ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2006

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.erndim.unibas.ch / www.ERNDIMQA.nl

2. *Participants*

A total of 193 laboratories from 26 countries subscribed to 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 117-123**	**Sample pair 119-122**	**Sample pair 118-121**	**Sample pair 120-124**
Alanine	A5824	103	222	460	937
Alpha-aminobutyric acid	A1879	18	10	25	6
Arginine	A5949	5	45	94	392
Asparagine	A8824	12	20	50	35
Aspartic acid	A8949	42	13	27	5
Citrulline	C7629	90	10	239	30
Cystine	C8755	9	119	59	29
Glutamine	(49419)	80	324	1122	598
Glutamic acid	G6904	165	204	294	109
Glycine	G7403	187	387	787	87
Histidine	H8000	297	19	58	138
1-Methyl Histidine	M9005	34	49	5	20
Hydroxyproline	H3656	16	28	40	4
Isoleucine	I7268	7	47	145	293
Leucine	L5652	1053	101	338	11
Lysine	L5501	104	462	223	24
Methionine	(64319)	57	117	2	896
Ornithine	O2375	197	8	78	137
Phenylalanine	(78020)	946	469	350	8
Proline	P8449	151	231	470	71
Serine	S4500	11	67	126	285
Taurine	(86329)	248	16	98	173
Threonine	T8534	131	191	16	72
Tyrosine	(93829)	471	232	24	83
Valine	V0258	266	581	59	148
Sulphocysteine	C2196	49	74	98	25
Cystathionine	C3633	4	9	23	45
Homocystine	H6010	99	10	25	50
Sarcosine	S7672	100	747	50	249

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

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 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 216 such Analyte-in-Detail-reports can be requested in the year 2006 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 2006). 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 2006). 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 2006

In this part the results as seen in the annual report 2006 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.

4.0 Hydrolysis of glutamine and asparagine

Although samples have been prepared exactly as in previous years some subtle changes appear to have occurred leading to hydrolysis of glutamine (to glutamic acid) and asparagine (to aspartic acid). The instability of these is reflected in the performance for these analytes in the annual report; poor reproducibility, linearity and deviating recovery. It is important to bear this in mind when inspecting your report.

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 434 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". Low recoveries are seen for the sulphur-containing amino acids: cystine, homocystine and sulphocysteine.

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 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 Valine (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 methionine (r = 0.9997). It must be born in mind that only a limited 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 best Interlab CV is seen for valine (median CV of 7.3%).

4.6 Number of Participating Labs and submitted results

Of the 193 subscribing labs 163 labs submitted sufficient results to allow complete evaluation of performance, 20 insufficient and 10 submitted no results..

For most of the individual amino acids results were submitted by more than 160 labs. For one amino acid there were just 103 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.

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 leucine all parameters indicate good performance: precision (CV = 5.4%), linearity (r = 0.9993), Recovery (96%) and Interlab Dispersion (Interlab CV 9.5%) and many labs (186) submitted results. The opposite is seen for sulfocysteine.

5. *Summary of performance*

The attached figure shows the overall performance by comparing precision (within lab variation) versus interlab variation for each amino acid. Generally three main groups are evident. There are ten amino acids with good precision and interlab CVs below 10%. Eleven amino acids show interlab CVs of about 10 – 20% with precison below 10% and there is a third group of 8 amino acids with clearly poor performance, shown here as interlab CV above 26% but note there is a wide range from 21% for +-methylhistidine to over 200% for homocysteine. It must be noted that in performance for glutamine, glutamic acid, asparagine and aspartic acid is clearly worse than in 2005 (see figure) for the reasons mentioned in 4.0 above.

Taking all parameters into account 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 and interlab CV as shown in the figure, 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).

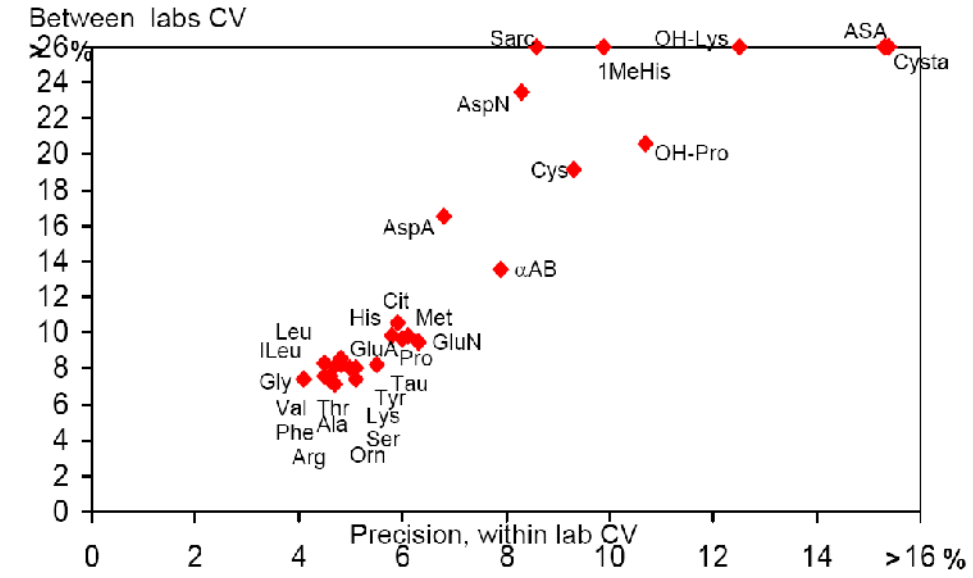
6. *Preview of the Scheme for 2007*

* 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 2007 the common amino acids remain although for some the range of concentrations have been modified compared with those in the 2006 scheme and four special amino acids are included.
* An important aim of the Scientific Advisory Board and ERNDIM Board is to introduce measures for the assessment of an individual laboratory's overall performance in all schemes both proficiency testing and quantitative. With this in mind a pilot judgment system for all quantitative ERNDIM schemes was developed in 2005 and this was presented at the last ERNDIM meeting in Prague. This has been applied to samples from 2006. It is intended by the ERNDIM board to provide each lab with a detailed listing of their own performance. It is also planned to indicate in participation certificates how many amino acids were analysed and for how many satisfactory performance was attained.

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)

Amino acid QC scheme 2005: precision vs. interlab variation



Amino acid QC scheme 2006: precision vs. interlab variation