Annual Report ERNDIM-EQAS Quantitative Amino Acids 2003

1. Purpose

The purpose of the ERNDIM External Quality Assurance Scheme for Quantitative Organic 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.ERNDIMQA.nl

2. Participants

171 laboratories from 26 countries submitted results within the Scheme.

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

The scheme consisted of 8 lyophilised samples, all prepared from the same basic human serum but with various amounts of added analytes. 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 amount of added analytes.

Table 1.		**Added quantities (micromol/L)**			
Analytes	**Source Sigma (Code)**	**Sample pair 93-98**	**Sample pair 94-96**	**Sample pair 95-100**	**Sample pair 97-99**
Alanine	A5824	400,0	119,8	1000	700
Alpha-aminobutyric acid	A1879	10,1	6,3	25,2	17,6
Arginine	A5949	303,9	212,9	121,6	15,3
Asparagine	A8824	20,1	12,6	50,4	35,2
Aspartic acid	A8949	20,1	12,6	50,4	35,2
Citrulline	C7629	10,4	148,7	104,2	59,4
Cystine	C8755	53,3	30,5	13,0	76,1
Glutamine	(49419)	83,5	1189,2	833,3	475,2
Glutamic acid	G6904	80,0	24,0	200,0	140,0
Glycine	G7403	355,6	203,0	86,5	507,5
Histidine	H8000	106,7	60,9	26,0	152,3
1-Methyl Histidine	M9005	50,4	37,4	20,2	5,6
Hydroxyproline	H3656	50,4	37,4	20,2	5,6
Isoleucine	I7268	142,2	81,2	34,6	203,0
Leucine	L5652	300,0	89,8	750,0	525,0
Lysine	L5501	213,4	121,8	51,9	304,5
Methionine	(64319)	13,9	198,2	138,9	79,2
Ornithine	O2375	202,6	142,0	81,0	10,2

Phenylalanine	(78020)	582,1	407,6	233,0	29,7
Proline	P8449	80,0	24,0	200,0	140,0
Serine	S8407	80,0	24,0	200,0	140,0
Taurine	(86329)	17,4	247,8	173,6	99,0
Threonine	T8534	80,0	24,0	200,0	140,0
Tyrosine	(93829)	405,2	283,9	162,1	20,4
Valine	V0258	284,5	162,4	69,2	406,0
l-Alloisoleucine	I8754	202,6	142,0	81,0	10,2
l-alpha-amino adipic acid	A7275	13,9	198,2	138,9	79,2
Phosphoethanolamine	P0503	71,1	40,6	17,3	101,5
Beta-alanine	A7752	100,7	74,7	40,4	11,3

Reports

All data-transfer, the submission of data as well as request and viewing of reports proceeded via the interactive website www.erndimqa.nl

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 technically reports could be immediately available a delay time of 14 days has been introduced to enable the scientific advisor to inspect the results and add his comment to the report.

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 232 such Analyte-in-Detail-reports can be requested in the year 2003 cycle for the 29 amino acids). A more condensed report is the "Current Report" which summarises the performance of all analytes in a specific sample (8 such Current-Reports can be requested in 2003). 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 2003). Depending on the responsibilities within the laboratory participants can choose to inspect the annual report (QC managers) or all (or part of) the 232 detailed reports (scientific staff).

4. Discussion of Results in the Annual Report 2003

In this part the results as seen in the annual report 2003 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 up to you 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 541 micromol/Liter with which you can compare the mean of your lab.

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 (do we have to add "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”. For 25 of the reported amino acids the median recovery is between 90 and 110%: e.g. 97% for alanine). Four amino acids are outside the 100 +/- 10% window. The 74% recovery of cystine is low and can be attribued to its binding to proteins in the sample. For asparagine and glutamic acid recoveries of 77% and 86% are seen. The recovery of phosphoethanolamine is low at 18%, probably due to lack of stability during the preparation of the lyophilised samples.

4.2 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 CV’s can be calculated. The column “Precision” in the annual report shows your CV’s for the respective amino acids in comparison to median values for all labs. The best median precision is observed for Leucine (CV 4.1%) and the worst for phosphoethanolamine (23.7%). A CV of greater than 10% is observed for only four amino acids.

4.3 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 tyrosine (r = 0.9982).

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 who submitted results is shown in the column “Data all labs” in the annual report. The best Interlab CV is seen for leucine (median CV of 6.7%) and the worst for hydroxyproline (97.2%).

4.6 Number of Participating Labs and submitted results

In total 173 laboratories received samples and 171 submitted results. For most of the individual amino acids results were submitted by more than 150 labs. For two amino acids there are less than 140 labs. 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. The ability to report on all 29 amino acids is a good test of your system. Deviations in obtained values from median values could indicate poor calibration pointing to the need for careful selection and testing of standards. In fact all but one of the 29 amino acids are available in the Sigma calibration mixture so that this should not be the cause of poor performance. Please note that not everything is what it seems to be. Some amino acids in the commercial calibration mixtures may not be stable (asparagine for example).

4.7 Interrelationships between quality parameters

The various parameters described above often have an interrelation in that usually more than one parameter points in the same direction towards either good or bad analytical quality.

For example for threonine all parameters indicate good performance: precision (CV = 4.3), linearity (r = 0.9980), Recovery (98%) and Interlab Dispersion (Interlab CV 6.9%) and many labs (170) submitted results. The opposite is seen for asparagine and hydroxyproline. For each of these the Interlab CV exceeds 20% and most other statistical parameters are also less satisfactory.

5. Summary

There is a large group (about 20 amino acids)of well-established amino acids for which there is overall good performance indicated by satisfactory values for all five analytical quality parameters. That is a precision below 8%, an interlab CV below 10%, a linearity exceeding 0.9, a recovery between 90 and 110% and a high degree of participation. Performance for the remaining 10 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 a better precision within the labs and/or standardization as referred to above (4.6).

6. Preview of the Scheme for 2004

* In 2003 some amino acids have been removed from the list of analytes and one new one has been added.
* The concentrations of a number of analytes have been modified compared with those in the 2003 scheme.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions 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)