ERNDIM
Quantitative Schemes
Amino Acids(serum)

ANNUAL REPORT 2021

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 298 datasets have been submitted, for 13 of them an annual report could not be generated due to insufficient data submission. 5 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.

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[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.

Analyte	Source	Added quantities (micromol/L)			
		Sample pair 2021. 01-07	Sample pair 2021. 02-08	Sample pair 2021. 03-06	Sample pair 2021. 04-05
2-aminobutyric acid	Sigma A1879	42.2	19.7	9.9	77.4
Alanine	Sigma 44526	777.0	389.3	193.8	1554.0
Alloisoleucine	Sigma I8754	146.0	97.3	48.7	194.7
Arginine	Sigma 90538	243.8	48.9	10.3	485.6
Arginino succinic acid	Sigma A5707	73.0	48.6	14.8	121.6
Asparagine	Sigma 51363	73.6	48.3	25.3	96.7
Aspartic acid	Sigma 51572	75.2	50.2	24.0	97.0
Citrulline	Sigma 1133842	970.9	48.9	9.9	1943.5
Cystine	Sigma 49603	96.6	29.6	9.7	145.6
Glutamic acid	Sigma 95436	146.0	96.7	48.3	194.4
Glutamine	Sigma 76523	1214.9	485.8	97.4	1700.7
Glycine	Sigma 76524	728.6	485.1	243.5	1457.2
Histidine	Sigma 73767	117.8	58.0	29.0	174.9
Homocitrulline	SC-269298	72.9	72.9	72.9	72.9
Hydroxyproline	Sigma PHR1939	21.0	10.0	4.4	38.7
Isoleucine	Sigma 56241	485.6	146.0	48.7	1458.0
Leucine	Sigma 76526	484.5	146.0	48.7	1458.0
Lysine	Sigma 67448	486.4	243.2	24.6	728.9
Methionine	Sigma 39496	485.4	97.3	9.7	728.6
Ornithine	Sigma O2375	390.0	195.4	19.8	777.3
Phenylalanine	Sigma 40451	583.4	349.7	116.8	1165.8
Proline	Sigma 93693	485.5	243.4	49.2	728.8
Pros-methylhistidine	Sigma M9005	14.6	9.4	5.1	24.0
Sarcosine	Sigma S7672	14.7	9.8	4.9	19.5
Serine	Sigma 54763	243.0	48.3	9.7	486.1
Sulphocysteine	Abcam Ab146303	73.1	48.7	9.9	96.9
Taurine	Sigma 93019	135.6	68.4	33.6	408.1
Tele-methylhistidine	Sigma 67520	9.4	15.4	24.0	5.1
Threonine	Sigma 61506	340.0	193.8	97.5	486.2
Thryptophan	Sigma 51145	121.5	73.2	48.3	145.7
Tyrosine	Sigma 91515	340.4	169.8	16.8	680.0
Valine	Sigma 50848	315.8	146.1	48.3	631.6

All amino acids used are of the highest purity commercially available. Concentrations < 100 micromol/L are given with one decimal place; 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 2021 cycle, 8 x 32 = 256 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 2021). 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 2021). 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 256 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	36.8	39.1	6.8%	7.2%	0.997	0.994	94%	97%	213	13.0%
Alanine	689	705	2.5%	5.2%	0.999	0.998	93%	94%	284	9.79%
Alloisoleucine	117	122	3.5%	6.2%	0.998	0.992	99%	101%	215	12.1%
Arginine	212	210	2.4%	5.5%	0.999	0.998	103%	101%	282	9.12%
Argininosuccinic acid		32.0		18.0%		0.959		57%	139	42.7%
Asparagine	64.1	68.6	1.2%	7.6%	1.000	0.988	113%	113%	257	20.8%
Aspartic Acid	57.4	56.2	3.2%	6.2%	0.996	0.982	81%	76%	270	16.7%
Citrulline	703	721	2.1%	5.9%	1.000	0.999	96%	97%	282	15.0%
Cystine	54.1	53.6	3.6%	8.4%	0.998	0.995	72%	73%	255	12.7%
Glutamic acid	123	131	6.8%	6.5%	0.990	0.991	104%	101%	281	10.3%
Glutamine	777	866	13.2%	6.1%	0.991	0.997	88%	98%	276	11.4%
Glycine	676	718	2.4%	4.8%	0.999	0.997	94%	97%	283	9.51%
Histidine	89.9	93.3	3.8%	5.5%	0.998	0.996	94%	95%	280	9.09%
Hydroxyproline	19.6	19.3	14.7%	16.0%	0.987	0.978	107%	102%	240	22.4%
Isoleucine	499	512	4.0%	4.5%	1.000	0.999	94%	95%	290	11.3%
Leucine	488	524	3.8%	4.6%	1.000	0.999	88%	96%	290	12.2%
Lysine	420	371	2.7%	5.3%	0.998	0.997	114%	98%	284	9.28%
Methionine	OR	328	OR	5.5%	OR	0.998	OR	99%	282	11.8%
Ornithine	339	343	3.1%	5.5%	0.999	0.998	100%	98%	285	10.2%
Phenylalanine	508	533	2.1%	4.4%	1.000	0.998	91%	95%	292	8.67%
Proline	348	356	5.2%	5.7%	0.997	0.996	98%	98%	270	9.71%
Serine	194	201	4.0%	4.9%	0.999	0.999	94%	95%	283	9.25%
Sulfocysteine		47.2		10.9%		0.983		87%	86	21.4%
Taurine	158	165	2.9%	5.1%	1.000	0.998	97%	101%	264	11.0%
Threonine	258	280	3.4%	4.8%	0.998	0.997	93%	97%	281	8.08%
Tryptophan	113	116	3.8%	7.8%	0.995	0.976	88%	94%	228	15.2%
Tyrosine	281	291	2.3%	4.5%	1.000	0.999	91%	95%	292	9.15%
Valine	286	288	1.6%	4.4%	0.999	0.999	103%	99%	291	7.80%
Overall	300	292	4.2%	6.7%	0.997	0.993	96%	95%	260	13.2%

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 2021

In this part the results as seen in the annual report 2021 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 705 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 23 analytes having a recovery within the range 90 - 110%. Poor recovery is evident for four analytes: argininosuccinic acid (57%), aspartic acid (76%), cystine (73%) and sulfocysteine (87%). Asparagine has a recovery of 113%.

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 4.4% for phenylalanine and valine to 18.0% for argininosuccinic acid. 17 amino acids demonstrated good performance with CVs < than 6%. The average intra-lab CV is 6.7%.

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 argininosuccinic acid (0.955) 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   Inter-lab 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 ten amino acids having an inter lab CV of <10% and eleven between 10 and 15%. However, six amino acids have a CV >15% with argininosuccinic acid having a CV of 42.7%.

4.6   Number of Participating Labs and submitted results

For 20 of the individual amino acids, results were submitted in at least 260 datasets (89% of the 293 datasets).

4.7   Inter-relationships 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 = 5.5%), linearity (r = 0.998), recovery (101%) and inter-lab variation (inter-lab CV 9.12) with the majority of labs (n=282 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 6% 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.

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	6%	6%
25%	1%	7%
20 – 25%	2%	9%
15 – 20%	5%	14%
10 – 15%	7%	21%
5 – 10%	15%	36%
0 – 5%	40%	76%
0%	24%	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

Pros and Tele methylhistidine, homocitrulline and sarcosine were present in the eight 2021 samples but were not included in the annual report. Sarcosine and homocitrulline were added to increase awareness of their potential to interfere with alanine (LC-MS/MS) and methionine (IEC). Pros and tele methylhistidine were included to raise awareness of the correct nomenclature for these two compounds. Education is a key aspect of the ERNIDM scheme.

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 (intra-lab variation) versus inter-lab variation for each amino acid reveals three main groups. There are twelve amino acids with good intra and inter-lab precision (<10%). Eleven amino acids show acceptable intea and inter-lab precision, (CVs between 10 – 15and there are five amino acids for which performance is poor, with inter-lab CV > 15% (range 15 – 42%).

Taking all parameters into account there is a group of 20 well-established amino acids for which there is good overall performance, reflected by satisfactory values for all five analytical quality parameters (acceptable precision and inter-lab CV, linearity exceeding 0.9, recovery between 90 and 110% and a high percentage of submitted results. There is also a group of five analytes, argininosuccinic acid, asparagine, aspartic acid, hydroxyproline and sulfocysteine, where perform is less than satisfactory.

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	Inter-lab CV
2-aminobutyric acid	39.1	7.2%	0.994	97%	213	13.0%
Alanine	705	5.2%	0.998	94%	284	9.79%
Alloisoleucine	122	6.2%	0.992	101%	215	12.1%
Arginine	210	5.5%	0.998	101%	282	9.12%
Arginino succinic acid	32.0	18.0%	0.959	57%	139	42.7%
Asparagine	68.6	7.6%	0.988	113%	257	20.8%
Aspartic acid	56.2	6.2%	0.982	76%	270	16.7%
Citrulline	721	5.9%	0.999	97%	282	15.0%
Cystine	53.6	8.4%	0.995	73%	255	12.7%
Glutamic acid	131	6.5%	0.991	101%	281	10.3%
Glutamine	866	6.1%	0.997	98%	276	11.4%
Glycine	718	4.8%	0.997	97%	283	9.51%
Histidine	93.3	5.5%	0.996	95%	280	9.09%
Hydroxyproline	19.3	16.0%	0.978	102%	240	22.4%
Isoleucine	512	4.5%	0.999	95%	290	11.3%
Leucine	524	4.6%	0.999	96%	290	12.2%
Lysine	371	5.3%	0.997	98%	284	9.28%
Methionine	328	5.5%	0.998	99%	282	11.8%
Ornithine	343	5.5%	0.998	98%	285	10.2%
Phenylalanine	533	4.4%	0.998	95%	292	8.67%
Proline	356	5.7%	0.996	98%	270	9.71%
Serine	201	4.9%	0.999	95%	283	9.25%
Sulphocysteine	47.2	10.9%	0.983	87%	86	21.4%
Taurine	165	5.1%	0.998	101%	264	11.0%
Threonine	280	4.8%	0.997	97%	281	8.08%
Thryptophan	116	7.8%	0.976	94%	228	15.2%
Tyrosine	291	4.5%	0.999	95%	292	9.15%
Valine	288	4.4%	0.999	99%	291	7.80%
Overall	292	6.7%	0.993	95%	260	13.2%

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 2022**
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 design of the 2022 scheme is essentially the same as in 2021.

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 6/1/22

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	6 January 2022	• 2021 annual report published

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