# Annual Report ERNDIM-EQAS Quantitative Amino Acids 2002

## *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.ERNDIMQA.nl

## *2. Participants*

160 laboratories from 26 countries submitted results within the Scheme.

## *3. Design*

The scheme has been designed, planned and co-ordinated by Dr. 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. A disadvantage of the scheme in previous years was that the lower concentration range for part of the amino acids was not covered. This has been improved in the 2002 scheme. As can be seen from table 1 the samples 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 (Code)** | **Sample pair 85-90** | **Sample pair 86-92** | **Sample pair 87-89** | **Sample pair 88-91** |
| Alanine | A5824 | 321.6 | 96.3 | 804.0 | 562.8 |
| Alpha-aminobutyric acid | A1879 | 9.96 | 6.21 | 24.8 | 17.4 |
| Arginine | A5949 | 299.1 | 209.4 | 119.6 | 14.9 |
| Asparagine | A8824 | 20.2 | 12.6 | 50.5 | 35.3 |
| Aspartic acid | A8949 | 20.1 | 12.5 | 50.2 | 35.1 |
| Citrulline | C7629 | 10.6 | 152.0 | 106.4 | 60.8 |
| Cystine | C8755 | 52.6 | 30.1 | 12.8 | 75.1 |
| Glutamine | (49419) | 84.0 | 1200.0 | 840.0 | 480.0 |
| Glutamic acid | G6904 | 80.2 | 24.0 | 200.6 | 140.4 |
| Glycine | G7403 | 348.3 | 199.0 | 84.5 | 497.5 |
| Histidine | H8000 | 103.1 | 58.9 | 25.0 | 147.3 |
| 1-Methyl Histidine | M9005 | 85.1 | 59.6 | 34.1 | 9.45 |
| Hydroxyproline | H3656 | 60 | 35.7 | 20.4 | 5.65 |
| Isoleucine | I7268 | 138.5 | 79.1 | 33.6 | 197.8 |
| Leucine | L5652 | 161.3 | 48.3 | 403.2 | 282.2 |
| Lysine | L5501 | 207.5 | 118.6 | 50.3 | 296.4 |

| | | | | | |
|---|---|---|---|---|---|
| Methionine | (64319) | 50.7 | 35.5 | 20.3 | 5.63 |
| Ornithine | O2375 | 200.2 | 140.1 | 80.1 | 10.0 |
| Phenylalanine | (78020) | 398.0 | 278.6 | 159.2 | 19.8 |
| Proline | P8449 | 80.6 | 24.1 | 201.4 | 141.0 |
| Serine | S8407 | 80.3 | 24.1 | 200.8 | 140.6 |
| Taurine | (86329) | 17.5 | 250.0 | 180.0 | 100.0 |
| Threonine | T8534 | 80.2 | 24.0 | 200.6 | 140.4 |
| Tyrosine | (93829) | 393.3 | 275.3 | 157.3 | 19.6 |
| Valine | V0258 | 277.0 | 158.3 | 67.2 | 395.6 |
| Arginino Succinic acid | A5707 | 396.4 | 277.5 | 158.6 | 19.7 |
| Sarcosine | S7672 | 35.2 | 503.5 | 352.4 | 201.4 |
| Hydroxylysine | H0377 | 12 | 7.50 | 30.0 | 21.0 |
| Cystathionine | C7505 | 8.02 | 5.00 | 20.0 | 14.0 |

***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 has been introduced to enable the scientific advisor to inspect the results and add his comment to the report.

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 (232 such Analyte-in-Detail-reports can be requested in the year 2002 cycle: 29 amino acids X 8 samples = 232). 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 2002). 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 2002). Depending on the responsibilities within the laboratory participants can choose to inspect the annual report (QC managers) or all (or part of) the 232 detailed reports (scientific staff).

## *4. Discussion of Results in the Annual Report 2002*

In this part the results as seen in the annual report 2002 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 456 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. 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 median outcome of all labs is shown in the column “Recovery”. For 26 of the reported amino acids the median recovery is between 90 and 110%: e.g. 97% for alanine). Three amino acids are outside the 100 +/- 10% window. The 65% recovery of cystine is low and must be related to its binding to proteins in the sample. For hydroxyproline and asparagine recoveries of 77% and 84% are seen. It is unlikely that this is due to erroneous weighing of these amino acids or impurity (purity is certified by manufacturer). Is seems more reasonable that either the calibration or the recovery of many participants is at the low edge. In 2001 the recovery for argininosuccinic acid was 70%; this year 94% is seen. This substantial improvement might reflect a learning effect of the scheme.

### *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 threonine (CV 3.8%) and the worst for asparagine (18.5%). A CV of greater than 10% is observed for only five amino acids.

### *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 threonine (r = 0.9981).

### *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 who submitted results is shown in the column "Data all labs" in the annual report. The best Interlab CV is seen for alanine (median CV of 6.8%) and the worst for argininosuccinic acid (65.1%).

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

In total 160 laboratories received samples and 158 submitted results. For most of the individual amino acids results were submitted by more than 140 labs. For three amino acids there are less than 130 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 (see attached chromatogram). 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 all but one of the 29 amino acids are available 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 so (asparagine for example). Also we have experienced a commercial preparation from a chemical company with the label "3-methylhistidine " which actually contained "1-Methylhistidine". Also a bottle of "glutamic acid" from our own collection of standards was found in fact to contain only glutamine.

### *4.7 Interrelationships between quality paramters*

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 quality.

For example for threonine all parameters indicate good performance: precision (CV = 3.8%), linearity (r = 0.9981), Recovery (99%) and Interlab Dispersion (Interlab CV 7.4%) and many labs (155) submitted results. The opposite is seen for argininosuccinic acid, asparagine, cystathionine, hydroxylysine and hydroxyproline. For each of these the Interlab CV exceeds 20% and most other statistical parameters are also less satisfactory.

## *5. Summary*

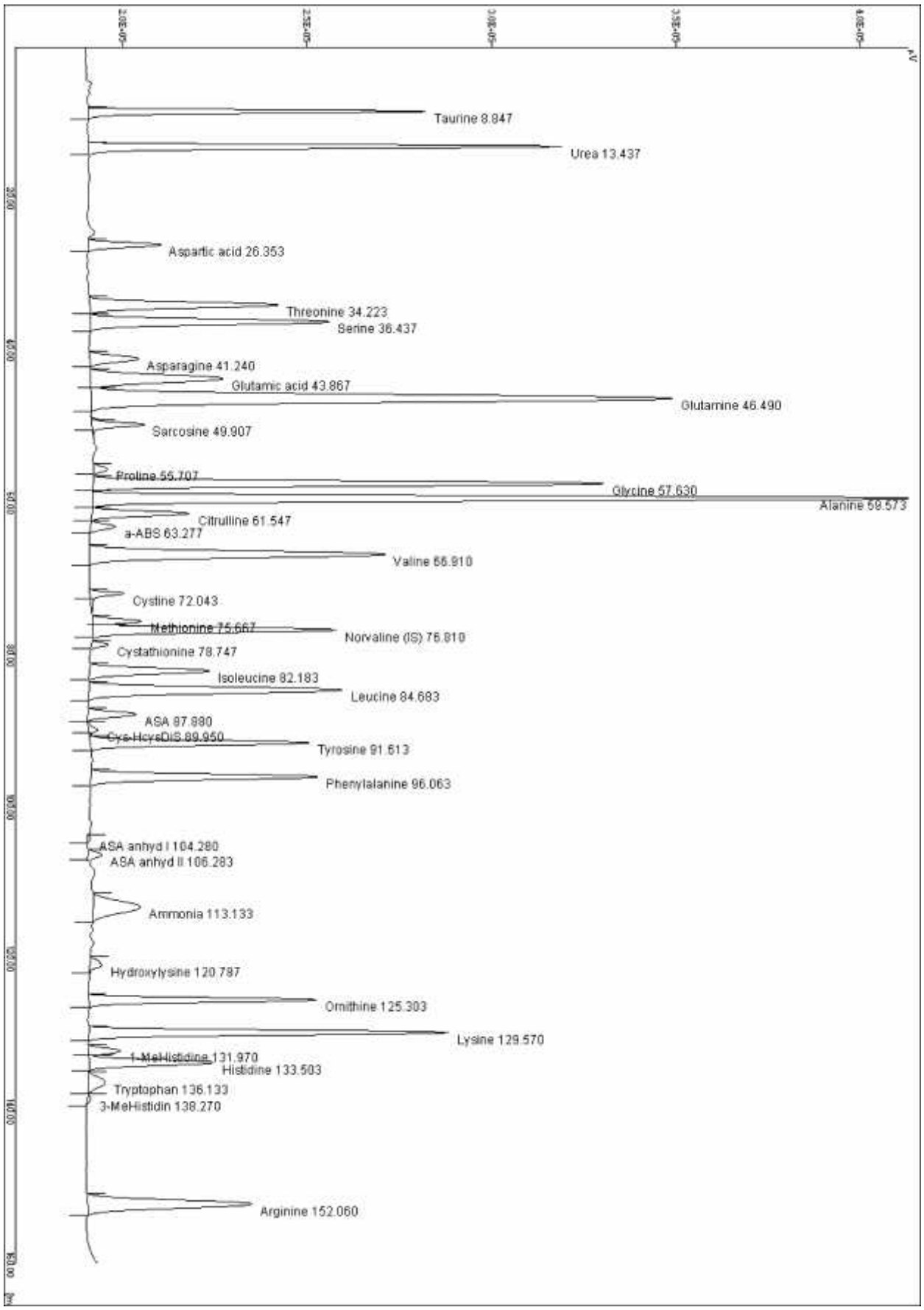
There is a large group (about 20 amino acids)of well-established amino acids for which there is overall good performance indicated by satisfactory values for all five analytical quality parameters. That is a precision below 8%, an interlab CV below 10%, a linearity exceeding 0.9, a recovery between 90 and 110% and a high degree of participation. Performance for the remaining 10 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 a better precision within the labs and/or standardization as referred to above (4.6).

## *6. Preview of the Scheme for 2003*

* In 2003 four amino acids have been removed from the list of analytes and four new ones have been added.
* The concentrations of a number of analytes have been changed to avoid levels too similar to those in the 2002 scheme.

## *7. Questions, Comments and Suggestions*

If you have any questions, comments or suggestions please address these to the scientific advisor of the scheme, Dr. Brian Fowler (Brian.Fowler@unibas.ch) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)


Taurine 8.847
Urea 13.437
Aspartic acid 26.353
Threonine 34.223
Serine 36.437
Asparagine 41.240
Glutamic acid 43.867
Glutamine 46.490
Sarcosine 49.907
Proline 55.707
Glycine 57.630
Alanine 59.573
Citrulline 61.547
a-ABS 63.277
Valine 66.910
Cystine 72.043
Methionine 75.667
Norvaline (IS) 76.810
Cystathionine 78.747
Isoleucine 82.183
Leucine 84.683
ASA 87.880
Cys-HcysDiS 89.950
Tyrosine 91.613
Phenylalanine 96.063
ASA anhyd I 104.280
ASA anhyd II 106.283
Ammonia 113.133
Hydroxylysine 120.787
Ornithine 125.303
Lysine 129.570
1-MeHistidine 131.970
Histidine 133.503
Tryptophan 136.133
3-MeHistidin 138.270
Arginine 152.060