ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2008

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 216 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 133-138**	**Sample pair 136-140**	**Sample pair 135-139**	**Sample pair 134-137**
Alanine	A5824	100	200	600	900
Alpha-aminobutyric acid	A1879	10	15	30	50
Arginine	A5949	20	60	180	360
Asparagine	A8824	25	50	400	10
Aspartic acid	A8949	20	35	50	12
Citrulline	C7629	15	30	150	600
Cystine	C8755	10	30	60	90
δ-aminolaevulinic acid	A3785	20	40	80	120
Glutamic acid	128430	50	100	200	25
Glutamine	(49419)	480	720	1200	240
Glycine	G7403	180	360	1080	60
Histidine	H8000	120	240	480	60
1-Methyl Histidine	M9005	20	40	60	5
Hydroxylysine	H0377	50	100	150	250
Hydroxyproline	H3656	30	40	60	20
Isoleucine	I7268	75	150	450	10
Leucine	L5652	250	1000	25	100
Lysine	L5501	300	600	50	150
Methionine	(64319)	120	800	5	30
Ornithine	O2375	144	360	12	72
Phenylalanine	(78020)	320	960	20	80
Proline	P8449	480	80	160	240
Sarcosine	S7672	40	80	160	320
Serine	S8407	210	10	52	105
Sulphocysteine	C2196	25	50	75	100
Taurine	(86329)	250	17	100	175
Threonine	T8534	280	30	80	140
Tyrosine	(93829)	700	30	60	240
Valine	V0258	50	150	400	800

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 2008 cycle, 8x29 = 232 such Analyte-in-Detail-reports can be requested). 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 2008). 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 2008). 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 2008

In this part the results as seen in the annual report 2008 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 429 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 acids: cystine (68%) and sulfocysteine (72%).

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 methionine (CV 4.8%).

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 = 1.000). 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 Tyrosine (8.73%).

4.6 Number of Participating Labs and submitted results

Of the 216 subscribing labs, 190 submitted sufficient results to allow complete evaluation of performance, 7 submitted insufficient results and 19 laboratories submitted no results.

For most of the individual amino acids results were submitted by more than 170 labs. For one amino acid (5-aminolevulinic acid) only 83 laboratories submitted results (see below). 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 methionine all parameters indicate good performance: precision (CV = 4.8%), linearity (r = 1.000), recovery (92%) and interlab dispersion (interlab CV 11.3% and many labs (203) submitted results. The opposite is seen for sulfocysteine.

4.8 Your performance: red and green flags

After some years of discussion and planning a system to judge performance of individual laboratories is implemented starting from 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. 16% 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 2% 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%	2%	2%
20 – 25%	4%	6%
15 – 20%	4%	10%
10 – 15%	12%	22%
5 – 10%	24%	46%
0 – 5%	38%	84%
0%	16%	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 and sulphocysteine, due to the known binding to protein and conversion to cysteine-homocysteine mixed disulphide. Borderline recoveries (between 80 and 90%) are seen for 2-aminobutyric acid, 5-Aminolevulinic acid, 1-Methyl-Histidine, Proline and Tyrosine. Such discrepancies may be attributable to problems with standardisation or low purity of the commercial amino acid products used.
One specific problem was clearly evident for 5-aminolevulinic acid. Only the very small number of 83 laboratories submitted results for this amino acid. This most likely reflects its lack of adequate separation from the closely eluting phenylalanine with many amino acid analysers. Hopefully the inclusion of this amino acid in this years scheme will have prompted laboratories to optimize their elution conditions to separate this amino acid. In this context an application note for a short programme for

rapid analysis of 5-aminolevulinic acid in an ion-exchange based amino acid analyser has been published by biochrom (see www.biochrom.co.uk).

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 twelve amino acids with good precision and interlab CVs of 10% or below. Thirteen amino acids show interlab CVs of about 11 – 20% 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%. 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 2009

* 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 2009 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2008 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)