ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2014

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 269 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.

Analyte	Source	Added quantities (micromol/L)			
		Sample pair 182-187	Sample pair 184-185	Sample pair 181-188	Sample pair 183-186
Alpha-aminobutyric acid	Sigma A1879	4,7	9,4	20,4	81,6
Alanine	Fluka 05129	76,3	229	450	1002
Alloisoleucine	Sigma I8754	80,2	160	19,7	39,5
Arginine	Sigma A6969	20,0	79,9	241	960
Asparagine	Roth KK37.1	125	175	49,6	75,5
Aspartic acid	Sigma A8949	59,6	80,2	19,5	40,1
Citrulline	Sigma C7629	11,1	24,9	200	1001
Cystine	Sigma C8755	10,8	24,9	75,4	101
Glutamic acid	Aldrich 128430	91,3	150	29,7	60,5
Glutamine	Sigma 49419	900	1500	300	600
Glycine	Sigma G7403	151	300	903	51,7
Histidine	Sigma H8000	100	201	300	51,1
Hydroxyproline	Roth 3893	49,4	76,5	100	25,9
Isoleucine	Roth 3922	50,6	250	751	9,9
Leucine	Roth 3984	419	800	38,2	80,2
Lysine	Sigma L5501	200	401	50,9	99,6
Methionine	Fluka 64319	242	900	7,6	32,5
Ornithine	Sigma O2375	1081	29,8	90,2	360
Phenylalanine	Fluka 78020	799	15,7	75,4	398
Phospho-ethanolamine	Sigma P0503	181	29,8	59,6	89,4
Proline	Roth T205	602	74,5	150	299
Saccharopine	Sigma S1634	50,4	100	150	300
Sarcosine	Sigma S7672	25,4	50,9	99,9	151
Serine	Merck 107769	15,4	60,0	240	420
Taurine	Fluka 86329	31,0	59,5	120	361
Threonine	Roth T206	102	200	300	50,3
Tyrosine	Fluka 93829	60,7	240	960	15,2
Valine	Roth 4879	240	399	900	80,1

All amino acids used are of the highest purity commercially available. Concentrations < 100 micromol/L 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 2014 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 2014). 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 2014). 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 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	31.8	29.6	11.6%	6.5%	0.995	0.996	95%	90%	214	13.3%
Alanine	398	414	5.2%	4.3%	0.998	0.999	89%	91%	265	8.16%
Alloisoleucine		72.3		5.4%		0.998		97%	181	12.0%
Arginine	303	307	8.6%	4.8%	0.998	1.000	87%	93%	262	11.6%
Asparagine		108		7.1%		0.989		115%	246	21.3%
Aspartic Acid	39.4	36.5	14.4%	9.1%	0.975	0.965	108%	92%	254	18.8%
Citrulline	307	298	3.9%	5.0%	0.999	1.000	97%	96%	259	11.6%
Cystine	32.5	36.4	31.7%	9.7%	0.942	0.991	59%	66%	242	16.6%
Glutamic acid	N/A	96.8	13.1%	8.7%	N/A	0.986	N/A	102%	263	13.7%
Glutamine	668	753	8.5%	5.4%	0.993	0.995	75%	98%	253	10.0%
Glycine	342	345	4.0%	4.6%	0.999	0.999	93%	96%	265	9.18%
Histidine	164	156	13.3%	4.9%	0.993	0.996	93%	93%	263	10.5%
Hydroxyproline	64.0	60.6	8.5%	8.8%	0.992	0.984	111%	99%	225	12.2%
Isoleucine	246	251	1.3%	4.3%	0.998	0.999	88%	95%	264	9.17%
Leucine	322	315	2.6%	4.7%	1.000	0.999	99%	95%	265	9.01%
Lysine	175	167	2.4%	4.1%	0.999	0.999	95%	87%	263	8.24%
Methionine	285	271	5.1%	5.0%	1.000	0.999	97%	92%	267	11.3%
Ornithine	398	379	17.4%	5.5%	0.993	0.999	101%	96%	265	12.2%
Phenylalanine	318	301	2.3%	4.4%	1.000	0.999	98%	93%	269	8.80%
Phospho ethanolamine		19.8		11.2%		0.942		51%	167	25.2%
Proline	252	262	4.3%	5.5%	0.995	0.998	85%	99%	250	9.18%
Saccharopine		147		5.2%		0.996		96%	96	14.2%
Sarcosine	68.6	78.0	12.0%	9.6%	0.936	0.987	78%	94%	181	16.6%
Serine	N/A	132	1.3%	5.2%	N/A	0.997	N/A	76%	264	8.95%
Taurine		141		4.8%		0.999		98%	247	8.76%
Threonine	N/A	158	9.9%	4.2%	N/A	0.998	N/A	98%	262	7.18%
Tyrosine	299	297	4.9%	4.8%	0.999	0.999	91%	92%	269	10.6%
Valine	401	389	3.8%	4.3%	0.998	0.999	97%	97%	267	7.98%
Overall	256	215	8.3%	6.0%	0.990	0.993	92%	92%	242	12.0%
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv

Example of part of an annual report. N/A means that no calculations could be made due to no results, too few results or too many outliers. In the next generation of the website this will be specified.

4. Discussion of Results in the Annual Report 2014

In this part the results as seen in the annual report 2014 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 414 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 phospho-ethanolamine (51%) possibly reflecting instability of the stored compound. The only other amino acids with recovery below 90% are cysteine (66%) due to binding of the compound to protein and serine (76%) which can not really be explained but may be due to impurity of the purchased compound.

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 lysine (CV 4.1%) compared with worst of 11.2% for phospho-ethanolamine.

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.

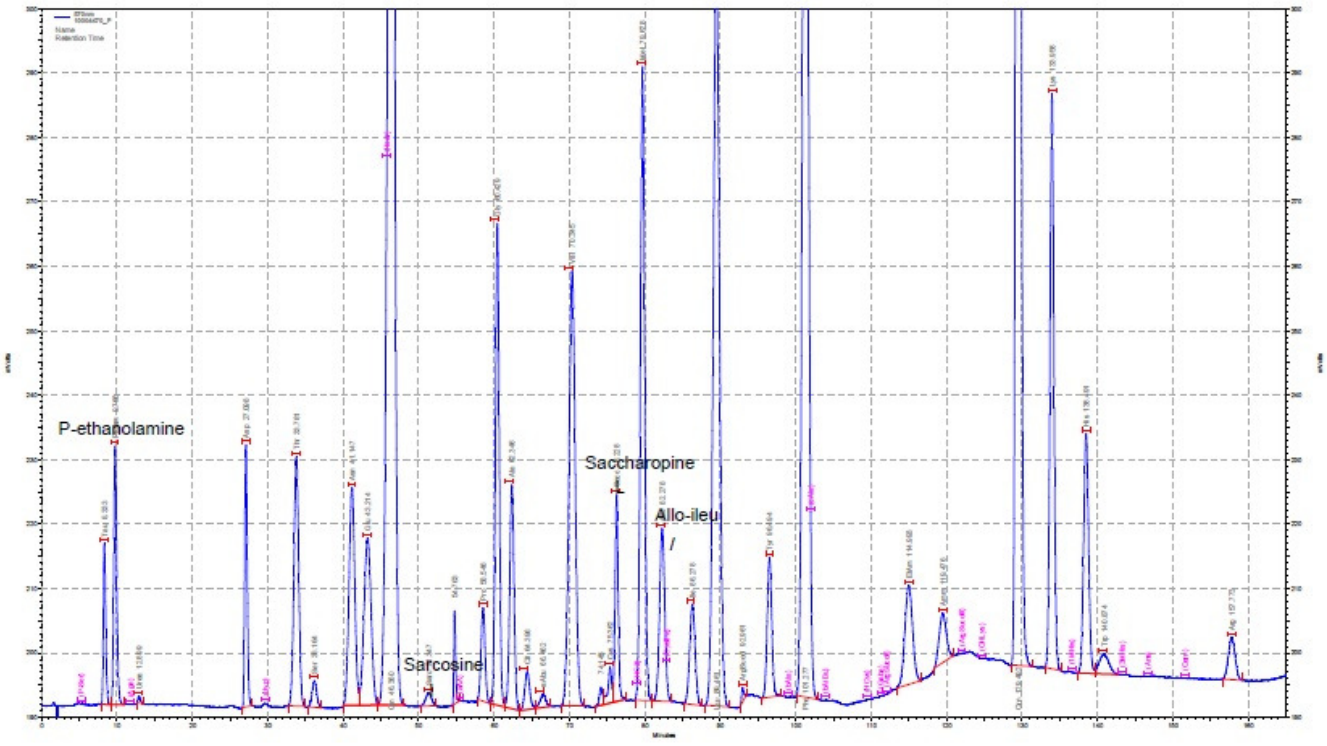
4.5 *Interlab CV*

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 7.18% for threonine to the worst of 25.2% for phospho-ethanolamine.

4.6 *Number of Participating Labs and submitted results*

Of the 269 submitted datasets, 254 allowed complete evaluation of performance.15 laboratories submitted no results.

For 22 of the individual amino acids results were submitted by more than 242 labs (90%). Of the others, results were submitted by over 70% of labs for two and less than 70% for four other amino acids. For saccharopine only 96 (36%) labs submitted results which is difficult to understand since at least using ion-exchange chromatography it can be separated from the closely eluting cystine (see figure) and authentic materials is available for standardisation.



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Chromatogram kindly provided by Prof. M. Hersberger, Dept. Clinical Chemistry, Univ. Child. Hosp. Zürich

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 (102%) and interlab dispersion (interlab CV 7.98%) and many labs (267) submitted results. The opposite is seen for phospho-ethanolamine.

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 red flags 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 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 (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. 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 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 issue a letter pointing out failure to achieve these levels 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%
20 – 25%	3%	7%
15 – 20%	6%	13%
10 – 15%	9%	22%
5 – 10%	20%	42%
0 – 5%	39%	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). Numbers of 2013 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 In 2014 (2013 in brackets)	Number of Labs with Excellent Performance Score 0% red flags In 2014 (2013 in brackets)
Long (Lab code <100)	3 (6)	17 (13)
Short (Lab code >300)	20 (19)	11 (11)

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. High level of performance cannot be taken for granted and 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 phospho ethanolamine (which in part may be related to instability of this compound).

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 nineteen amino acids with good precision and interlab CVs of 12% or below. Five amino acids show interlab CVs of about 12 – 15% with precison below 12% and there is a third group of five amino acids with clearly poor performance, shown here as interlab CV above 16%. This is very similar to performance in 2013.
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.

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	29.6	6.5%	0.996	90%	214	13.3%
Alanine	414	4.3%	0.999	91%	265	8.2%
Alloisoleucine	72.3	5.4%	0.998	97%	181	12.0%
Arginine	307	4.8%	1.000	93%	262	11.6%
Asparagine	108	7.1%	0.989	115%	246	21.3%
Aspartic acid	36.5	9.1%	0.965	92%	254	18.8%
Citrulline	298	5.0%	1.000	96%	259	11.6%
Cystine	36.4	9.7%	0.991	66%	242	16.6%
Glutamic acid	96.8	8.7%	0.986	102%	263	13.7%
Glutamine	753	5.4%	0.995	98%	253	10.0%
Glycine	345	4.6%	0.999	96%	265	9.2%
Histidine	156	4.9%	0.996	93%	263	10.5%
Hydroxyproline	60.6	8.8%	0.984	99%	225	12.2%
Isoleucine	251	4.3%	0.999	95%	264	9.2%
Leucine	315	4.7%	0.999	95%	265	9.0%
Lysine	167	4.1%	0.999	87%	263	8.2%
Methionine	271	5.0%	0.999	92%	267	11.3%
Ornithine	379	5.5%	0.999	96%	265	12.2%
Phenylalanine	301	4.4%	0.999	93%	269	8.8%
Phospho ethanolamine	19.8	11.2%	0.942	51%	167	25.2%
Proline	262	5.5%	0.998	99%	250	9.2%
Saccharopine	147	5.2%	0.996	96%	96	14.2%
Sarcosine	78.0	9.6%	0.987	94%	181	16.6%
Serine	132	5.2%	0.997	76%	264	9.0%
Taurine	141	4.8%	0.999	98%	247	8.8%
Threonine	158	4.2%	0.998	98%	262	7.2%
Tyrosine	297	4.8%	0.999	92%	269	10.6%
Valine	389	4.3%	0.999	97%	267	8.0%
Overall	215	6.0%	0.993	92%	242	12.0%

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 2015*

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 2015 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2014 scheme and four special amino acids are included. We are very pleased to announce that Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) has taken over the role of deputy scientific advisor for this scheme and we thank Rachel Webster for her input in this role over the past two years.

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)