ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2010

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 235 datasets from laboratories in 43 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 (Merck)**	**Sample pair 149-156**	**Sample pair 150-153**	**Sample pair 151-155**	**Sample pair 152-154**
Alanine	A5824	107	960	1333	320
Alpha-aminobutyric acid	A1879	7	40	80	13
Allo-isoleucine	I8754	33	133	267	67
Arginine	A5949	20	160	800	60
Arginino succinic acid	A5707	133	400	533	267
Asparagine	A8824	27	107	53	213
Aspartic acid	A8949	13	40	20	80
Citrulline	C7629	13	267	1067	33
Cystine	C8755	27	80	120	53
Glutamic acid	128430	27	80	53	240
Glutamine	(49419)	333	1000	667	1333
Glycine	G7403	1440	480	960	240
Histidine	H8000	960	107	320	53
Hydroxyproline	H3656	200	67	100	33
Isoleucine	I7268	480	43	171	11
Leucine	L5652	427	53	107	1280
Lysine	L5501	480	40	120	960
Methionine	(64319)	192	11	32	768
Ornithine	O2375	333	33	100	667
Phenylalanine	(78020)	533	27	120	1600
Phospho Ethanolamine	P0503	33	133	267	67
Proline	P8449	160	960	80	480
Saccharopine	S1634	40	120	160	80
Serine	(107769)	67	400	13	267
Taurine	(86329)	120	400	20	160
Threonine	T8534	267	100	33	167
Tryptophan	T9753	200	67	133	33
Tyrosine	(93829)	867	60	20	233
Valine	V0258	1200	200	100	400

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 29 amino acids in the year 2010 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 2010). 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 2010). 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).

4. Discussion of Results in the Annual Report 2010

In this part the results as seen in the annual report 2010 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 658 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 recoveries are seen for the sulphur-containing amino acid cystine (56%) and for phospho-ethanolamine (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 phenylalanine (CV 4.3%).

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 11 amino acids (r = 0.999). It must be born in mind that only a limited concentration range is tested in this scheme.

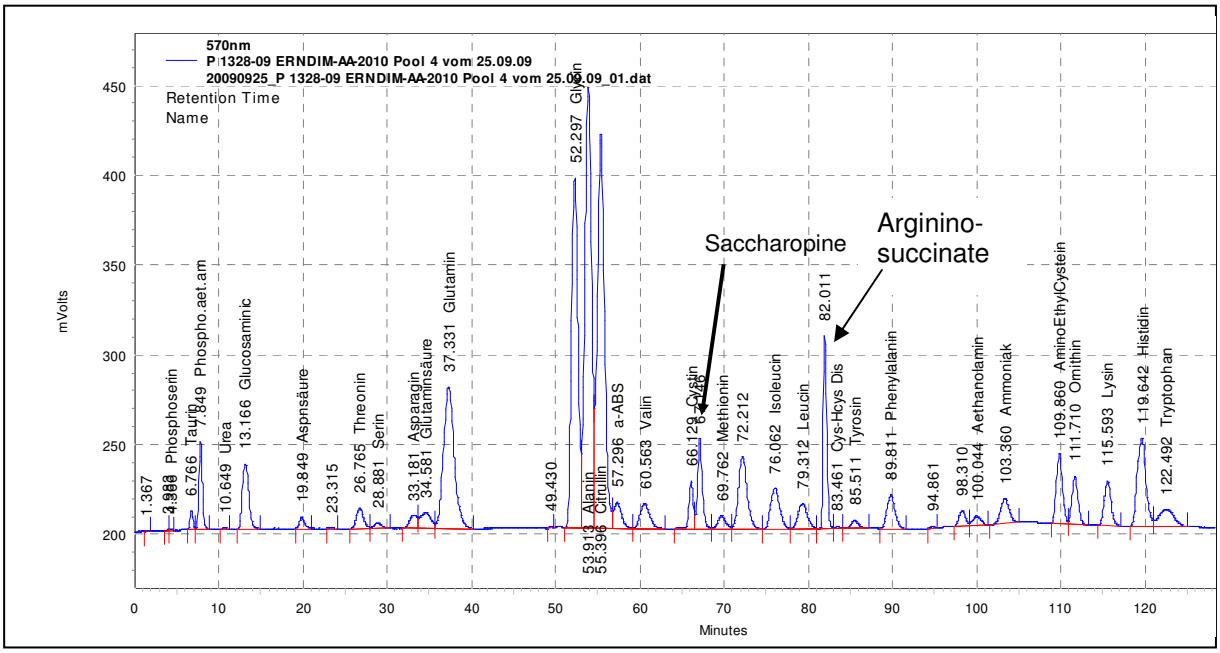
4.5 Interlab CV

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 Interlaboratory 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 8.66% for threonine to the worst of 26.9% for arginino-succinic acid.

4.6 Number of Participating Labs and submitted results

Of the 235 labs, 223 submitted sufficient results to allow complete evaluation of performance, 7 submitted insufficient results and 5 laboratories submitted no results. This is an improvement on 2009 when 8 submitted insufficient results and 22 submitted no results

For 21 of the individual amino acids results were submitted by more than 211 labs (90%). Of the others, results were submitted by over 80% of labs for three and over 70% for three other amino acids. For saccharopine only 113 laboratories (48%) and for arginino-succinate only 142 (60%) 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. 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 2009 results suggested that some laboratories experienced difficulties in the separation of cystine and saccharopine. The separation obtained using the Biochrom ion exchange system is shown in the figure below. See chromatogram below.



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 (94%) and interlab dispersion (interlab CV 9.1% and many labs (234) submitted results. The opposite is seen for argininosuccinic acid.

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. 17% 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 4% 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%	4%	4%
20 – 25%	2%	6%
15 – 20%	5%	11%
10 – 15%	7%	18%
5 – 10%	26%	44%
0 – 5%	39%	83%
0%	17%	100%

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) and phospho-ethanolamine. Borderline recoveries (between 80 and 85%) are seen for tryptophan and serine. Such discrepancies may be attributable to problems with standardisation or low purity of the commercial amino acid products used.

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 eighteen amino acids with good precision and interlab CVs of 12% or below. Seven amino acids show interlab CVs of about 12 – 15% with precison below 10% and there is a third group of 4 amino acids with clearly poor performance, shown here as interlab CV above 20%. This is very similar to performance in 2009.
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).

6. Preview of the Scheme for 2011

* 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 2011 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@unibas.ch) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)