme. The 2016 scheme will retain the mix of artifical matrix and pooled CSF to investigate this further.

6. *Preview Scheme 2015*

The design of the 2016 scheme is similar to the 2015 scheme.

7. *Questions, Remarks, Suggestions*

If you have any questions, remarks or suggestions please address to the scientific advisor Prof. Simon Heales (simon.heales@gosh.nhs.uk) Or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).