ERNDIM
Quantitative Schemes
Neurotransmitters in CSF

ANNUAL REPORT 2021

Scheme Organiser	Scientific Advisors	Website for reporting results	Administration office
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Published: London-Winterswijk, 12th January 2022[1]

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 36 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 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.

[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

Analyte	Source	Estimate Quantities in nmol/liter			
		Sample Pair 2021. 01-08	Sample Pair 2021. 02-07	Sample Pair 2021. 03-06	Sample Pair 2021. 04-05
3-methyl dopa	Sigma-Aldrich M4255	14.7	13.9	1518	28.8
5HIAA	Sigma-Aldrich H9772	91.5	94	135	370
5-OH-Tryptophan	Sigma-Aldrich H1252	2.92	3.1	13.8	18.0
Homovanillic acid	Sigma-Aldrich H8876	212	198	201	560
HVA:5HIAA ratio	Not applicable	2.3	2.2	1.5	1.5

Samples 03, 06, 04 and 05 were made in artificial matrix and samples 01, 08, 02 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 2021 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 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 40 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 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 376 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 97% for 3-methyl dopa to 108% for 5-HTTP. 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 4.1% for 5-HIAA to 16.2% for 5-HTTP. The overall intralab CV is 9.6%.

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 0.974 for 5-HTTP to 0.998 for 3-MD and 5-HIAA. 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=37) whereas only 27 labs assayed 5-HTTP. The Interlab CV ranges from 7.17% for HVA to 41.7% for 3-MD.The mean Interlab CV for all analytes is 21.6%.

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. 69% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 14% 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%	14%	14%
25%	0%	14%
20 – 25%	3%	17%
15 – 20%	6%	23%
10 – 15%	5%	28%
5 – 10%	3%	31%
0 – 5%	0%	31%
0%	69%	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 2021. 01-08 (7y-7y)	Pair 2021. 02-07 (3mo-2mo)	Pair 2021. 03-06 (6y-7y)	Pair 2021. 04-05 (3mo-3mo)
No obvious disorder of serotonin or dopamine metabolism.	29 - 29	2 – 1	1 - 0	30 – 33
A patient with AADC deficiency.	0 - 0	1 – 1	4 - 1	1 – 0
A patient with pterin disorder not on treatment	2 - 3	26 – 30	0 -1	1 - 0
A patient with tyrosine hydroxylase deficiency on treatment.	2 – 0	0 – 0	27 - 30	0 – 0
A patient with a dopamine transporter defect..	0 – 0	0 – 0	0 – 0	1 - 0

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 7 were identical but were given ages of 3 and 2 months)

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.

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 01-08 –No obvious disorder of serotonin or dopamine metabolism. The metabolite concentrations were within the typical age-related reference ranges.

Samples 02-07 – A patient with pterin disorder not on treatment. The homovanillic acid and 5HIAA were below the age-related reference ranges suggestive of a metabolic impairment. 3-methyl dopa and 5-hydroxytryptophan were not elevated. They would be elevated in aromatic amino acid decaboxylase deficiency. Therefore, a pterin-related defect is the most likely cause.

Samples 03-06 – A patient with tyrosine hydroxylase deficiency on treatment. The homovanillic acid and 5HIAA were within the age-related reference ranges, making a diagnosis of amino acid decaboxylase deficiency or a pterin-related defect unlikely. The elevated 3-methyl dopa is indicative of L-dopa treatment. 5-hydroxytryptophan is not elevated. Therefore, a patient with tyrosine hydroxylase deficiency on treatment is the most likely cause of this metabolite profile.

Samples 04-05 –No obvious disorder of serotonin or dopamine metabolism. The metabolite concentrations were within the typical age-related reference ranges.

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. During 2022, interpretation will be scored and this will be reviewed at the end of the year and will be commented on in performance support letters.
A new scoring system has been agreed for these mixed quantitative-qualitative schemes. 2 points will be awarded for a correct answer and 0 for an incorrect answer. Occasionally, where a participant is partially correct a score of 1 point will be given. At the end of the year, a final score out of 16 (2 points x 8 submissions) will be calculated. A score of 10/16 or above will be considered to be satisfactory and a score below 10/16 will be considered to be poor performance.

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,12th January, 2022

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.

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APPENDIX 1. Change log (changes since the last version)

Version Number	Published	Amendments
1	12th Jan 2022	• 2021 annual report published

END