	ERNDIM - Quantitative Schemes Amino Acids		
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Annual Report ERNDIM-EQAS 2009

1. **Purpose**

The ERNDIM External Quality Assurance Scheme for Quantitative Amino Acids fulfils the purpose of monitoring 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 241 laboratories in 27 countries subscribed to the scheme and received samples. Of these, 219 laboratories submitted results.

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 reduce the levels of amino acids present as much as possible 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. Analytes	Source Sigma (Merck)	Added quantities (micromol/L)			
		Sample pair 142-145	Sample pair 141-146	Sample pair 143-148	Sample pair 144-147
Alanine	A5824	100	200	600	900
Alpha-aminobutyric acid	A1879	10	15	30	50
Allo-isoleucine	I8754	100	50	25	200
Arginine	A5949	15	45	120	600
Arginino succinic acid	A5707	300	200	100	400
Asparagine	A8824	20	160	80	40
Aspartic acid	A8949	10	60	30	15
Citrulline	C7629	15	30	150	600
Cystine	C8755	20	40	60	90
Glutamic acid	128430	20	180	60	40
Glutamine	(49419)	250	1000	750	500
Glycine	G7403	180	360	1080	60
Histidine	H8000	80	240	720	40
1-Methyl Histidine	M9005	20	40	60	5
Hydroxyproline	H3656	50	75	150	25
Isoleucine	I7268	32	128	360	8
Leucine	L5652	250	1000	25	100
Lysine	L5501	360	720	30	90
Methionine	(64319)	144	576	8	24
Ornithine	O2375	144	360	12	72
Phenylalanine	(78020)	320	960	20	80
Phospho Ethanolamine	P0503	100	50	25	200
Proline	P8449	720	60	120	360
Saccharopine	S1634	90	60	30	120
Serine	S8407	300	15	75	150
Taurine	(86329)	300	15	90	120
Threonine	T8534	200	25	75	125
Tyrosine	(93829)	650	15	45	175
Valine	V0258	800	50	150	400

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 2009 cycle, $8 \times 29 = 232$ such Analyte-in-Detail-reports can be requested). A more condensed report is the "Cycle Review" which summarises the performance of all analytes in a specific sample (8 such Cycle Reviews can be requested in 2009). 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 2009). 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). More detailed information on for example, analysis of long term trends or comparison of results obtained with different methods can also be called up when required.

4. Discussion of Results in the Annual Report 2009

In this part the results as seen in the annual report 2009 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 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". Lowest recoveries are seen for the sulphur-containing amino acids: cystine (49%) and Phospho ethanolamine (64%).

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 CVs can be calculated. The column "Precision" in the annual report shows your CVs for the respective amino acids in comparison to median values for all labs. The best median precision is observed for alanine (CV 4.3%).

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 borne in mind that although a range of concentrations is tested in this scheme, the extent of variation of concentrations may be limited as shown in table 1.

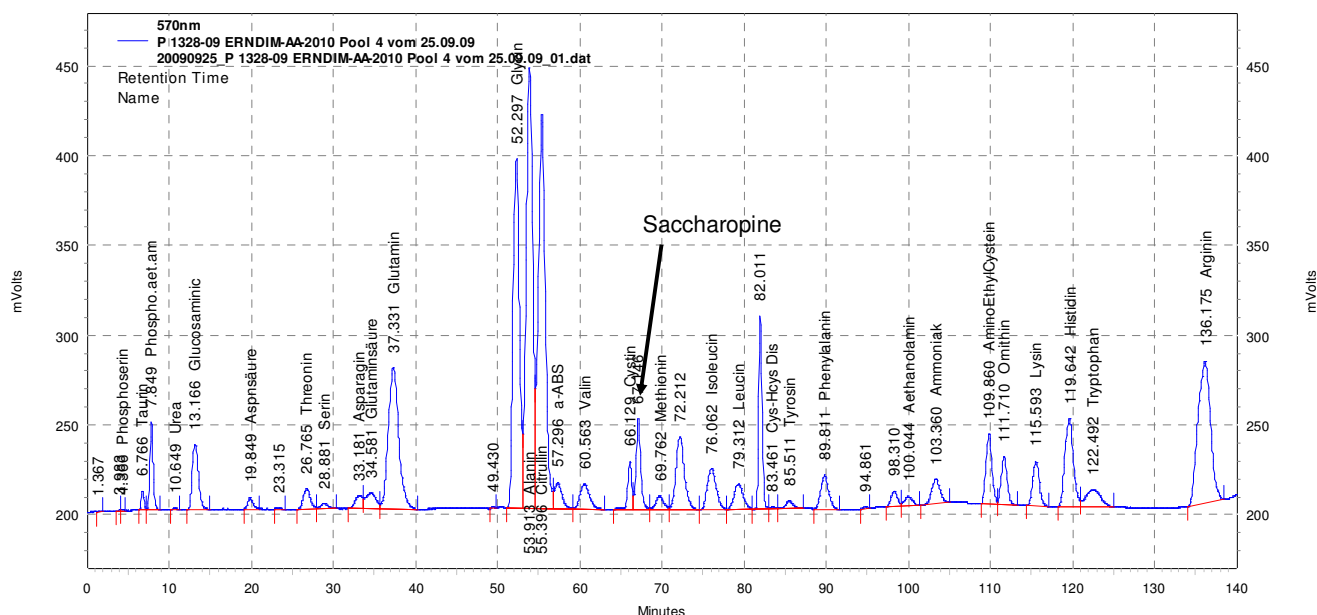
4.5 Interlab CV

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 threonine (8.06%).

4.6 Number of Participating Labs and submitted results

Of the 241 labs, 211 submitted sufficient results to allow complete evaluation of performance, 8 submitted insufficient results and 22 laboratories submitted no results.

For most of the individual amino acids results were submitted by more than 200 labs. For one amino acid (saccharopine) only 107 laboratories submitted results. With modern amino acid analysers employing ion-exchange chromatography a complete separation and quantitation of all the amino acids present in the distributed samples should be 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. Results suggested that some laboratories experienced difficulties in the separation of cystine and saccharopine. The separation obtained using the Biochrom ion exchange system is shown in the figure below.



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 **alanine** all parameters indicate good performance: precision (CV = 4.3%), linearity ($r = 0.998$), recovery (93%) and interlab dispersion (interlab CV 8.27% and many labs (216) submitted results. In contrast poor values for these parameters were found for **argininosuccinic acid**: precision (CV = 12.8%), linearity ($r = 0.967$), recovery (84%) and interlab dispersion (interlab CV 32% and only 128 labs submitted results.

4.8 **Your performance: red and green flags**

After some years of discussion and planning, a system to judge performance of individual laboratories was implemented. 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. 18% 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. For 2009 it is expected that 13 laboratories will receive such letters.

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%	16%	38%
0 – 5%	44%	82%
0%	18%	100%

4.10 **Certificates**

As for other schemes performance as 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 (due to the known binding to protein and conversion to cysteine-homocysteine mixed disulphide) and phospho ethanolamine. Borderline recoveries (between 80 and 90%) are seen for argininosuccinic acid and serine. Such discrepancies may be attributable to problems with standardisation or low purity of the commercial amino acid products used.

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 seven amino acids with good precision and interlab CVs of 10% or below. Nineteen amino acids show interlab CVs of about 10% – 14% with precision below 10% and there is a third group of 4 amino acids with clearly poor performance, shown here as an 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 2010

- * 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 from year to year.
- * Thus for 2010 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2009 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)