ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2019

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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London - Winterswijk, 7 February 2020

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 293 datasets have been submitted, for 13 of them an annual report could not be generated due to insufficient data submission. 8 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. Cas Weykamp as scheme organiser (subcontractor on behalf of the SKML), 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, SKML prepare and dispatch 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 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 2019. 01-05**	**Sample pair 2019. 02-06**	**Sample pair 2019. 03-08**	**Sample pair 2019. 04-07**
2-aminobutyric acid	Sigma A1879	100	4,3	24,6	50,7
Alanine	Sigma 44526	1499	50,3	500	1001
Alloisoleucine	Sigma I8754	250	0,0	26,2	125
Arginine	Sigma 90538	751	24,8	75,1	225
Arginino succinic acid	Sigma A5707	200	9,8	50,5	100
Asparagine	Sigma 51363	100	24,9	49,8	75,8
Aspartic acid	Sigma 51572	100	24,7	51,6	75,2
Citrulline	Sigma 1133842	1200	29,8	300	600
Cystine	Sigma 49603	150	9,9	30,5	99,5
Glutamic acid	Sigma 95436	200	39,6	80,2	120
Glutamine	Sigma 76523	1501	250	500	1000
Glycine	Sigma 76524	1281	79,6	320	640
Histidine	Sigma 73767	480	40,4	79,9	160
Histidine 3-Methyl	Sigma M9005	50,3	50,3	50,3	50,3
Hydroxyproline	Sigma PHR1939	91,1	14,8	29,6	60,4
Isoleucine	Sigma 56241	1099	10,2	100	299
Leucine	Sigma 76526	901	14,8	150	301
Lysine	Sigma 67448	500	19,6	101	250
Methionine	Sigma 39496	750	11,0	50,1	500
Ornithine	Sigma O2375	750	24,8	75,3	225
Phenylalanine	Sigma 40451	1000	15,4	149	300
Proline	Sigma 93693	750	50,6	250	500
Serine	Sigma 54763	500	25,6	49,7	250
Sulphocysteine	Ab146303 (Abcam)	89,0	9,0	44,8	66,9
Taurine	Sigma 93019	499	25,1	51,3	249
Threonine	Sigma 61506	500	25,1	50,2	250
Thryptophan	Sigma 51145	180	30,0	60,0	120
Tyrosine	Sigma 91515	600	19,8	100	300
Valine	Sigma 50848	800	49,7	99,5	300

All amino acids used are of the highest purity commercially available. Concentrations < 100 micromol/L are given with one decimal; 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 long-term 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 28 amino acids in the year 2019 cycle, 8 x 28 = 224 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 2019). 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 2019). 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 224 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	FR	42.5	FR	7.6%	FR	0.996	FR	96%	220	13.6%
Alanine	774	723	5.4%	5.4%	0.993	0.997	98%	94%	288	9.09%
Alloisoleucine	MP	97.0	14.2%	7.0%	0.988	0.998	111%	97%	206	12.9%
Arginine	287	276	6.2%	5.6%	0.998	0.999	101%	97%	283	9.86%
Argininosuccinic acid		48.8		14.8%		0.985		63%	151	50.5%
Asparagine	ORFR	64.7	ORFR	7.9%	ORFR	0.987	ORFR	101%	262	17.6%
Aspartic Acid	74.6	55.9	12.2%	6.7%	0.943	0.992	107%	89%	275	16.1%
Citrulline	576	512	11.1%	6.8%	0.990	0.997	104%	95%	284	11.4%
Cystine	59.5	55.2	20.5%	8.3%	0.982	0.994	86%	74%	260	14.1%
Glutamic acid	119	116	8.2%	7.7%	0.972	0.989	86%	92%	283	10.4%
Glutamine	FR	778	FR	6.2%	FR	0.995	FR	94%	280	11.3%
Glycine	593	556	6.8%	5.2%	0.994	0.998	95%	94%	288	10.1%
Histidine	172	169	4.8%	6.0%	0.998	0.997	84%	83%	283	10.5%
Hydroxyproline		44.5		11.8%		0.982		96%	242	15.3%
Isoleucine	371	353	8.5%	6.6%	0.998	0.999	94%	93%	291	11.8%
Leucine	357	324	8.4%	5.6%	0.997	0.999	105%	94%	290	9.86%
Lysine	228	214	11.1%	5.2%	0.993	0.998	104%	96%	286	8.57%
Methionine	331	307	8.1%	5.9%	0.995	0.998	99%	94%	290	9.73%
Ornithine	278	259	6.2%	5.8%	0.999	0.999	101%	95%	288	12.0%
Phenylalanine	364	341	6.6%	5.5%	0.998	0.999	97%	91%	293	9.83%
Proline	FR	366	FR	6.0%	FR	0.996	FR	95%	272	9.87%
Serine	213	198	9.7%	5.3%	0.995	0.999	101%	95%	284	9.31%
Sulfocysteine		41.8		11.8%		0.978		84%	88	20.4%
Taurine	208	202	4.1%	6.2%	0.997	0.998	96%	97%	264	10.1%
Threonine	223	200	13.4%	5.1%	0.991	0.999	104%	96%	285	9.72%
Tryptophan	111	103	10.2%	8.1%	0.983	0.989	93%	87%	210	15.7%
Tyrosine	258	239	3.9%	5.3%	0.999	0.998	96%	92%	292	9.07%
Valine	308	301	3.5%	5.2%	0.999	0.999	95%	95%	292	8.50%
Overall	295	250	8.7%	7.0%	0.991	0.995	98%	92%	262	13.1%

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 2019

In this part the results as seen in the annual report 2019 will be discussed. Please print out 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 723 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 falling within the range 90 - 110% for all but two amino acids. Under recovery is seen for 6 analytes: argininosuccinic acid (63%), aspartic acid (89%), cystine (74%), histidine (83%), sulfocysteine (84%) and Tryptophan(87%).

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 5.1% for Threonine to 14.8% for argininosuccinic acid. Performance was particularly good for 12 amino acids with CVs < than 6%. The average intralab CV is 7.0%.

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 glutamic acid (0.978) to 8 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 eleven amino acids having an inter lab CV of <10% and ten between 10 and 15%. However, seven amino acids have a CV >15% with argininosuccinic acid having a CV of 50.5%.

4.6 Number of Participating Labs and submitted results

For 21 of the individual amino acids, results were submitted in at least 262 datasets (89% of the 293 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 = 5.6%), linearity (r = 0.999), recovery (97%) and interlab dispersion (interlab CV 9.86%) and many labs (n=283 datasets) submitted 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 (different colours starting from this year) 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. 32% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme 7% 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 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 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%	7%	7%
25%	1%	8%
20 – 25%	3%	11%
15 – 20%	4%	15%
10 – 15%	4%	19%
5 – 10%	10%	29%
0 – 5%	39%	68%
0%	32%	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

Our intention was for the 2019 sample set to include 3-methylhistidine. Unfortunately we experienced some difficulties with the 3-methylhisitidine and as such we did not include the 3-methylhisitidine results in the 2019 scoring. We would like to apologise for any confusion this may have caused participants and use this as a learning opportunity.

The 2019 ERNDIM samples should have included 3-methylhistidine. The material used to prepare them was purchased from Sigma and was labelled as 3-methylhisitidine. In the pre-distribution check, both laboratories reported the expected concentration of 3-methylhistidine. However, it transpires that there is some confusion/dispute as to the nomenclature of 1 and 3-methylhistidine and the material Sigma sell as 3-methylhistidine was identified as 1-methylhistidine by the majority of ERNIDM participants. The two compounds are readily identifiable, the issue is with the nomenclature. We believe the correct nomenclauture is as described below.

Tele-methylhistidine (abbreviated tau-methylhistidine) is the compound traditionally designated by biochemists as ‘3-methylhistidine’ and used as a measure of muscle turnover.CAS No 368-16-1, IUPAC (2*S*)-2-amino-3-(3-methylimidazol-4-yl)propanoic acid.

Pros-methylhistidine (abbreviated pi-methylhistidine) is the compound traditionally designated by biochemists as ‘1-methylhistidine’ and comes predominantly from ingested poultry. Cas No 332-80-9, IUPAC (2*S*)-2-amino-3-(1-methylimidazol-4-yl)propanoic acid.

Tele and pros methylhistidine can be readily identified analytically. The Sigma material added to the 2019 ERNDIM samples was subsequently analysed by mass spectrometry which confirmed it was Pros-methylhistidine, MRM 170.1>126 (tele-methylhistidine has the MRM 170.1>124).

Additional information can be found on the following web sites:
https://folk.uib.no/mfapu/Pages/BV/BVSite/analyteinfo/mHist.html
https://goldbook.iupac.org/terms/view/P04890

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 4).
The overall performance evaluated by comparing precision (within lab variation) versus interlab variation for each amino acid reveals three main groups. There are 19 amino acids with good precision and interlab CVs of 12% or below. Four amino acids show interlab CVs of about 12 – 15% with precision below 12% and there are five 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 aspartate. Aspartate inter-laboratory variation has reduced from 26% in 2008 to 16% in 2018 and 2019.

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, hydroxyproline, sulfocyteine and tryptophan is less satisfactory and this is reflected by more than one analytical quality parameter. Cystine and hydroxyproline demonstrate less satisfactory recovery, but other parameters are adequate. Measurement of these amino acids should be improved.

Table 4. 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	42.5	7.6%	0.996	96%	220	13.6%
Alanine	723	5.4%	0.997	94%	288	9.09%
Alloisoleucine	97.0	7.0%	0.998	97%	206	12.9%
Arginine	276	5.6%	0.999	97%	283	9.86%
Arginino succinic acid	48.8	14.8%	0.985	63%	151	50.5%
Asparagine	64.7	7.9%	0.987	101%	262	17.6%
Aspartic acid	55.9	6.7%	0.992	89%	275	16.1%
Citrulline	512	6.8%	0.997	95%	284	11.4%
Cystine	55.2	8.3%	0.994	74%	260	14.1%
Glutamic acid	116	7.7%	0.989	92%	283	10.4%
Glutamine	778	6.2%	0.995	94%	280	11.3%
Glycine	556	5.2%	0.998	94%	288	10.1%
Histidine	169	6.0%	0.997	83%	283	10.5%
Hydroxyproline	44.5	11.8%	0.982	96%	242	15.3%
Isoleucine	353	6.6%	0.999	93%	291	11.8%
Leucine	324	5.6%	0.999	94%	290	9.86%
Lysine	214	5.2%	0.998	96%	286	8.57%
Methionine	307	5.9%	0.998	94%	290	9.73%
Ornithine	259	5.8%	0.999	95%	288	12.0%
Phenylalanine	341	5.5%	0.999	91%	293	9.83%
Proline	366	6.0%	0.996	95%	272	9.87%
Serine	198	5.3%	0.999	95%	284	9.31%
Sulphocysteine	41.8	11.8%	0.978	84%	88	20.4%
Taurine	202	6.2%	0.998	97%	264	10.1%

Threonine	200	5.1%	0.999	96%	285	9.72%
Thryptophan	103	8.1%	0.989	87%	210	15.7%
Tyrosine	239	5.3%	0.998	92%	292	9.07%
Valine	301	5.2%	0.999	95%	292	8.50%
Overall	250	7.0%	0.995	92%	262	13.1%

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 2020

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 homocitrulline and sarcosine from 2020 onwards. Three selected special amino acids are also included in the 2020 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. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).

London, 07/02/20

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.