ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2018

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
Dr. C. Weykamp Queen Beatrix Hospital MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail: c.w.weykamp@skbwinterswijk.nl	Dr. Rachel Carling Biochemical Sciences, Viapath 4th Floor, North Wing St Thomas' Hospital London SE1 7EH e-mail: Rachel.Carling@viapath.co.uk Prof. B. Fowler University Children's Hospital Basel CH – 4031 Basel Switzerland e-mail : brian.fowler@ukbb.ch	Mrs. Irene de Graaf Queen Beatrix Hospital MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail : i.degraaf@skbwinterswijk.nl	ERNDIM Administration Office Manchester Centre for Genomic Medicine 6th Floor, St Mary's Hospital, Oxford Road, Manchester M13 9WL, United Kingdom. e-mail: admin@erndim.org

London - Winterswijk, 14th December 2018

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 274 datasets have been submitted, for 8 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.

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 2018. 01-06**	**Sample pair 2018. 02-05**	**Sample pair 2018. 03-07**	**Sample pair 2018. 04-08**
2-aminobutyric acid	Sigma A1879	52,2	26,1	5,8	100
5-aminolevulinic acid	Sigma A3785	25,0	150	101	50,8
Alanine	Fluka 05129	500	248	52,0	1001
Arginine	Sigma A6969	200	80,1	19,8	600
Arginino succinic acid	Aldrich A5707	50,1	20,1	4,9	100
Asparagine	Roth KK37.1	120	90,6	30,8	15,9
Aspartic acid	Sigma A8949	198	97,6	50,5	24,7
Citrulline	Sigma C7629	751	251	16,2	7,7
Cystine	Sigma C8755	9,3	149	100	30,5
Glutamic acid	Sigma G1251	125	199	151	99,5
Glutamine	Sigma 49419	1297	851	420	101
Glycine	Sigma G7403	99,4	700	499	300
Histidine	Sigma H8000	50,0	300	150	101
Hydroxyproline	Roth 3893.1	39,9	400	202	80,9
Isoleucine	Roth 3922.1	202	50,1	1000	501
Leucine	Roth 3984.1	200	50,1	1001	498
Lysine	Sigma L5501	79,7	21,5	1000	199
Methionine	Fluka 64319	250	50,1	750	499
Ornithine	Sigma O2375	201	79,7	20,4	1000
Phenylalanine	Fluka 78019	1195	19,9	90,4	398
Proline	Roth T205.1	301	200	101	600
Saccharopine	Sigma S1634	24,3	5,4	10,3	49,7
Serine	Merck 1.07769	252	49,7	25,6	502
Sulphocysteine	Abcam Ab146303	99,7	60,9	30,8	87,7
Taurine	Fluka 86329	50,1	100	251	500
Threonine	Roth T206.1	50,2	102	250	500
Tyrosine	Fluka 93829	719	360	121	30,5
Valine	Roth 4879.1	49,7	249	500	749

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 2018 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 2018). 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 2018). 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 200 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	44.4	45.5	11.2%	7.2%	0.988	0.995	83%	92%	220	10.8%
5-Aminolevulinic acid		74.5		8.2%		0.988		93%	52	20.7%
Alanine	415	423	6.4%	5.0%	0.996	0.998	94%	94%	282	9.35%
Arginine	219	217	3.4%	5.3%	0.999	0.999	97%	96%	281	9.93%
Argininosuccinic acid		31.4		19.8%		0.974		74%	140	56.2%
Asparagine	22.0	22.2	33.2%	23.3%	0.967	0.971	52%	51%	254	42.4%
Aspartic Acid	85.8	88.6	9.8%	6.9%	0.987	0.988	81%	85%	268	16.5%
Citrulline	264	247	9.6%	5.9%	0.998	0.999	102%	96%	277	12.4%
Cystine	46.4	52.0	34.6%	8.9%	0.917	0.993	62%	63%	252	13.5%
Glutamic acid	142	155	12.5%	9.1%	0.945	0.946	107%	112%	278	14.0%
Glutamine	543	573	6.9%	8.3%	0.994	0.994	82%	89%	271	14.0%
Glycine	398	386	5.6%	5.2%	0.996	0.996	101%	93%	282	7.92%
Histidine	130	140	4.3%	6.1%	0.998	0.996	85%	91%	280	9.99%
Hydroxyproline	MP	170	10.4%	8.6%	0.992	0.995	99%	94%	241	14.8%
Isoleucine	417	411	6.1%	5.4%	0.998	0.999	99%	93%	283	9.30%
Leucine	363	414	44.6%	5.1%	0.900	0.998	72%	94%	285	9.04%
Lysine	293	286	0.7%	5.4%	1.000	0.999	89%	86%	281	11.6%
Methionine	369	367	3.4%	5.1%	0.999	0.998	93%	93%	284	8.42%
Ornithine	319	311	2.0%	5.2%	1.000	0.999	97%	94%	284	12.8%
Phenylalanine	442	401	3.9%	5.5%	0.998	0.999	86%	90%	283	12.2%
Proline	278	265	7.4%	6.5%	0.995	0.996	99%	94%	269	9.57%
Saccharopine	ORFR	21.7	ORFR	8.6%	ORFR	0.994	ORFR	92%	89	17.3%
Serine	187	188	3.7%	5.0%	0.998	0.999	95%	95%	279	9.33%
Sulfocysteine	FR	49.2	FR	9.8%	FR	0.972	FR	71%	78	25.9%
Taurine	230	222	4.7%	6.2%	0.999	0.997	104%	95%	262	9.91%
Threonine	225	220	2.4%	4.7%	0.999	0.999	98%	97%	279	8.42%
Tyrosine	287	288	2.6%	4.4%	1.000	0.999	89%	92%	286	9.36%
Valine	378	374	4.8%	5.0%	0.999	0.998	97%	96%	285	7.90%
Overall	265	230	9.8%	7.5%	0.986	0.992	90%	90%	247	14.8%

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 (Interactive Website / Explanation Annual Report)

4. Discussion of Results in the Annual Report 2018

In this part the results as seen in the annual report 2017 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 423 micromol/Liter, 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 7 analytes: asparagine (51%), cytine (63%), sulfocysteine (71%), argininosuccinic acid (74%), aspartic acid (85%), lysine (86%) and glutamine (89%).

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 tyrosine to 23.3% for asparagine. All analytes showed reasonable precision with CVs of < 10%. Performance was particularly good for 14 amino acids with CVs < than 6%. The overall intralab CV is 7.5%.

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 (0.946) to 9 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, with thirteen amino acids having an inter lab CV of <10%, nine between 10 and 15% and six have a CV >15% with 56.2% for argininosuccinic acid.

4.6 Number of Participating Labs and submitted results

For 22 of the individual amino acids, results were submitted by at least 247 labs (90%). Of the others, results were submitted by over 70% of labs for 2 amino acids.

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 = 5.3%), linearity (r = 0.999), recovery (96%) and interlab dispersion (interlab CV 9.93%) and many labs (n=281 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; interactive 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. 27% 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 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%	5%	5%
25%	1%	6%
20 – 25%	2%	8%
15 – 20%	4%	12%
10 – 15%	9%	21%
5 – 10%	15%	36%
0 – 5%	37%	73%
0%	27%	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

Recovery: The low recovery seen for some analytes was not entirely unexpected and is likely a reflection of stability. Glutamine is deaminated to glutamate and asparagine to aspartate, although this does not explain the under recovery of aspartate. Cystine is unstable unless the sample is immediately deproteinised after collection and argininousuccinic acid will readily convert to the two argininosuccinic acid anhydrides.

For educational purposes, the 2018 sample set contained 5-aminolevulinc acid (5-ALA) to highlight its potential to interfere with phenylalanine in samples measured by IEC. 5-ALA co-elutes with phenylalanine on most IEC systems and this was evident in the bimodal distribution of results returned by many laboratories. Careful inspection of the chromatogram may reveal a shoulder visible on the phenylalanine peak and equally, this may be identified by assessment of the 440:570 ratio.
Likewise, the 2018 sample set contained sarcosine. Sarcosine is isobaric with alanine and is a potential interference for mass spectrometry methods unless the two analytes are resolved.

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 15 amino acids with good precision and interlab CVs of 12% or below. Seven amino acids show interlab CVs of about 12 – 15% with precision below 12% and then there are six amino acids which perform poorly, shown here as interlab CV above 15%. This is slightly weakened in comparison to performance in 2017. However, 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.

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, asparagine, sulfocyteine, glutamic acid and aspartic acid is less satisfactory and this is reflected by more than one analytical quality parameter. Lysine, glutamine, cystine 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	45.5	7.2%	0.995	92%	220	10.8%
5-aminolevulinic acid	74.5	8.2%	0.988	93%	52	20.7%
Alanine	423	5.0%	0.998	94%	282	9.35%
Arginine	217	5.3%	0.999	96%	281	9.93%
Arginino succinic acid	31.4	19.8%	0.974	74%	140	56.2%
Asparagine	22.2	23.3%	0.971	51%	254	42.4%
Aspartic acid	88.6	6.9%	0.988	85%	268	16.5%
Citrulline	247	5.9%	0.999	96%	277	12.4%
Cystine	52.0	8.9%	0.993	63%	252	13.5%
Glutamic acid	155	9.1%	0.946	112%	278	14.0%
Glutamine	573	8.3%	0.994	89%	271	14.0%
Glycine	386	5.2%	0.996	93%	282	7.92%
Histidine	140	6.1%	0.996	91%	280	9.99%
Hydroxyproline	170	8.6%	0.995	94%	241	14.8%
Isoleucine	411	5.4%	0.999	93%	283	9.30%
Leucine	414	5.1%	0.998	94%	285	9.04%
Lysine	286	5.4%	0.999	86%	281	11.6%
Methionine	367	5.1%	0.998	93%	284	8.42%
Ornithine	311	5.2%	0.999	94%	284	12.8%
Phenylalanine	401	5.5%	0.999	90%	283	12.2%
Proline	265	6.5%	0.996	94%	269	9.57%
Saccharopine	21.7	8.6%	0.994	92%	89	17.3%
Serine	188	5.0%	0.999	95%	279	9.33%
Sulphocysteine	49.2	9.8%	0.972	71%	78	25.9%
Taurine	222	6.2%	0.997	95%	262	9.91%
Threonine	220	4.7%	0.999	97%	279	8.42%
Tyrosine	288	4.4%	0.999	92%	286	9.36%
Valine	374	5.0%	0.998	96%	285	7.90%
Overall	230	7.5%	0.992	90%	247	14.8%

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 2019

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 sulphocysteine and allisoleucine from 2019 onwards. Three selected special amino acids are also included in the 2019 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, 14/12/18

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.