ERNDIM
Quantitative Schemes
Neurotransmitters in CSF

ANNUAL REPORT 2020

Scheme Organiser	Scientific Advisors	Website for reporting results	Administration office
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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*

A total of 35 datasets were submitted.

3. *Design*

The Scheme has been designed, planned and co-ordinated by Dr. Simon Pope and Prof. Simon Heales as scientific advisors and Dr. Eline van der Hagen as scheme organizer (on behalf of the MCA Laboratory), each 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. 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 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

[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

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 2020. 01-05	Sample Pair 2020. 02-06	Sample Pair 2020. 03-08	Sample Pair 2020. 04-07
3-methyl dopa	Sigma-Aldrich M4255	29	14.0	25	1580
5HIAA	Sigma-Aldrich H9772	272	17.0	178	219
5-OH-Tryptophan	Sigma-Aldrich H1252	2.89	1.0	7	195
Homovanillic acid	Sigma-Aldrich H8876	580	45.4	1810	465
HVA:5HIAA ratio	Not applicable	2.18	2.7	10.2	2.2

Samples 02, 06, 03 and 08 were made in artificial matrix and samples 01, 05, 04 and 07 were made in pooled CSF.

Unfortunately the exact concentration 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 from the 1st round of results. Duplicate samples gave consistent 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 2020. 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 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 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 2020 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 2020). 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 2020). 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 2020*

In this part the results as seen in the annual report 2020 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 402 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 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 95% for to 102% for 5-HTTP and HVA. 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 6.9% for HVA to 21.3% for 5-HTTP. The overall intralab CV is 10.9%.

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 0.995 for 5HIAA to 1.000 for 3-MD. 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 5-HIAA and HVA (n=35) whereas only 24 labs assayed 5-HTTP. The Interlab CV ranges from 11.3% for HVA to 69.1% for 5-HTTP.The mean Interlab CV for all analytes is 31.8%.

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. *Your perfomance: 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 of 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 red flag indicates that your laboratory has failed to attain satisfactory performance. Criteria for 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. 55% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 6% of laboratories with more than 25% 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 Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	6%	6%
25%	3%	9%
20 – 25%	3%	12%
15 – 20%	9%	21%
10 – 15%	9%	30%
5 – 10%	15%	45%
0 – 5%	0%	45%
0%	55%	100%

4.9. *Interpretation*

In this scheme we also requested the interpretation of test results. 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 2020. 01-05 (4y-3y)	Pair 2020. 02-06 (3y-3y)	Pair 2020. 03-08 (4y-3y)	Pair 2020. 04-07 (3y-3y)
No obvious disorder of serotonin or dopamine metabolism.	33 - 33	0 - 0	0 - 0	2 - 3
A patient with a pterin disorder on treatment.	0 - 0	1 - 2	1 - 0	26 - 29
A patient with a pterin disorder not on treatment.	0 - 0	29 - 29	0 -1	0 - 1
A patient with tyrosine hydroxylase deficiency not on treatment.	0 – 0	0 - 0	0 - 0	0 – 0
A patient with a dopamine transporter defect.	0 – 0	0 – 1	31 – 31	0 - 0
Other	0 – 0	3 – 2	0 – 1	2 – 1

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 1 and 5 were identical but were given ages of 4 and 3 years)

4.10 Certificates

Neurotransmitters are included in the certificates.

4.11 Additional Specific Remarks of the Scientific Advisor

NA

5. Summary

Since starting the CSF neurotransmitters scheme in 2014 there has been an increase in participants each year and generally the results returned have shown a good

degree of consistency between the laboratories around the world. Similar to previous years, many red flags are due to not submitting results, especially for the minor metabolites, 3-methyl dopa and 5-hydroxytryptophan, and the HVA:5HIAA ratio. We would advise reporting these values even if they are of low concentration.

The interpretive part is included to see how different laboratories, with different CSF collection protocols/fractions, reference ranges and populations, interpret the results. We believe the interpretation is very important and we try to make the samples so that they reflect actual patient samples we have seen in the laboratory. We would encourage all participants to choose an interpretive comment and regularly review their results versus the other participants.
A brief discussion of each of the duplicate samples is given below.

Samples 01-05 – A patient with no obvious disorder of serotonin or dopamine metabolism. This sample had metabolite concentrations within the age-related reference ranges.

Samples 02-06 – A patient with a pterin disorder not on treatment. This sample had low concentrations of all the metabolites.

Samples 03-08 – A patient with a dopamine transporter defect. This sample had an elevated homovanillic acid and HVA:5HIAA ratio.

Samples 04-07 – A patient with a pterin disorder on treatment. This sample had homovanillic acid and 5HIAA within the reference range and elevated 3-methyl dopa and 5-hydroxytryptophan, consistent with treatment with L-dopa and 5-hydroxytryptophan.

6. *Preview Scheme 2021*

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. During 2021, interpretation will be scored and this will be reviewed at the end of the year and will be commented on in performance support letters. We are still in the process of agreeing the best way to score interpretation for these mixed quantitative-qualitative schemes and will give an update later in the year.

7. *Questions, Remarks, Suggestions*

If you have any questions, remarks or suggestions please address to the scientific advisors Prof. Simon Heales (simon.heales@gosh.nhs.uk) and Dr Simon Pope (simonpope@nhs.net) or the scheme organiser Dr. Eline van der Hagen (E.vanderHagen@skbwinterswijk.nl).

London,19 th January, 2021

Simon Heales Simon Pope

Scientific Advisors

Please note:
This annual report is intended for participants of the ERNDIM CSF Neurotransmitters 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	19th Jan 2021	• 2020 annual report published

END