ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2013

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 283 datasets from laboratories in 46 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 (subcontractor on behalf of the SKML), both 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 Sigma (Aldrich)**	**Sample pair 173-179**	**Sample pair 175-178**	**Sample pair 174-177**	**Sample pair 176-180**
Alpha-aminobutyric acid	A1879	61	40	22	5
Alanine	A5824	808	406	202	101
Arginine	A5949	970	242	81	21
Asparagine	A8824	50	177	127	76
Aspartic acid	A8949	20	80	61	41
Aspartylglucosamine	A6681	18	14	9	5
Citrulline	C7629	91	31	15	363
Cystathionine	C3633	5	81	40	10
Cystine	C8755	76	25	10	101
Glutamic acid	(128430)	30	152	91	60
Glutamine	(49419)	303	1514	909	606
Glycine	G7403	253	102	1265	507
Histidine	H8000	101	51	303	203
Histidine 1-Methyl (CAS number 332-80-9)	(67520)	5	61	41	20
Histidine 3-Methyl (CAS number 368-16-1)	M9005	76	25	10	51
Hydroxyproline	H3656	51	25	102	76
Isoleucine	I7268	52	11	757	253
Leucine	L5652	30	1211	323	121
Lysine	L5501	50	404	202	101
Methionine	(64319)	9	909	242	32
Ornithine	O2375	50	808	404	101
Phenylalanine	(78020)	26	1060	353	75
Pipecolic acid	P1393	121	91	60	30
Proline	P8449	606	303	152	75
Sarcosine	S7672	52	25	152	102
Serine	(107769)	447	111	56	13
Taurine	(86329)	364	121	60	31
Threonine	T8534	202	101	51	303
Tyrosine	(93829)	61	15	157	98
Valine	V0258	101	757	505	253

All amino acids used are of the highest purity commercially available. Samples have been tested for stability and homogeneity according to ISO 13528.

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 anonimised 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 30 amino acids in the year 2013 cycle, 8 x 30 = 240 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 2013). 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 2013). 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 240 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	32.4	33.4	13.2%	6.5%	0.978	0.994	94%	104%	213	9.94%
Alanine	351	371	7.6%	4.2%	0.998	0.999	89%	96%	259	8.59%
Arginine	296	316	12.5%	5.1%	0.997	0.999	85%	94%	257	11.3%
Asparagine	103	116	14.2%	6.3%	0.973	0.991	114%	108%	239	21.4%
Aspartic Acid	51.7	47.9	10.6%	5.6%	0.943	0.967	110%	86%	252	18.0%
Aspartyl glucosamine		7.36		19.7%		0.944		73%	47	34.7%
Citrulline	111	120	15.6%	5.4%	0.995	0.999	88%	95%	253	10.3%
Cystathionine	28.3	32.9	9.8%	7.8%	0.997	0.996	89%	99%	186	18.0%
Cystine	37.8	41.4	15.9%	9.0%	0.986	0.992	68%	74%	232	13.9%
Glutamic acid	92.4	93.5	9.9%	6.5%	0.987	0.993	114%	99%	259	10.9%
Glutamine	852	785	2.8%	5.6%	0.998	0.996	112%	95%	246	10.2%
Glycine	449	511	6.3%	4.3%	0.997	0.999	81%	94%	259	10.1%
Histidine	144	154	8.9%	6.1%	0.991	0.996	84%	91%	255	9.85%
Histidine 1-Methyl	29.4	28.1	9.9%	6.1%	0.989	0.995	100%	86%	193	12.9%
Histidine 3-Methyl	30.5	32.4	11.7%	7.3%	0.982	0.994	69%	79%	193	14.6%
Hydroxyproline	59.5	60.6	4.9%	8.8%	0.992	0.985	88%	93%	218	13.3%
Isoleucine	244	255	10.5%	4.6%	0.997	0.999	89%	93%	262	10.5%
Leucine	384	396	8.8%	4.8%	0.999	0.999	94%	92%	262	10.3%
Lysine	165	171	9.3%	4.3%	0.997	0.998	87%	85%	257	7.48%
Methionine	271	273	8.2%	4.5%	1.000	1.000	92%	91%	262	10.3%

Example of part of an annual report

4. *Discussion of Results in the Annual Report 2013*

In this part the results as seen in the annual report 2013 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 371 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 aspartyl glucosamine (73%).

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 threonine (CV 3.9%).

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 14 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 7.03% for threonine to the worst of 34.7% for aspartyl glucosamine.

4.6 Number of Participating Labs and submitted results

Of the 283 labs, 253 submitted sufficient results to allow complete evaluation of performance, 13 submitted insufficient results and 17 laboratories submitted no results. The number of poor and non-submitters is somewhat higher than in 2012. For 20 of the individual amino acids results were submitted by more than 253 labs (90%). Of the others, results were submitted by over 80% of labs for two and over 70% for three other amino acids. For aspartyl glucosamine only 47 (18%) 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 56 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 = 4.0%), linearity (r = 0.999), recovery (98%) and interlab dispersion (interlab CV 7.29%) and many labs (264) 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. 20 of the laboratories have no red flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are 2% 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%	2%	2%
20 – 25%	4%	6%
15 – 20%	7%	13%
10 – 15%	6%	19%
5 – 10%	18%	37%
0 – 5%	43%	80%
0%	20%	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 of 2012 are in brackets.

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)	6 (1)	13 (12)
Short (Lab code >300)	19 (19)	11 (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. It is of note that the number of poor performing labs within the experienced group increased from 1 to 6- This emphasises that high level of performance cannot be taken for granted. We suggest that this may for example depend on replacement of retired persons by less experienced new staff pointing to the need for well-planned and timely succession.

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 amino acids with good precision and interlab CVs of 12% or below. Four amino acids show interlab CVs of about 12 – 15% with precison below 12% 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 2012.

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	33.4	6.5%	0.994	104%	213	9.94%
Alanine	371	4.2%	0.999	96%	259	8.59%
Arginine	316	5.1%	0.999	94%	257	11.3%
Asparagine	116	6.3%	0.991	108%	239	21.4%
Aspartic Acid	47.9	5.6%	0.967	86%	252	18.0%
Aspartyl glucosamine	7.36	19.7%	0.944	73%	47	34.7%
Citrulline	120	5.4%	0.999	95%	253	10.3%
Cystathionine	32.9	7.8%	0.996	99%	186	18.0%
Cystine	41.4	9.0%	0.992	74%	232	13.9%
Glutamic acid	93.5	6.5%	0.993	99%	259	10.9%
Glutamine	785	5.6%	0.996	95%	246	10.2%
Glycine	511	4.3%	0.999	94%	259	10.1%
Histidine	154	6.1%	0.996	91%	255	9.85%
Histidine 1-Methyl	28.1	6.1%	0.995	86%	193	12.9%
Histidine 3-Methyl	32.4	7.3%	0.994	79%	193	14.6%
Hydroxyproline	60.6	8.8%	0.985	93%	218	13.3%
Isoleucine	255	4.6%	0.999	93%	262	10.5%
Leucine	396	4.8%	0.999	92%	262	10.3%
Lysine	171	4.3%	0.998	85%	257	7.48%
Methionine	273	4.5%	1.000	91%	262	10.3%
Ornithine	327	4.3%	0.999	95%	260	9.28%
Phenylalanine	350	4.2%	0.999	91%	264	9.79%
Pipecolic acid	69.4	10.8%	0.974	99%	56	27.0%
Proline	243	5.1%	0.998	85%	240	9.58%
Sarcosine	79.2	10.9%	0.988	98%	171	17.5%
Serine	150	4.9%	0.999	96%	258	9.29%
Taurine	140	4.8%	0.999	96%	238	8.90%
Threonine	160	3.9%	0.998	96%	258	7.03%
Tyrosine	82.6	4.8%	0.997	97%	265	8.86%
Valine	389	4.0%	0.998	95%	264	7.29%
Overall	**195**	**6.3%**	**0.993**	**93%**	**229**	**12.7%**

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 2014

Our continuing 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 2014 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2013 scheme and four special amino acids are included. We are very pleased to announce that Dr. Rachel Webster (e-mail: rachel.webster@uhb.nhs.uk) has taken on the role of deputy scientific advisor for this 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, Prof. Brian Fowler (Brian.Fowler@ukbb.ch) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)