ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2020

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 Quantitative Amino Acids is the monitoring of the analytical quality of the quantitative assay of amino acids in plasma 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 295 datasets have been submitted, for 6 of them an annual report could not be generated due to insufficient data submission. 9 laboratories did not submit results at all.

3. *Design*

The scheme has been designed, planned and co-ordinated by Dr. Rachel Carling and Prof. Brian Fowler as scientific advisors and Dr. Eline van der Hagen as scheme organiser (on behalf of the MCA Laboratory), each appointed by and according to procedures laid down by the ERNDIM Board. The design includes special attention to sample content and to the layout of reports. Samples are produced with amino acids in concentrations that are found in physiological samples and reflect findings in inborn errors of metabolism. Low levels of amino acids are sometimes included to mimic those seen in pathological states or in treated patients. 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.

[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

Samples

The scheme consisted of 8 lyophilised samples, all prepared from the same basic human serum which has been treated to remove most of the amino acids present and to which various amounts of analytes are added. As can be seen from table 1 the added quantities were identical in pairs of the samples. The nature, source and the added amounts of the analytes are also summarised in table 1.

Table 1. Pair identification, source and amounts of added analytes.

		Added quantities (micromol/L)			
Analyte	**Source**	**Sample pair 2020. 01-07**	**Sample pair 2020. 02-08**	**Sample pair 2020. 03-05**	**Sample pair 2020. 04-06**
2-aminobutyric acid	Sigma A1879	10.3	50.7	75.3	24.6
Alanine	Sigma 44526	101	600	900	300
Alloisoleucine	Sigma I8754	0.0	50.1	125	25.5
Arginine	Sigma 90538	5.2	150	500	75.5
Arginino succinic acid	Sigma A5707	9.8	29.9	50.1	20.1
Asparagine	Sigma 51363	10.9	30.4	50.4	21.1
Aspartic acid	Sigma 51572	11.0	150	201	50.5
Citrulline	Sigma 1133842	5.3	1000	1500	50.5
Cystine	Sigma 49603	25.0	75.2	100	49.8
Glutamic acid	Sigma 95436	500	400	200	300
Glutamine	Sigma 76523	201	1000	1501	500
Glycine	Sigma 76524	150	601	800	301
Histidine	Sigma 73767	125	50.0	100	251
Hydroxyproline	Sigma PHR1939	25.5	75.2	100	50.5
Isoleucine	Sigma 56241	29.6	350	750	151
Leucine	Sigma 76526	60.3	700	1501	301
Lysine	Sigma 67448	20.0	200	501	100
Methionine	Sigma 39496	10.5	200	499	50.4
Ornithine	Sigma O2375	100	500	751	250
Phenylalanine	Sigma 40451	50.1	750	1001	151
Proline	Sigma 93693	50.9	251	500	1250
Pros-methylhistidine	Sigma M9005	25.0	75.0	99.8	50.3
Sarcosine	Sigma S7672	102	50.3	26.0	77.0
Serine	Sigma 54763	99.9	500	752	250
Sulphocysteine	Abcam Ab146303	9.6	35.8	72.1	18.3
Taurine	Sigma 93019	75.4	300	501	151
Threonine	Sigma 61506	51.2	250	501	126
Thryptophan	Sigma 51145	25.0	75.3	100	50.0
Tyrosine	Sigma 91515	50.3	400	801	200
Valine	Sigma 50848	99.4	400	599	201

All amino acids used are of the highest purity commercially available. Concentrations < 100 micromol/L are given with one decimal place; otherwise without decimal. Samples have been tested for stability and homogeneity according to ISO 13528 in which requirements for regulatory purposes of quality management systems for medical devices are described.

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 on the eight individual specimens are available two weeks after the submission deadline and provide up-to-date information on analytical performance. Although it is technically possible to produce reports immediately there is a delay of 14 days to enable the scientific advisor to inspect the results and add comments to the report when appropriate.
The ***annual report*** summarises the results of the whole year.

A second important characteristic of the website is the different levels of detail of results which allows individual laboratories the choice of fully detailed and/or summarised reports. The “Analyte in Detail” is the most detailed report and shows results of a specific analyte in a specific sample. Thus for the 31 amino acids in the year 2020 cycle, 8 x 31 = 248 such Analyte-in-Detail-reports can be requested. A more condensed report is the “Cycle Review” which summarises the performance of all analytes in a specific sample (8 such Cycle Reviews can be requested in 2020). The Annual Report summarizes all results giving an indication of overall performance for all analytes in all 8 samples (1 such Annual-Report can be requested in 2020). Depending on the responsibilities within the laboratory, participants can choose to inspect the annual report (e.g. Quality Managers) or all (or part of) the 248 detailed reports (e.g. scientific staff).

Analyte	Accuracy (mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (%added analyte)		Data all labs	
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
2-Aminobutyric acid	ORFR	39.8	ORFR	7.9%	ORFR	0.989	ORFR	100%	213	13.1%
Alanine	440	449	2.7%	4.4%	0.999	0.998	96%	93%	286	8.30%
Alloisoleucine	FR	45.7	FR	6.4%	FR	0.998	FR	92%	215	16.9%
Arginine	179	187	6.5%	5.8%	0.999	0.999	94%	97%	285	10.5%
Argininosuccinic acid	13.5	14.5	52.3%	20.9%	0.872	0.956	73%	70%	139	47.4%
Asparagine		27.1		11.4%		0.981		98%	257	23.5%
Aspartic Acid	77.5	76.3	7.3%	6.2%	0.997	0.996	72%	68%	270	19.1%
Citrulline	400	605	10.3%	5.5%	0.983	0.998	58%	95%	284	14.0%
Cystine	44.0	44.9	4.0%	11.1%	0.995	0.974	76%	72%	257	15.0%
Glutamic acid	313	352	8.5%	6.1%	0.957	0.973	61%	82%	283	9.12%
Glutamine	654	721	9.2%	6.4%	0.987	0.996	89%	89%	275	11.0%
Glycine	450	449	4.0%	4.3%	0.996	0.997	99%	95%	285	7.76%
Histidine	124	125	11.0%	5.7%	0.991	0.995	90%	91%	283	9.53%
Hydroxyproline	71.6	59.0	22.9%	10.5%	0.936	0.978	111%	98%	240	14.7%
Isoleucine	293	300	1.6%	4.7%	0.999	0.999	95%	94%	290	8.75%
Leucine	628	593	11.3%	4.8%	0.993	0.999	105%	92%	290	10.3%
Lysine	212	214	2.0%	4.6%	0.997	0.993	98%	95%	286	8.87%
Methionine	176	186	4.9%	6.6%	0.998	0.998	96%	94%	283	16.9%
Ornithine	325	373	3.8%	4.2%	0.993	0.996	87%	91%	288	8.85%
Phenylalanine	462	453	1.4%	4.6%	1.000	0.999	95%	92%	293	9.01%
Proline	470	474	2.8%	6.4%	1.000	0.998	95%	95%	272	11.4%
Sarcosine		61.0		12.4%		0.970		98%	155	20.5%
Serine	MP	353	7.1%	4.2%	0.995	0.998	86%	86%	284	7.91%
Sulfocysteine	30.2	26.9	17.2%	13.3%	0.992	0.986	82%	78%	88	25.4%
Taurine	297	253	8.5%	4.9%	0.992	0.997	112%	97%	266	9.50%
Threonine	213	225	6.5%	4.5%	0.998	0.999	95%	96%	284	7.87%
Tryptophan		76.4		8.3%		0.973		90%	220	16.8%
Tyrosine	335	342	4.0%	4.5%	0.999	0.999	96%	93%	293	8.86%
Valine	309	313	5.9%	4.4%	0.995	0.998	101%	96%	290	7.54%
Overall	283	256	9.0%	7.1%	0.986	0.991	90%	91%	257	13.7%

See this example of part of an annual report.

As agreed in 2016, the flagging system has been changed. The explanation of the flags can be found in the General information section (Use Website / Explanation Annual Report)

4. Discussion of Results in the Annual Report 2020

In this part the results as seen in the annual report 2020 will be discussed. Please keep at hand your annual report from the website when you follow the various aspects below and keep in mind that we only discuss the results of “all labs”. It is your responsibility to inspect and interpret the results of your own laboratory.

4.1 Accuracy

A first approach to evaluating your performance in terms of accuracy is comparison of your mean values for each amino acid in the eight samples with those of all labs. This is shown in the columns "Your Lab" and "All Labs" under the heading "Accuracy". For example, for alanine, the mean for all labs is 449 micromol/Litre, with which you can compare the mean of your lab.

4.2 Recovery

A second approach to describe performance is the percentage recovery of added analyte. In this approach the amounts of weighed quantities added to the samples are the assumed target values after adjustment for blank values. The correlation between weighed amounts (on the x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the resulting relation (a in y = ax + b) in this formula multiplied by 100% is your recovery of the added amounts. The outcome for your lab in comparison to the median outcome of all labs is shown in the column “Recovery”. The recovery is generally acceptable with 22 analytes having a recovery within the range 90 - 110%. Poor recovery is evident for seven analytes: argininosuccinic acid (70%), aspartic acid (68%), cystine (72%), glutamic acid (82%), glutamine (89%), serine (86%) and sulfocysteine (78%).

4.3 Precision

Reproducibility is an important parameter for the analytical performance of a laboratory and is addressed in the schemes’ design. Samples provided in pairs can be regarded as duplicates from which CVs can be calculated. The column “Precision” in the annual report shows your CVs for the respective amino acids in comparison to median values for all labs. Precision ranges from 4.2% for serine to 20.9% for argininosuccinic acid. 15 amino acids demonstrated good performance with CVs < than 6%. The average intra-lab CV is 7.1%.

4.4 *Linearity*

Linearity over the whole relevant analytical range is another important parameter for analytical quality and is also examined within the schemes. A comparison of the weighed quantities on the x-axis and your measured quantities on the y-axis allows calculation of the coefficient of regression (**r**). The column “Linearity” in the annual report shows your **r** values for the respective amino acids in comparison to the median **r** values for all labs. Ideally the **r** value is close to 1.000 and ranges from arginine succinic acid (0.956) to 6 amino acids that give an excellent r value (r = 0.999). It must be remembered that only a limited concentration range is tested in this scheme.

4.5 Interlab CV

For comparison of amino acid levels for diagnosis and monitoring of treatment for one patient in different hospitals and for use of shared reference values it is essential to have a high degree of harmonization between results of laboratories. Part of the schemes’ design is to monitor this by calculating the inter-laboratory CV. This, along with the number of laboratories that submitted results is shown in the column “Data all labs” in the annual report. Agreement between laboratories is reasonable for most amino acids, with thirteen amino acids having an inter lab CV of <10% and eight

between 10 and 15%. However, nine amino acids have a CV >15% with argininosuccinic acid having a CV of 47.4%.

4.6 Number of Participating Labs and submitted results

For 22 of the individual amino acids, results were submitted in at least 253 datasets (86% of the 295 datasets).

4.7 Interrelationships between quality parameters

The various parameters described above often have an interrelationship: usually more than one parameter points in the same direction towards either good or bad analytical performance.
For example for arginine all parameters indicate good performance: precision (CV = 5.8%), linearity (r = 0.999), recovery (97%) and interlab variation (interlab CV 10.5%) with the majority of labs (n=285 datasets) submitting results.

4.8 Your performance: red and green flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags in case of poor performance for accuracy, precision, linearity and recovery. Amino acids with satisfactory performance for at least three of the four parameters (thus no or only one flag) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular amino acid. Criteria for flags can be found in the general information on the website (on this website under general information; use website, explanation annual report).

4.9 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 flag at all and thus have attained excellent overall performance. In contrast, at the other extreme 5% of laboratories have 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 agreed 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 are 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 this will be ratified by the SAB. A letter pointing out failure to achieve these levels will be issued 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.

If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter.

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	5%	5%
25%	1%	6%
20 – 25%	3%	9%
15 – 20%	2%	11%
10 – 15%	6%	17%
5 – 10%	17%	34%
0 – 5%	36%	70%
0%	30%	100%

4.10 Certificates

As for other schemes, the performance, as indicated by the flags in the individual laboratories annual report, is summarised in the annual participation certificate. The certificate lists the total number of amino acids 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.

4.11 Additional Specific Remarks of the Scientific Advisor

Homocitrulline was present in the eight 2020 samples at a constant concentration. This was to demonstrate the impact of its interference on methionine when measured by ion exchange chromatography. Education is a key aspect of the ERNIDM scheme and the intention here was to raise awareness. As such, homocitrulline has not been included in the performance report.

5. Summary of performance

General comments

The results obtained this year agree fairly well with those expected. Some discrepancies with calculated recoveries are evident for a few amino acids.

Quantitative comparisons (see table 3).

The overall performance evaluated by comparing precision (within lab variation) versus interlab variation for each amino acid reveals three main groups. There are 13 amino acids with good precision and interlab CVs of 10% or below. Eight amino acids show interlab CVs of about 10 – 15% with precision below 12% and there are nine amino acids which perform poorly, shown here as interlab CV above 15%. A review of inter-laboratory CVs for each analyte for the past 10 years revealed no significant changes, with the exception of aspartic acid. Aspartic acid inter-laboratory variation has reduced from 26% in 2008 to 16% in 2018 and 2019 and 19% in 2020.

Taking all parameters into account there is a large group of well-established amino acids (about 20) for which there is good overall performance, reflected by satisfactory values for all five analytical quality parameters (acceptable precision and interlab CV, linearity exceeding 0.9, recovery between 90 and 110% and a high percentage of submitted results. Performance for argininosuccinic acid, aspartic acid, glutamic acid, cysteine and sulfocyteine is less satisfactory and this is reflected by more than one analytical quality parameter. Glutamine and serine demonstrate less satisfactory recovery, but other parameters are adequate. Measurement of these amino acids should be improved.

Table 3. Summary of results of all laboratories

Analyte	Accuracy (mean µmol/L)	Precision (CV% duplicates)	Linearity (r)	Recovery (%added analyte)	Data all labs	
	All labs	All labs	All labs	All labs	n	Interlab CV
2-aminobutyric acid	39.8	7.9%	0.989	100%	213	13.1%
Alanine	449	4.4%	0.998	93%	286	8.30%
Alloisoleucine	45.7	6.4%	0.998	92%	215	16.9%
Arginine	187	5.8%	0.999	97%	285	10.5%
Arginino succinic acid	14.5	20.9%	0.956	70%	139	47.4%
Asparagine	27.1	11.4%	0.981	98%	257	23.5%
Aspartic acid	76.3	6.2%	0.996	68%	270	19.1%
Citrulline	605	5.5%	0.998	95%	284	14.0%
Cystine	44.9	11.1%	0.974	72%	257	15.0%
Glutamic acid	352	6.1%	0.973	82%	283	9.12%
Glutamine	721	6.4%	0.996	89%	275	11.0%
Glycine	449	4.3%	0.997	95%	285	7.76%
Histidine	125	5.7%	0.995	91%	283	9.53%
Hydroxyproline	59.0	10.5%	0.978	98%	240	14.7%
Isoleucine	300	4.7%	0.999	94%	290	8.75%
Leucine	593	4.8%	0.999	92%	290	10.3%
Lysine	214	4.6%	0.993	95%	286	8.87%
Methionine	186	6.6%	0.998	94%	283	16.9%
Ornithine	373	4.2%	0.996	91%	288	8.85%
Phenylalanine	453	4.6%	0.999	92%	293	9.01%
Proline	474	6.4%	0.998	95%	272	11.4%
Sarcosine	61.0	12.4%	0.970	98%	155	20.5%
Serine	353	4.2%	0.998	86%	284	7.91%
Sulphocysteine	26.9	13.3%	0.986	78%	88	25.4%
Taurine	253	4.9%	0.997	97%	266	9.50%
Threonine	225	4.5%	0.999	96%	284	7.87%
Thryptophan	76.4	8.3%	0.973	90%	220	16.8%
Tyrosine	342	4.5%	0.999	93%	293	8.86%
Valine	313	4.4%	0.998	96%	290	7.54%
Overall	256	7.1%	0.991	91%	257	13.7%

Educational Effect of ERNDIM

Greater experience of amino acid analysis as reflected by longer participation in ERNDIM schemes clearly seems to contribute to improved performance. Beyond this the learning/educational effect of EQA as provided by ERNDIM is undoubtedly a major factor in improving performance.

6. Preview of the Scheme for 2021

Our policy is to include the same common amino acids in each year's samples as well as a few unusual ones which are selected year to year.

The common amino acids have been updated to include Tele-Methylhistidine from 2021 onwards. Three selected special amino acids are also included in the 2021 scheme.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions in addition to specific user comments please address these to the scientific advisor of the scheme, Dr.Rachel Carling (Rachel.Carling@viapath.co.uk) and/or the scheme organiser Dr. Eline van der Hagen (E.vanderHagen@skbwinterswijk.nl).

London 18/1/21

Dr. Rachel Carling
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Amino Acids (serum). 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	18 January 2021	2020 annual report published
2	8th February 2021	Page 5, Poor Performance Policy, information for appeal of poor performance added.

END