ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2012

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 255 datasets from laboratories in 45 countries were submitted.

3. *Design*

The scheme has been designed, planned and co-ordinated by Prof. Brian Fowler as scientific advisor and Dr. Cas Weykamp as scheme organiser, both appointed 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.

Table 1.		**Added quantities (micromol/L)**			
Analytes	**Source Sigma (Aldrich)**	**Sample pair 165-172**	**Sample pair 168-170**	**Sample pair 166-169**	**Sample pair 167-171**
Alpha-aminobutyric acid	A1879	5	20	40	60
Alanine	A5824	100	200	400	800
Arginine	A5949	30	60	240	720
Asparagine	A8824	50	100	200	25
Aspartic acid	A8949	25	50	100	15

Aspartylglucosamine	A6681	15	30	45	60
Citrulline	C7629	30	90	360	15
Cystathionine	C3633	80	5	10	40
Cystine	C8755	30	60	120	15
Glutamic acid	(128430)	50	100	250	25
Glutamine	(49419)	400	800	1200	200
Glycine	G7403	500	1250	100	250
Histidine	H8000	270	540	30	90
Hydroxyproline	H3656	90	180	30	60
Isoleucine	I7268	180	540	15	45
Leucine	L5652	810	30	90	270
Lysine	L5501	810	45	90	270
Methionine	(64319)	750	10	50	250
Ornithine	O2375	800	50	100	400
Phenylalanine	(78020)	1050	25	75	350
Pipecolic acid	P1393	20	40	60	80
Proline	P8449	50	150	300	900
Serine	(107769)	12	48	96	384
Taurine	(86329)	50	100	200	400
Threonine	T8534	60	180	360	30
Tryptophan	T9753	100	150	25	50
Tyrosine	(93829)	200	800	10	50
Valine	V0258	750	100	250	500

All amino acids used are of the highest purity commercially available.

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).

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 2012 cycle, 8 x 29 = 232 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 2012). 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 2012). Depending on the responsibilities within the laboratory participants can choose to inspect the annual report (e.g. QC managers) or all (or part of) the 232 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	33.8	30.9	7.5%	6.8%	0.988	0.992	95%	92%	204	11.5%
Alanine	379	373	3.9%	4.2%	0.999	0.998	99%	95%	252	8.13%
Arginine	268	258	2.4%	4.4%	1.000	0.999	99%	95%	247	9.50%
Asparagine	122	99.6	6.2%	6.7%	0.997	0.996	134%	107%	236	24.2%
Aspartic Acid	40.2	46.4	7.4%	6.8%	0.990	0.993	81%	95%	245	19.1%
Aspartyl glucosamine		29.3		7.7%		0.989		86%	49	17.1%
Citrulline	122	122	3.4%	4.7%	1.000	0.999	96%	97%	245	9.44%
Cystathionine	17.4	30.4	6.9%	8.7%	0.992	0.996	50%	91%	181	21.9%
Cystine	44.0	44.8	7.2%	7.4%	0.996	0.994	69%	74%	229	12.6%
Glutamic acid	117	118	3.9%	5.8%	0.999	0.998	99%	99%	251	10.5%
Glutamine	689	619	11.1%	5.6%	0.991	0.996	110%	95%	241	9.40%
Glycine	517	513	2.9%	4.1%	1.000	0.999	97%	96%	252	9.81%
Histidine	243	229	4.0%	5.6%	0.998	0.996	100%	94%	247	11.1%
Histidine 3-Methyl	33.2	28.9	18.8%	7.4%	0.971	0.992	97%	89%	188	13.3%
Hydroxyproline	85.8	86.8	2.5%	7.6%	0.999	0.995	101%	98%	213	12.5%
Isoleucine	192	190	3.2%	4.0%	1.000	0.999	97%	95%	253	9.17%
Leucine	290	290	8.0%	3.9%	0.998	0.999	94%	94%	253	8.71%

Example of part of an annual report

4. *Discussion of Results in the Annual Report 2012*

In this part the results as seen in the annual report 2012 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 373 micromol/Liter, with which you can compare the mean of your lab.

4.2 *Recovery*

A second approach to describe accuracy 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”. Lowest recovery is seen for the sulphur-containing amino acid cystine (74%) which is likely due to binding of this amino acid to protein.

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. The best median precision is observed for valine (CV 3.7%).

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 this is indeed observed for all amino acids; the best **r** value is seen for 13 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. The interlab CV ranges widely from the best of 7.30% for valine to the worst of 27.8% for pipecolic acid.

4.6 Number of Participating Labs and submitted results

Of the 255 labs, 239 submitted sufficient results to allow complete evaluation of performance, 8 submitted insufficient results and 8 laboratories submitted no results. This is quite similar to that seen in 2011.

For 21 of the individual amino acids results were submitted by more than 230 labs (90%). Of the others, results were submitted by over 80% of labs for three and over 70% for two other amino acids. For aspartyl glucosamine only 49 (19%) of labs submitted results. In ion-exchange based systems this substance elutes just before urea and thus should be visible on careful examination of the chromatogram. Similarly only 62 labs reported pipecolic acid, possibly due to inadequate resolution and / or low sensitivity with ninhydrin. We recognise that conventional amino acid analysis is not the method of choice for this compound but nevertheless included this as one of the special amino acids since it may be detectable or even interfere with other amino acids.

Even with those amino acids present at concentrations close to the limit of detection in the basal sample these should be easily measurable in those samples with additions.

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 = 3.7%), linearity (r = 0.998), recovery (97%) and interlab dispersion (interlab CV 7.3%) and many labs (253) submitted results. The opposite is seen for aspartyl glucosamine.

4.8 Your performance: red and green flags

After some years of discussion and planning a system to judge performance of individual laboratories was implemented in January 2009. In the annual report of an

individual laboratory red flags indicate 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 red flag or no result) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular amino acid while a red flag indicates that your laboratory has failed to attain satisfactory performance. Criteria for red flags can be found in the general information on the website (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. 19% of the laboratories have no red flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 3% of laboratories with 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 been able to agree 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 issue a letter of advice of failure to achieve satisfactory performance 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%	3%	3%
20 – 25%	4%	7%
15 – 20%	8%	15%
10 – 15%	6%	21%
5 – 10%	19%	40%
0 – 5%	41%	81%
0%	19%	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).

Table 3. Performance in relation to length of ERNDIM history

ERNDIM Participation	Number of Labs with Poor Performance Score >15% red flags	Number of Labs with Excellent Performance Score 0% red flags
Long (Lab code <100)	1	12
Short (Lab code >300)	19	9

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 nearly exclusively seen in the more recent subscribers. This supports the idea that alongside greater experience participation in EQA probably plays an important role in improving performance and reinforces the educational role of ERNDIM.

4.10 Certificates

As for other schemes the performance, as it is indicated by the red/green 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

First, the results obtained this year agree fairly well with those expected.
Second, some discrepancies with calculated recoveries are evident for a few amino acids with low values for cystine (due to the known binding to protein and conversion to cysteine-homocysteine mixed disulphide) and poor recognition of aspartylglucosamine and pipecolic acid*.*

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 twenty one amino acids with good precision and interlab CVs of 12% or below. Three amino acids show interlab CVs of about 12 – 15% with precison below 10% and there is a third group of three amino acids with clearly poor performance, shown here as interlab CV above 20%. This is very similar to performance in 2011.
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 remaining amino acids is less satisfactory as indicated mostly by more than one analytical quality parameter. Improvement of quality for these analytes needs to be achieved by either better precision within the labs and/or improved standardization as referred to above (4.6).

Table 4. Summary of results of all laboratories

Analyte	Accuracy (mean)	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	30.9	6.8%	0.992	92%	204	11.5%
Alanine	373	4.2%	0.998	95%	252	8.13%
Arginine	258	4.4%	0.999	95%	247	9.50%
Asparagine	99.6	6.7%	0.996	107%	236	24.2%
Aspartic Acid	46.4	6.8%	0.993	95%	245	19.1%

Aspartyl glucosamine	29.3	7.7%	0.989	86%	49	17.1%
Citrulline	122	4.7%	0.999	97%	245	9.44%
Cystathionine	30.4	8.7%	0.996	91%	181	21.9%
Cystine	44.8	7.4%	0.994	74%	229	12.6%
Glutamic acid	118	5.8%	0.998	99%	251	10.5%
Glutamine	619	5.6%	0.996	95%	241	9.40%
Glycine	513	4.1%	0.999	96%	252	9.81%
Histidine	229	5.6%	0.996	94%	247	11.1%
Histidine 3-Methyl	28.9	7.4%	0.992	89%	188	13.3%
Hydroxyproline	86.8	7.6%	0.995	98%	213	12.5%
Isoleucine	190	4.0%	0.999	95%	253	9.17%
Leucine	290	3.9%	0.999	94%	253	8.71%
Lysine	276	4.5%	0.999	87%	252	9.81%
Methionine	246	4.9%	0.999	92%	253	9.86%
Ornithine	330	4.7%	0.999	96%	252	9.78%
Phenylalanine	354	4.5%	0.999	92%	254	8.91%
Pipecolic acid	46.7	13.1%	0.960	101%	62	27.8%
Proline	304	5.6%	0.999	85%	234	10.5%
Serine	143	4.4%	0.999	102%	251	9.64%
Taurine	187	4.4%	0.998	97%	230	8.32%
Threonine	159	4.1%	0.999	98%	250	7.30%
Tryptophan	98.6	6.4%	0.989	87%	191	11.9%
Tyrosine	234	3.9%	0.999	83%	254	10.4%
Valine	393	3.7%	0.998	97%	253	7.30%
Overall	**203**	**5.7%**	**0.996**	**93%**	**225**	**12.1%**

Educational Effect of ERNDIM

Longer participation in ERNDIM schemes clearly seems to contribute to improved performance. This is probably due to the learning/educational effect of EQA as provided by ERNDIM.

6. *Preview of the Scheme for 2013*

Our continuing policy is to include the same common amino acids in each years samples as well as a few unusual ones which are selected year to year.
Thus for 2013 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2012 scheme and three 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, Prof. Brian Fowler (Brian.Fowler@ukbb.ch) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)