

# Annual Report ERNDIM-EQAS Quantitative Amino Acids 2005

## 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/](http://www.erndim.unibas.ch/) / [www.ERNDIMQA.nl](http://www.ERNDIMQA.nl)

## 2. *Participants*

182 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. Analytes	Source Sigma (Merck)	Added quantities (micromol/L)			
		Sample pair 109-116	Sample pair 110-114	Sample pair 111-113	Sample pair 112-115
Alanine	A5824	124	791	396	198
Alpha-aminobutyric acid	A1879	13	4	25	15
Arginine	A5949	124	62	7	247
Asparagine	A8824	15	9	50	30
Aspartic acid	A8949	15	9	50	30
Citrulline	C7629	150	105	60	3
Cystine	C8755	53	30	13	75
Glutamine	(49419)	72	1206	844	482
Glutamic acid	G6904	11	189	133	76
Glycine	G7403	199	65	497	348
Histidine	H8000	51	322	161	81
1-Methyl Histidine	M9005	24	10	2	40
Hydroxyproline	H3656	30	12	3	50
Isoleucine	I7268	202	101	12	404
Leucine	L5652	155	989	495	247
Lysine	L5501	144	47	359	251
Methionine	(64319)	200	140	80	4

Ornithine	O2375	196	137	78	4
Phenylalanine	(78020)	404	202	24	808
Proline	P8449	75	482	241	121
Saccharopine	S1634	12	200	140	80
Serine	S8407	195	98	49	14
Taurine	(86329)	15	254	178	102
Threonine	T8534	79	26	196	138
Tyrosine	(93829)	265	152	64	379
Valine	V0258	91	585	292	146
l-alpha-amino adipic acid	A7275	241	120	60	17
Beta-alanine	A7752	125	62	31	9
L-allo-isoleucine	I8754	12	203	142	81

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](http://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 2005 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 2005). 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 2005). 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 2005**

In this part the results as seen in the annual report 2005 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 403 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 27 of the reported amino acids the median recovery is between 90 and 110%: e.g. 98% for alanine). Two amino acids are outside the 100 +/- 10% window. The 76% recovery of cystine is low and can be attributed to its binding to proteins in the sample. For proline a recovery of 89% 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 Tyrosine (CV 4.1 %) and the worst for hydroxyproline (18.0%). A CV of greater than 10% is observed for only four amino acids, asparagine, beta-alanine, cystine and hydroxyproline.

##### **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 phenylalanine ( $r = 0.9990$ ). It must be borne 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 6.4%) and the worst for hydroxyproline (253%).

With Hydroxyproline, the shape of the results is not symmetrical. The results from the majority of labs show a gaussian distribution but there are two substantial group of laboratories with results at the extreme low end and at the extreme high end. These are too many labs to be considered as outliers. These extreme groups cause a high interlab CV and indicate that a substantial number of labs have problems in determining hydroxyproline correctly.

The interlab CV of cystine is probably (like last year) related to interference by saccharopine (quite a number of labs measure saccharopine as cystine).

#### **4.6 *Number of Participating Labs and submitted results***

In total 185 laboratories received samples and 182 submitted results. For most of the individual amino acids results were submitted by more than 160 labs. For one amino acid 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 29 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 performance.

For example for leucine all parameters indicate good performance: precision (CV = 4.5%), linearity ( $r = 0.9951$ ), Recovery (96%) and Interlab Dispersion (Interlab CV 7.4%) and many labs (182) submitted results. The opposite is seen for hydroxyproline.

### **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 2006*

- \* 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 2006 the common amino acids remain although for some the range of concentrations have been modified compared with those in the 2005 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 judgement scheme is currently under evaluation by scientific advisors.

## 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](mailto:Brian.Fowler@unibas.ch)) and/or the scheme organiser Dr. Cas Weykamp ([c.w.weykamp@skbwinterswijk.nl](mailto:c.w.weykamp@skbwinterswijk.nl))