ERNDIM - Quantitative Schemes
Amino Acids

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

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

Annual Report ERNDIM-EQAS 2011

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 245 datasets from laboratories in 44 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 159-161**	**Sample pair 157-163**	**Sample pair 160-164**	**Sample pair 158-162**
Alpha-aminobutyric acid	A1879	5	10	30	60
5-aminolaevulinic acid	A3785	25	50	100	150
Alanine	A5824	80	240	720	1000
Allo-isoleucine	I8754	10	40	120	360
Arginine	A5949	30	60	240	720

Asparagine	A8824	25	200	100	50
Aspartic acid	A8949	15	100	50	25
Aspartylglucosamine	A6681	8	20	40	60
Citrulline	C7629	10	25	200	800
Cystine	C8755	15	30	60	120
Glutamic acid	(128430)	25	250	100	50
Glutamine	(49419)	200	1200	800	400
Glycine	G7403	1080	180	360	720
Histidine	H8000	540	30	90	270
Hydroxyproline	H3656	180	30	60	90
Isoleucine	I7268	540	15	45	180
Leucine	L5652	320	960	40	80
Lysine	L5501	270	810	45	90
Methionine	(64319)	250	750	10	50
Ornithine	O2375	250	500	25	75
Phenylalanine	(78020)	400	1200	20	90
Proline	P8449	150	300	900	50
Serine	(107769)	50	200	300	10
Taurine	(86329)	100	200	400	50
Threonine	T8534	360	180	60	30
Tryptophan	T9753	150	25	50	100
Tyrosine	(93829)	800	200	50	10
Valine	V0258	900	300	150	75

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 2011 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 2011). 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 2011). 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	26.3	25.2	1.7%	7.9%	0.998	0.995	92%	92%	199	15.5%
5-Aminolevulinic acid	80.6	74.5	5.1%	7.6%	0.997	0.993	101%	95%	76	19.3%
Alanine	502	492	3.4%	4.7%	0.998	0.998	97%	98%	242	9.10%
Alloisoleucine	129	124	3.3%	5.9%	0.999	0.999	93%	93%	181	12.6%
Arginine	262	250	3.0%	4.9%	1.000	0.999	98%	96%	237	10.3%
Asparagine	96.2	99.8	3.4%	7.8%	0.999	0.994	100%	107%	231	23.8%
Aspartic Acid	41.7	38.4	2.0%	7.6%	0.980	0.981	77%	75%	238	20.0%
Aspartyl glucosamine	31.3	26.9	6.2%	10.4%	0.997	0.988	101%	93%	55	38.6%
Citrulline	252	252	5.0%	5.5%	0.999	0.999	93%	96%	233	12.1%
Cystine	41.6	40.8	3.1%	6.8%	1.000	0.997	74%	74%	217	12.4%
Glutamic acid	114	110	3.4%	6.2%	0.996	0.996	94%	94%	241	11.8%
Glutamine	563	596	1.8%	6.2%	0.998	0.996	88%	95%	234	11.0%
Glycine	546	562	1.9%	4.5%	1.000	0.998	89%	95%	242	8.65%
Histidine	213	220	1.0%	5.6%	1.000	0.998	92%	94%	238	10.2%
Hydroxyproline	77.5	87.6	15.6%	7.2%	0.983	0.995	86%	99%	203	11.3%
Isoleucine	182	174	1.4%	4.8%	1.000	0.999	94%	88%	243	8.42%
Leucine	326	324	3.4%	4.4%	0.999	0.999	91%	91%	242	8.94%

Example of an annual report

4. *Discussion of Results in the Annual Report 2011*

In this part the results as seen in the annual report 2011 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 492 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 leucine (CV 4.5%).

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 12 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 8.02% for valine to the worst of 38.6% for aspartylglucosamine.

4.6 Number of Participating Labs and submitted results

Of the 245 labs, 233 submitted sufficient results to allow complete evaluation of performance, 8 submitted insufficient results and 4 laboratories submitted no results. This is similar to that seen in 2010.

For 21 of the individual amino acids results were submitted by more than 220 labs (90%). Of the others, results were submitted by over 80% of labs for three and over 70% for two other amino acids. For 5-amino laevulinic acid only 76 laboratories (31%) and for aspartyl glucosamine only 55 (23%) of labs submitted results. With modern amino acid analysers employing ion-exchange chromatography a separation and quantitation of all the amino acids present in the distributed samples is possible. However one difficulty with the samples this year is the closeness of elution times of 5-amino laevulinic acid and phenylalanine in ion-exchange systems. This can be overcome by running a special programme for separation of 5-amino laevulinic acid. It is reflected in the higher than usual interlab CV for phenylalanine.

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. As in 2010 results suggested that some laboratories experienced difficulties in the separation of cystine and saccharopine.

There has been confusion on the nomenclature of methylhistidines. Thus histidine-3-methyl has the CAS-number 368-16-1 and will be included in 2012 samples whereas the previously included "methyl-histidine that was included has the CAS-number 332-80-9

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 alanine all parameters indicate good performance: precision (CV = 4.7%), linearity (r = 0.998), recovery (98%) and interlab dispersion (interlab CV 9.1%)

and many labs (242) 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. 16% 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%	2%	5%
15 – 20%	7%	12%
10 – 15%	10%	22%
5 – 10%	19%	41%
0 – 5%	43%	84%
0%	16%	100%

Performance is also related to experience. Table 3 shows the number of labs with a poor and an excellent performance in relation to the time they have participated in ERNDIM schemes: labs with the longest history of participation (ERNDIM number <100) and labs with the shortest history of participation (ERNDIM number >300).

Table 3. Performance in relation to length of ERNDIM history

ERNDIM History	Number of Labs with Poor Performance Score >15% red flags	Number of Labs with Excellent Performance Score 0% red flags
Long History (Lab code <100)	6	18
Short History (Lab code >300)	17	3

Poor and excellent performance is seen in both groups but the prevalence of excellent performance is 6 times higher in the longer standing participants whereas the prevalence of poor performance is 3 times higher in the more recent subscribers. This provides good evidence that participation in EQA does indeed improve 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 new style of 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).

Quantitative comparisons

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 10% and there is a third group of four amino acids with clearly poor performance, shown here as interlab CV above 20%. This is very similar to performance in 2010.
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).

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 2012

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 2012 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2010 scheme and four 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)