ERNDIM - Quantitative Schemes

Amino Acids

Dr. Rachel Carling
Biochemical Sciences, Viapath
4th Floor, North Wing
St Thomas' Hospital
London SE1 7EH
Rachel.Carling@viapath.co.uk

Dr. C.W. Weykamp
Queen Beatrix Hospital
MCA Laboratory
P.O. Box 9005
NL – 7100 GG Winterswijk
The Netherlands
e-mail: c.w.weykamp@skbwinterswijk.nl

Prof. B. Fowler
University Children's Hospital Basel
CH – 4031 Basel
Switzerland
e-mail : brian.fowler@ukbb.ch

London / Winterswijk, 18 January 2018

Annual Report ERNDIM-EQAS 2017

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 272 datasets have been submitted, for 14 of them an annual report could not be generated due to insufficient data submission. 7 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 2017. 01-07**	**Sample pair 2017. 02-08**	**Sample pair 2017. 03-06**	**Sample pair 2016. 04-05**
Alpha-aminobutyric acid	Sigma A1879	100	26,1	52,2	4,3
Alanine	Fluka 05129	964	179	320	93,9
Alloisoleucine	Sigma I8754	100	14,8	50,1	4,6
Arginine	Sigma A6969	801	101	251	19,8
Asparagine	Roth KK37.1	15,9	89,6	120	29,9
Aspartic acid	Sigma A8949	25,8	101	200	50,5
Citrulline	Sigma C7629	15,3	751	2000	250
Cystine	Sigma C8755	9,9	100	150	29,8
Glutamic acid	Sigma G1251	101	201	49,8	151
Glutamine	Sigma 49419	425	1300	100	850
Glycine	Sigma G7403	149	748	77,6	450
Histidine	Sigma H8000	100	300	50,0	150
Homocystine	Sigma H6010	5,0	15,6	20,0	10,0
Hydroxyproline	Roth 3893.1	75,2	26,2	50,1	99,1
Isoleucine	Roth 3922.1	1500	100	501	2000
Leucine	Roth 3984.1	480	50,1	100	1200
Lysine	Sigma L5501	599	90,9	300	30,6
Methionine	Fluka 64319	498	49,1	151	12,0
Ornithine	Sigma O2375	750	75,3	225	25,7
Phenylalanine	Fluka 78019	1001	100	400	19,9
Proline	Roth T205.1	60	360	720	119
Serine	Merck 1.07769	9,9	202	399	38,4
Taurine	Fluka 86329	100	299	50,1	150
Threonine	Roth T206.1	151	500	50,2	300
Tyrosine	Fluka 93829	120	720	29,7	360
Valine	Roth 4879.1	99,5	800	49,7	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 25 amino acids in the year 2017 cycle, 8 x 25 = 200 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 2017). 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 2017). 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	47.2	49.4	10.0%	6.5%	0.985	0.983	86%	92%	217	10.5%
Alanine	383	378	12.0%	5.1%	0.998	0.999	95%	94%	278	9.72%
Alloisoleucine	ORFR	44.9	ORFR	6.9%	ORFR	0.995	ORFR	92%	202	12.5%
Arginine	281	284	17.9%	5.4%	0.998	0.999	91%	95%	276	10.1%
Asparagine	55.5	64.2	27.4%	7.9%	0.934	0.993	114%	97%	255	18.6%
Aspartic Acid	75.1	79.1	17.2%	5.5%	0.984	0.997	78%	83%	266	16.2%
Citrulline	653	712	11.1%	5.4%	0.995	0.999	93%	94%	274	14.5%
Cystine	FR	42.4	FR	8.2%	FR	0.992	FR	56%	250	12.4%
Glutamic acid	150	136	10.8%	6.7%	0.979	0.988	108%	100%	278	10.6%
Glutamine	601	627	4.3%	5.8%	0.998	0.997	84%	93%	266	11.0%
Glycine	320	347	1.6%	4.4%	0.999	0.998	83%	95%	277	8.28%
Histidine	126	139	2.4%	5.5%	0.988	0.993	78%	94%	272	10.2%
Hydroxyproline	ORFR	60.2	ORFR	9.5%	ORFR	0.978	ORFR	102%	240	13.5%
Isoleucine	910	964	5.8%	4.9%	0.996	0.998	83%	93%	280	11.6%
Leucine	413	439	3.0%	5.0%	0.999	0.999	85%	95%	282	9.99%
Lysine	214	235	37.6%	5.0%	0.982	0.998	84%	90%	275	9.78%
Methionine	MP	165	21.2%	5.4%	0.987	0.999	71%	91%	277	10.2%
Ornithine	251	258	28.5%	5.1%	0.987	0.999	87%	93%	279	11.3%
Phenylalanine	354	355	6.2%	4.8%	1.000	0.999	88%	91%	282	9.53%
Proline	338	292	21.5%	6.3%	0.982	0.998	113%	94%	264	10.6%
Serine	154	159	17.8%	4.8%	0.987	0.999	101%	96%	275	8.61%
Taurine	128	150	7.9%	4.6%	0.996	0.997	67%	95%	259	8.30%
Threonine	222	246	3.2%	4.3%	0.999	0.998	81%	97%	272	7.54%
Tyrosine	257	287	2.6%	4.4%	0.999	0.999	80%	93%	282	8.15%
Valine	271	307	3.5%	4.3%	0.999	0.999	80%	96%	283	7.88%
Overall	295	273	12.4%	5.7%	0.990	0.996	88%	92%	266	10.9%

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 2017

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 378 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 cystine (56%) and aspartate (83%).

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.
All analytes showed reasonable precision with CVs of < 10%. Performance was particularly good for eighteen amino acids with CVs < than 6%.

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 this is indeed observed for all amino acids; 10 amino acids 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 ten amino acids having an inter lab CV of <10% and thirteen between 10 and 15%.

4.6 Number of Participating Labs and submitted results

For 22 of the individual amino acids, results were submitted by more than 245 labs (90%). Of the others, results were submitted by over 70% of labs for 3 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 valine all parameters indicate good performance: precision (CV = 4.3%), linearity (r = 0.999), recovery (96%) and interlab dispersion (interlab CV 7.88%) and many labs (n=283) 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. 34% 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%	7%	22%
5 – 10%	12%	34%
0 – 5%	32%	66%
0%	34%	100%

Performance is also related to experience. Table 3 shows the number of labs with poor and excellent performance in relation to the time they have participated in ERNDIM schemes: labs with the longest participation (ERNDIM number <100) and labs with the shortest participation (ERNDIM number >300). Numbers from 2015 are shown in brackets for comparison.

Table 3. Performance in relation to length of ERNDIM history

ERNDIM Participation	Number of Labs with Poor Performance Score >15% red flags in 2017 (2016 in brackets)	Number of Labs with Excellent Performance Score 0% red flags in 2017 (2016 in brackets)
Long (Lab code <100)	3 (7)	34 (23)
Short (Lab code >300)	28 (31)	19 (16)

Poor and excellent performance is seen in both groups but the prevalence of excellent performance is higher in the longer standing participants whereas the prevalence of poor performance is higher in the more recent subscribers. This supports the idea that alongside experience, participation in EQA probably plays an important role in improving performance. This reinforces the educational role of ERNDIM.

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.

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. Low values for cystine presumably reflect the known binding to protein and conversion to cysteine-homocysteine mixed disulphide. Few laboratories reported phosphoethanolamine results. This may be due to poor recognition of this compound and/or its unstable nature.

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 then there are two amino acids which perform poorly, shown here as interlab CV above 15%. This is very similar to the performance in 2016.

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 glutamic acid, cystine, hydroxyproline, methionine and aspartate is less satisfactory and this is reflected by more than one analytical

quality parameter. Measurement of these amino acids should be improved. Clinically, the most important one is methionine.

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
Alpha-aminobutyric acid	49.4	6.5%	0.983	92%	217	10.5%
Alanine	378	5.1%	0.999	94%	278	9.72%
Alloisoleucine	44.9	6.9%	0.995	92%	202	12.5%
Arginine	284	5.4%	0.999	95%	276	10.1%
Asparagine	64.2	7.9%	0.993	97%	255	18.6%
Aspartic acid	79.1	5.5%	0.997	83%	266	16.2%
Citrulline	712	5.4%	0.999	94%	274	14.5%
Cystine	42.4	8.2%	0.992	56%	250	12.4%
Glutamic acid	136	6.7%	0.988	100%	278	10.6%
Glutamine	627	5.8%	0.997	93%	266	11.0%
Glycine	347	4.4%	0.998	95%	277	8.28%
Histidine	139	5.5%	0.993	94%	272	10.2%
Hydroxyproline	60.2	9.5%	0.978	102%	240	13.5%
Isoleucine	964	4.9%	0.998	93%	280	11.6%
Leucine	439	5.0%	0.999	95%	282	9.99%
Lysine	235	5.0%	0.998	90%	275	9.78%
Methionine	165	5.4%	0.999	91%	277	10.2%
Ornithine	258	5.1%	0.999	93%	279	11.3%
Phenylalanine	355	4.8%	0.999	91%	282	9.53%
Proline	292	6.3%	0.998	94%	264	10.6%
Serine	159	4.8%	0.999	96%	275	8.61%
Taurine	150	4.6%	0.997	95%	259	8.30%
Threonine	246	4.3%	0.998	97%	272	7.54%
Tyrosine	287	4.4%	0.999	93%	282	8.15%
Valine	307	4.3%	0.999	96%	283	7.88%
Overall	273	5.7%	0.996	92%	266	10.9%

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 2018

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.
Thus for 2018 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2017 scheme and two selected special amino acids are included.

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).