Annual Report ERNDIM-EQAS Quantitative Amino Acids 2004

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

172 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 (Merck)**	**Sample pair 101-106**	**Sample pair 102-105**	**Sample pair 103-108**	**Sample pair 104-107**
Alanine	A5824	397	119	991	694
Alpha-aminobutyric acid	A1879	10	6	25	18
Arginine	A5949	298	208	74	12
Asparagine	A8824	20	12	50	35
Aspartic acid	A8949	20	12	49	34
Citrulline	C7629	150	105	38	6
Cystine	C8755	52	30	13	74
Glutamine	(49419)	84	1202	838	479
Glutamic acid	G6904	80	24	200	140
Glycine	G7403	349	199	85	498
Histidine	H8000	107	61	26	153
1-Methyl Histidine	M9005	50	35	20	6
Hydroxyproline	H3656	50	35	20	6
Isoleucine	I7268	77	23	192	134
Leucine	L5652	773	541	193	30
Lysine	L5501	210	120	51	300
Methionine	(64319)	198	138	50	8
Ornithine	O2375	201	141	50	8

Phenylalanine	(78020)	98	29	245	171
Proline	P8449	79	24	198	138
Saccharopine	S1634	16	228	159	91
Serine	S8407	80	24	199	139
Taurine	(86329)	18	254	177	101
Threonine	T8534	80	24	200	140
Tyrosine	(93829)	266	152	64	379
Valine	V0258	281	160	68	401
l-alpha-amino adipic acid	A7275	10	6	25	18

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 enables the scientific advisor to inspect the results and add 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 216 such Analyte-in-Detail-reports can be requested in the year 2004 cycle for the 27 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 2004). 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 2004). Depending on the responsibilities within the laboratory participants can choose to inspect the annual report (QC managers) or all (or part of) the 216 detailed reports (scientific staff).

4. Discussion of Results in the Annual Report 2004

In this part the results as seen in the annual report 2004 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 563 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 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 26 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 serine a recovery of 88% is seen.

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 4.3 %) and the worst for cystine (12.4%). 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 ornithine (r = 0.9985).

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 phenylalanine (median CV of 6.8%) and the worst for cystine (206%) and saccharopine (110.2%). The interlab CV of cystine is remarkable: we did not see this in previous years. Inspection of the individual samples, especially the histograms in the analyte in detail report, shows that in specimen 102/105 some 30 labs observe a cystine between 206 and 260 micromol/liter whereas only 30 micromol/liter cystine is added. A similar phenomenon is seen in specimen 103/108: 26 labs measure cystine between 115 and 160 where only 13 micromol/liter cystine was added. The only difference with the specimens of last year is that the 2004-series contained also saccharopine. In specimen 102/105 228 micromol/liter was added and in 103/108 159 micromol/liter: about the amounts measured for cystine by some 30 labs. It has to be assumed that the separation and identification of cystine and saccharopine by these laboratories is unsatisfactory.

4.6 Number of Participating Labs and submitted results

In total 174 laboratories received samples and 172 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 27 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 the amino acids are present 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 leucine all parameters indicate good performance: precision (CV = 4.3%), linearity (r = 0.9983), Recovery (97%) and Interlab Dispersion (Interlab CV 7.6%) and many labs (171) 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 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 2005

* 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 2005 the common amino acids remain although for some the range of concentrations have been modified compared with those in the 2004 scheme and four special amino acids are included.

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)