ERNDIM - Quantitative Schemes

Amino Acids

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Annual Report ERNDIM-EQAS 2016

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 280 datasets from laboratories in 46 countries were submitted.

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 2016. 01-06**	**Sample pair 2016. 02-05**	**Sample pair 2016. 03-07**	**Sample pair 2016. 04-08**
Alpha-aminobutyric acid	Sigma A1879	26,1	5,1	50,7	100
Alanine	Fluka 05129	179	90,5	320	961
Alloisoleucine	Sigma I8754	161	39,8	21,6	79,7
Arginine	Sigma A6969	60,3	9,9	320	640
Asparagine	Roth KK37.1	199	49,8	24,9	101
Aspartic acid	Sigma A8949	181	31,4	15,7	60,6
Citrulline	Sigma C7629	250	15,3	751	2000
Cystine	Sigma C8755	72,1	144	24,2	8,1
Glutamic acid	Aldrich 128430	201	80,2	39,6	120
Glutamine	Sigma 49419	1200	401	199	899
Glycine	Sigma G7403	450	752	149	75,6
Histidine	Sigma H8000	160	480	79,9	41,4
Homocitrulline	Bio Connect SC-269298	150	60,0	30,0	90,0
Hydroxyproline	Roth 3893	60,4	91,1	29,6	16,0
Isoleucine	Roth 3922	480	25,0	8,0	72,9
Leucine	Roth 3984	1200	100	50,1	479
Lysine	Sigma L5501	60,3	300	601	150
Methionine	Fluka 64319	30,0	30,0	30,0	31,0
Ornithine	Sigma O2375	25,7	225	750	75,3
Phenylalanine	Fluka 78019	19,9	400	1000	100
Phospho-ethanolamine	Sigma P0503	60,3	29,6	101	122
Proline	Roth T205	251	50,6	500	750
Saccharopine	Sigma S1634	29,7	20,0	120	238
Serine	Merck 1.07769	41,2	11,4	199	399
Taurine	Fluka 86329	150	451	76,4	26,2
Threonine	Roth T206	240	360	120	40,1
Tyrosine	Fluka 93829	250	750	50,3	9,1
Valine	Roth 4879	300	800	99.5	49.7

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 2016 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 2016). 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 2016). 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	47.0	45.8	10.3%	7.1%	0.976	0.997	81%	101%	217	13.7%
Alanine	354	357	15.3%	5.2%	0.990	0.998	84%	95%	272	9.04%
Alloisoleucine	47.4	72.5	22.8%	7.0%	0.973	0.996	67%	95%	194	11.7%
Arginine	238	249	12.9%	4.4%	0.990	0.999	83%	96%	269	8.65%
Asparagine	90.6	88.8	28.6%	10.2%	0.949	0.991	98%	97%	251	19.8%
Aspartic Acid	63.7	49.2	14.1%	9.0%	0.995	0.993	96%	67%	262	19.3%
Citrulline	661	719	12.8%	6.2%	0.985	0.998	75%	95%	266	13.4%
Cystine	OR	38.2	OR	9.2%	OR	0.990	OR	59%	241	13.7%
Glutamic acid	184	120	17.2%	10.2%	0.957	0.987	216%	112%	271	15.3%
Glutamine	587	614	29.2%	7.1%	0.950	0.994	94%	94%	262	10.8%
Glycine	374	350	13.5%	4.8%	0.983	0.998	98%	97%	271	8.81%
Histidine	186	178	10.7%	6.6%	0.995	0.998	92%	91%	266	11.2%
Homocitrulline	ORFR	71.2	ORFR	6.9%	ORFR	0.995	ORFR	87%	118	15.5%
Hydroxyproline	49.2	47.4	7.3%	10.9%	0.835	0.986	79%	94%	232	13.6%
Isoleucine	166	141	28.2%	5.6%	0.989	0.999	115%	94%	271	11.7%
Leucine	486	431	22.5%	5.5%	0.983	0.999	116%	93%	275	10.3%
Lysine	274	261	5.3%	5.3%	0.997	0.998	97%	90%	269	8.18%
Ornithine	269	266	1.8%	4.9%	1.000	0.999	97%	96%	272	10.4%
Phenylalanine	372	354	7.3%	4.9%	0.998	0.999	100%	92%	276	9.48%
Proline	346	346	8.4%	6.5%	0.995	0.996	94%	97%	257	9.54%
Saccharopine	ORFR	99.8	ORFR	5.6%	ORFR	0.998	ORFR	97%	101	11.0%
Serine	148	146	12.4%	5.5%	0.994	0.997	92%	97%	271	9.14%
Taurine	OR	173	OR	5.5%	OR	0.999	OR	96%	255	9.15%
Threonine	202	187	14.9%	4.5%	0.970	0.998	97%	97%	268	7.39%
Tyrosine	257	247	7.8%	5.1%	0.996	0.999	90%	92%	276	9.75%
Valine	318	303	13.1%	5.0%	0.991	0.999	97%	96%	276	8.61%
Overall	260	229	14.4%	6.5%	0.977	0.996	98%	93%	248	11.5%

See this example of part of an annual report.

As agreed last year, the flagging system has been changed. The explanation of the flags can be found in the general information section (Interactive Website / Explanation Annual Report) and is also explained in the mail we sent you on 10th December 2016.

4. Discussion of Results in the Annual Report 2016

In this part the results as seen in the annual report 2016 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 357 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 four amino acids. Under recovery is seen for cystine (59%), aspartate (67%) and homocitrulline (87%). Over recovery is noted for glutamic acid (112%), probably reflecting the breakdown of glutamine to glutamic acid). The recovery of phophoethanolamine has not been included in the report; very few labs report results on this analyte and those that do achieve poor recovery due to stability issues.

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.

With the exception of hydroxyproline, glutamic acid and asparagine, all analytes showed reasonable precision with CVs of < 10%. Performance was particularly good for fourteen 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; the best **r** value is seen for 8 amino acids (r = 0.999). It must be born in mind 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 generally good, with

eleven amino acids having an inter lab CV of <10% and eleven of between 10 and 15%. Methionine performs poorly with an interlab CV of 35.2%, reflecting the inability of many labs to separate methionine from homocitrulline. This is also illustrated by the fact that all labs reporting a high methionine did not report homocitrulline (thus their methionine is the sum of methionine and homocitrulline)

4.6 Number of Participating Labs and submitted results

Of the 280 submitted datasets, 268 allowed complete evaluation of performance. 7 laboratories submitted no results.
For 19 of the individual amino acids, results were submitted by more than 252 labs (90%). Of the others, results were submitted by over 70% of labs for 5 and less than 70% for 3 other 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 = 5.0%), linearity (r = 0.999), recovery (96%) and interlab dispersion (interlab CV 8.61%) and many labs (276) submitted results. Conversely the parameters for methionine indicate performance is less than satisfactory with linearity = 0.436 and interlab CV 36%. The within laboratory precision of 9% suggests that performance of a given laboratory is consistent.

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 flags observed. 23% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are 4% of laboratories with more than 25% 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%	4%	4%
25%	6%	10%
20 – 25%	3%	13%
15 – 20%	5%	18%
10 – 15%	7%	25%
5 – 10%	19%	44%
0 – 5%	33%	77%
0%	23%	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 2015 (2014 in brackets)	Number of Labs with Excellent Performance Score 0% red flags In 2015 (2014 in brackets)
Long (Lab code <100)	7 (3)	23 (16)
Short (Lab code >300)	31 (15)	16 (13)

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 the known binding to protein and conversion to cysteine-homocysteine mixed disulphide. Few laboratories reported phospho ethanolamine results. This may be due to poor recognition of this compound and/or it's unstable nature.

The inability of many laboratories to separate methionine and homocitrulline has been highlighted by the distribution of a sample set containing fixed amounts of methionine and variable concentrations of Homocitrulline.

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 18 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 is a third group of five amino acids with clearly poor performance, shown here as interlab CV above 15%. This is very similar to performance in 2015.

Taking all parameters into account there is a large group of well-established amino acids (about 20) for which there is good overall performance indicated by satisfactory values for all five analytical quality parameters. That is satisfactory precision and interlab CV, linearity exceeding 0.9, recovery between 90 and 110% and a high percentage of submitted results. Performance for the glutamic acid, cystine, hydroxyproline, methionine and aspartate is less satisfactory as indicated mostly by more than one analytical quality parameter. Measurement of these amino acids should be improved and 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	45.8	7.1%	0.997	101%	217	13.7%
Alanine	357	5.2%	0.998	95%	272	9.04%
Alloisoleucine	72.5	7.0%	0.996	95%	194	11.7%
Arginine	249	4.4%	0.999	96%	269	8.65%
Asparagine	88.8	10.2%	0.991	97%	251	19.8%
Aspartic acid	49.2	9.0%	0.993	67%	262	19.3%
Citrulline	719	6.2%	0.998	95%	266	13.4%
Cystine	38.2	9.2%	0.990	59%	241	13.7%
Glutamic acid	120	10.2%	0.987	112%	271	15.3%
Glutamine	614	7.1%	0.994	94%	262	10.8%
Glycine	350	4.8%	0.998	97%	271	8.81%
Histidine	178	6.6%	0.998	91%	266	11.2%
Homocitrulline	71.2	6.9%	0.995	87%	118	15.5%
Hydroxyproline	47.4	10.9%	0.986	94%	232	13.6%
Isoleucine	141	5.6%	0.999	94%	271	11.7%
Leucine	431	5.5%	0.999	93%	275	10.3%
Lysine	261	5.3%	0.998	90%	269	8.18%
Ornithine	266	4.9%	0.999	96%	272	10.4%
Phenylalanine	354	4.9%	0.999	92%	276	9.48%
Proline	346	6.5%	0.996	97%	257	9.54%
Saccharopine	99.8	5.6%	0.998	97%	101	11.0%

Serine	146	5.5%	0.997	97%	271	9.14%
Taurine	173	5.5%	0.999	96%	255	9.15%
Threonine	187	4.5%	0.998	97%	268	7.39%
Tyrosine	247	5.1%	0.999	92%	276	9.75%
Valine	303	5.0%	0.999	96%	276	8.61%
Overall	222	6.6%	0.975	93%	249	12.4%

Interference of homocitrulline and methionine

As we remarked in detail last year we wish to emphasise that a substantial number of labs use a method where homocitrulline interferes with methionine. (Please see information on this in the annual report of 2015).

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 2017

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 2017 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2016 scheme and two selected special amino acids are included. We are very pleased to announce that Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) has been confirmed by the ERNDIM board as scientific advisor for this scheme.
One important change is to include all potential special amino acids on the reporting list without specifying which are actually included in the yearly cycle. Thus at least for the first analysis participants will be challenged to identify which special amino acids are present.

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, Prof. Brian Fowler (Brian.Fowler@ukbb.ch), Dr.Rachel Carling (Rachel.Carling@viapath.co.uk) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).