ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2007

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 205 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 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 125-131**	**Sample pair 127-132**	**Sample pair 128-130**	**Sample pair 126-129**
Alanine	A5824	104	224	704	944
Alpha-aminobutyric acid	A1879	30	10	40	6
Arginine	A5949	15	45	245	395
Asparagine	A8824	10	25	75	50
Aspartic acid	A8949	43	13	28	5
Citrulline	C7629	100	10	500	20
Cystathionine	C3633	5	10	50	100
Cystine	C8755	9.5	89.5	59.5	29.5
Glutamic acid	G6904	10	35	185	85
Glutamine	(49419)	240	480	1200	720
Glycine	G7403	187	387	987	37
Histidine	H8000	459	39	99	219
1-Methyl Histidine	M9005	35	50	5	20
Homocystine	H6010	200	20	50	100
Hydroxyproline	H3656	20	35	50	5
Isoleucine	I7268	7.5	72.5	147.5	447.5
Leucine	L5652	1061	101	341	11
Lysine	L5501	134	584	284	34
Methionine	(64319)	27	117	2	797
Ornithine	O2375	198	8	78	138
Phenylalanine	(78020)	793	393	73	8
Proline	P8449	152	232	472	72
Sarcosine	S7672	100	750	50	250
Serine	S4500	11	67	127	187
Sulphocysteine	C2196	50	75	100	25
Taurine	(86329)	249	16	99	174
Threonine	T8534	132.5	292.5	16.5	72.5
Tyrosine	(93829)	594	234	24	54
Valine	V0258	390	690	60	150

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.unibas.ch).

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 of 14 days enables 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 216 such Analyte-in-Detail-reports can be requested in the year 2007 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 2007). 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 2007). 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 216 detailed reports (e.g. scientific staff).

4. Discussion of Results in the Annual Report 2007

In this part the results as seen in the annual report 2007 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 Losses of glutamine and asparagine

This year's samples revealed very poor recoveries of glutamine and asparagine with concomitant increases of glutamic acid and aspartic acid. These changes were due to a problem in the manufacture of samples and unfortunately this was the case for all the samples for 2007. We apologise for this and hope for your understanding. Detailed studies of possible reasons for this allowed us to identify the cause and we have been able to produce samples with the correct "physiological" levels of these amino acids for 2008. This manufacturing problem is reflected in the performance for these analytes as shown in the annual report, especially poor reproducibility, linearity and deviating recovery. Please 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 446 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 as well as glutamine and asparagine, with high recoveries of glutamic acid and aspartic acid (see above).

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 4.2%).

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 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 best Interlab CV is seen for valine (median CV of 7.0%).

4.6 Number of Participating Labs and submitted results

Of the 205 subscribing labs, 172 submitted sufficient results to allow complete evaluation of performance, 22 insufficient and 11 submitted no results..

For most of the individual amino acids results were submitted by more than 160 labs. For one amino acid (sulfocysteine) there were just 105 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 valine all parameters indicate good performance: precision (CV = 4.2%), linearity (r = 0.9984), recovery (96%) and interlab dispersion (interlab CV 7.0%) and many labs (204) submitted results. The opposite is seen for sulfocysteine.

5. Summary of performance

General comments

First, the results obtained this year agree fairly well with those expected with the exception of the problems with glutamine / glutamic acid and asparagine / aspartic acid which has already been explained and corrected for future samples.
Second, some discrepancies with calculated recoveries are evident for a few amino acids with low values for homcystine (due to the known binding to protein and conversion to cysteine-homocysteine mixed disulphide), and also for arginine, cystathionine, proline, serine and sulphocysteine. Such discrepancies may be attributable to problems with standardisation or low purity of the commercial amino

acid products used. The latter possibility will be investigated.
Third, most amino acids are reported by nearly all labs except for cystathionine (76%), homocystine (74%), hydroxyproline (82%), sarcosine (78%) and sulphocysteine (45%). We would like to receive feedback from labs unable to report these amino acids to ascertain the reasons for this. Also there are several labs which register for the scheme and receive samples but do not submit any results.
It should be noted that the mixed disulphide of cysteine and homocysteine has a similar elution time to that of nor-leucine which is sometimes used as internal standard. The presence of this disulphide, which is even present in normal plasma (2-3 µmol/L) may therefore lead to errors in quantitation.

Quantitative comparisons

The overall performance evaluated by comparing precision (within lab variation) versus interlab variation for each amino acid reveals three main groups. Excluding glutamine and asparagine (see 4.0 above) there are ten amino acids with good precision and interlab CVs below 10%. Twelve amino acids show interlab CVs of about 10 – 20% with precison below 10% and there is a third group of 5 amino acids with clearly poor performance, shown here as interlab CV above 20% but note there is a wide range from 20.3% for aspartic acid to 155% for hydroxyproline. 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 2008

* 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 2008 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2007 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 2006 ERNDIM meeting in Prague (see also the ERNDIM website (http://www.erndim.unibas.ch/) under meetings and reports . This will be applied to samples from 2007. 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 the participation certificates how many amino acids were analysed and for how many satisfactory performance was attained. These modifications will be implemented in the course of 2008, along with an update of the website which will allow laboratories to see their own relative performance for each individual amino acid. We will inform you about these changes in due course.
* Fourth, we have prepared a questionnaire on the scheme and hope that all participants will complete this. It will provide valuable information on many aspects of amino acid analysis which should benefit all of us. We apologise to labs which did not receive the questionnaire when we first circulated it in October. So far 62% of participating labs have replied. Thanks for taking part in this exercise which will provide a unique overview of current practice which we plan to circulate as soon as possible.

7. Questions, Comments and Suggestions

User comments: the opportunity exists within the "reports" page to make comments on specific findings. This year 8 comments were submitted: one referring to glutamine/asparagine stability; one pointing out analytical difficulties using HPLC; one reporting an additional peak following leucine which is likely to be due to cysteine-homocysteine disulphide; two result corrections; two pointing out that they had not received the amino acid questionnaire; two pointing out possible anomalies in the evaluation of results. One laboratory questioned how precision and recovery are calculated and wondered whether this is related to their apparent general overestimation of values.
In the future we will endeavour to respond to such comments more promptly.
Please do not give in both your name and ERNDIM number in order to maintain confidentiality.

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)