ERNDIM - Quantitative Schemes
Amino Acids

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Annual Report ERNDIM-EQAS 2015

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 271 datasets from laboratories in 46 countries were submitted.

3. *Design*

The scheme has been designed, planned and co-ordinated by Prof. Brian Fowler as scientific advisor with support from Dr. Rachel Carling and Dr. Cas Weykamp as scheme organiser (subcontractor on behalf of the SKML), each appointed by and according to procedures laid down 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.

		Added quantities (micromol/L)			
Analyte	**Source**	**Sample pair 2015. 01-08**	**Sample pair 2015. 02-05**	**Sample pair 2015. 03-07**	**Sample pair 2015. 04-06**
Alpha-aminobutyric acid	Sigma A1879	79,7	5,2	10,3	21,2
Alanine	Fluka 05129	1000	76,1	225	449
Alloisoleucine	Sigma I8754	20,5	40,2	81,2	161
Arginine	Sigma A6969	640	9,9	60,3	320
Asparagine	Roth KK37.1	49,9	99,8	200	25,2
Aspartic acid	Sigma A8949	30,4	59,5	180	14,6
Citrulline	Sigma C7629	1000	10,6	24,7	200
Cystine	Sigma C8755	145	8,1	24,4	72,4
Glutamic acid	Aldrich 128430	80,3	120	200	40,0
Glutamine	Sigma 49419	401	900	1201	200
Glycine	Sigma G7403	50,9	150	301	901
Histidine	Sigma H8000	39,9	79,9	160	480
Homocitrulline	Bio Connect SC-269298	151	30,2	60,0	90,2
Hydroxyproline	Roth 3893	14,2	29,6	60,7	90,0
Isoleucine	Roth 3922	24,5	71,7	480	8,4
Leucine	Roth 3984	80,8	420	799	39,8
Lysine	Sigma L5501	149	300	599	61,0
Methionine	Fluka 64319	40,3	160	601	10,0
Ornithine	Sigma O2375	360	1080	30,7	91,7
Phenylalanine	Fluka 78019	400	801	15,6	76,4
Phospho-ethanolamine	Sigma P0503	29,1	60,9	100	120
Proline	Roth T205	499	750	50,1	251
Saccharopine	Sigma S1634	20,5	29,7	121	241
Serine	Merck 1.07769	241	422	15,6	61,0
Taurine	Fluka 86329	453	24,9	76,6	150
Threonine	Roth T206	360	41,1	125	243
Tyrosine	Fluka 93829	752	9,9	50,9	251
Valine	Roth 4879	80,9	239	400	902

All amino acids used are of the highest purity commercially available. Concentrations < 100 micromol/L are given with one decimal; otherwise without decimal. Samples have been tested for stability and homogeneity according to ISO 13528 in which requirements for regulatory purposes of quality management systems for medical devices are described.

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). The results of your laboratory are confidential and only accessible to you (with your name and password). The anonymised mean results of all labs are accessible to all participants. Statistics of the respective reports are explained in the general information section of the website.

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 28 amino acids in the year 2015 cycle, 8 x 28 = 224 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 2015). 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 2015). 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 224 detailed reports (e.g. scientific staff).

Analyte	Accuracy (mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (%added analyte)		Data all labs	
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
2-Aminobutyric acid	27.6	27.8	2.9%	8.3%	1.000	0.998	96%	95%	213	13.9%
Alanine	405	416	2.2%	4.3%	1.000	0.999	91%	93%	266	8.65%
Alloisoleucine	71.2	71.2	5.6%	5.7%	0.999	0.997	106%	99%	188	11.2%
Arginine	246	249	1.8%	4.3%	1.000	0.999	95%	96%	264	8.29%
Asparagine	92.2	92.9	23.5%	8.6%	0.936	0.994	89%	100%	246	19.5%
Aspartic Acid	59.1	57.1	2.5%	7.5%	0.996	0.997	87%	81%	255	18.0%
Citrulline	292	299	1.5%	4.7%	1.000	1.000	93%	96%	258	11.7%
Cystine	39.9	41.5	3.5%	7.2%	0.999	0.996	63%	64%	237	13.7%
Glutamic acid	152	126	6.2%	8.4%	0.962	0.991	147%	111%	264	13.2%
Glutamine	628	623	14.7%	5.7%	0.981	0.995	97%	94%	250	10.8%
Glycine	337	346	3.5%	4.4%	0.999	0.999	94%	96%	265	9.02%
Histidine	154	180	5.0%	4.9%	1.000	0.999	80%	94%	261	11.4%
Homocitrulline		78.4		6.2%		0.993		96%	99	17.3%
Hydroxyproline	54.4	47.4	10.7%	8.4%	0.992	0.991	113%	96%	222	14.4%
Isoleucine	144	141	5.7%	4.0%	0.999	1.000	97%	96%	267	10.3%
Leucine	313	321	6.7%	4.2%	0.998	0.999	93%	95%	268	8.56%
Lysine	256	260	3.8%	4.0%	0.999	0.999	89%	91%	264	8.00%
Methionine	195	198	6.5%	7.0%	0.999	0.995	98%	90%	262	28.7%
Ornithine	371	380	8.8%	4.4%	0.998	0.999	93%	96%	264	11.4%
Phenylalanine	295	307	5.3%	3.9%	0.999	0.999	89%	93%	270	8.14%
Phospho ethanolamine	5.75	11.6	34.8%	14.2%	0.887	0.888	15%	30%	160	32.8%
Proline	N/A	358	7.1%	5.1%	N/A	0.997	N/A	97%	249	9.65%
Saccharopine	105	102	1.9%	5.3%	0.999	0.998	100%	97%	99	14.3%
Serine	169	174	4.7%	4.6%	0.999	0.998	93%	97%	266	8.32%
Taurine	169	171	2.2%	4.8%	0.999	0.999	95%	97%	251	8.79%
Threonine	186	191	2.6%	4.3%	0.998	0.998	91%	93%	262	7.39%
Tyrosine	234	250	10.1%	4.7%	0.997	0.999	86%	92%	270	9.17%
Valine	389	394	2.9%	4.2%	1.000	0.999	97%	97%	269	8.30%
Overall	207	211	6.9%	5.8%	0.990	0.993	92%	92%	240	12.7%
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
Analyte	Accuracy		Precision		Linearity		Recovery		Data all labs	

See this example of part of an annual report.

N/A indicates that no calculations could be made due to no results, too few results or too many outliers. In the next generation of the website this will be specified (see below).

4. Discussion of Results in the Annual Report 2015

In this part the results as seen in the annual report 2015 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 416 micromol/Liter, with which you can compare the mean of your lab.

4.2 Recovery

A second approach to describe performance 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 recovery is seen for phospho-ethanolamine (30%) possibly reflecting instability of the stored compound. The only other amino acids with recovery below 90% are cysteine (64%) due to binding of the compound to protein and aspartic acid (81%).

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 phenylalanine (CV 3.9%) compared with worst of 14.2% for phospho-ethanolamine.

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 13 amino acids (r = 0.999). 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 inter-laboratory CV. This, along with the number of laboratories that submitted results is shown in the column “Data all labs” in the annual report. The interlab CV ranges widely from the best of 7.39% for threonine to the worst of 32.8% for phospho-ethanolamine.

4.6 Number of Participating Labs and submitted results

Of the 271 submitted datasets, 260 allowed complete evaluation of performance.18 laboratories submitted no results.
For 21 of the individual amino acids, results were submitted by more than 244 labs (90%). Of the others, results were submitted by over 70% of labs for 5 and less than 70% for 2 other amino acids.

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 = 0.999), recovery (97%) and interlab dispersion (interlab CV 8.30%) and many labs (269) submitted results. The opposite is seen for phospho-ethanolamine.

4.8 Your performance: red and green flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include red flags in case of 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 (on this website under 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. 20 of the laboratories have no red flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are 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 agreed 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 this will be ratified by the SAB. A letter pointing out failure to achieve these levels will be issued 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%	3%	3%
20 – 25%	2%	5%
15 – 20%	6%	11%
10 – 15%	11%	22%
5 – 10%	20%	42%
0 – 5%	39%	81%
0%	19%	100%

Performance is also related to experience. Table 3 shows the number of labs with poor and excellent performance in relation to the time they have participated in ERNDIM schemes: labs with the longest participation (ERNDIM number <100) and labs with the shortest participation (ERNDIM number >300). Numbers of 2013 are in brackets.

Table 3. Performance in relation to length of ERNDIM history

ERNDIM Participation	Number of Labs with Poor Performance Score >15% red flags In 2015 (2014 in brackets)	Number of Labs with Excellent Performance Score 0% red flags In 2015 (2014 in brackets)
Long (Lab code <100)	3 (3)	16 (17)
Short (Lab code >300)	15 (20)	13 (11)

Poor and excellent performance is seen in both groups but the prevalence of excellent performance is higher in the longer standing participants whereas the prevalence of poor performance is nearly exclusively seen in the more recent subscribers. This supports the idea that alongside greater experience participation in EQA probably plays an important role in improving performance and reinforces the educational role of ERNDIM. High level of performance cannot be taken for granted and may for example depend on replacement of retired persons by less experienced new staff pointing to the need for well-planned and timely succession.

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 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 poor recognition of phospho ethanolamine (which in part may be related to instability of this compound).

Quantitative comparisons (see table 4).

The overall performance evaluated by comparing precision (within lab variation) versus interlab variation for each amino acid reveals three main groups. There are 18 amino acids with good precision and interlab CVs of 12% or below. Five amino acids show interlab CVs of about 12 – 15% with precision below 12% and there is a third group of five amino acids with clearly poor performance, shown here as interlab CV above 16%. This is very similar to performance in 2014.
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.

Table 4. Summary of results of all laboratories

Analyte	Accuracy (mean µmol/L)	Precision (CV% duplicates)	Linearity (r)	Recovery (%added analyte)	Data all labs	
	All labs	All labs	All labs	All labs	n	Interlab CV
Alpha-aminobutyric acid	27.8	8.3%	0.998	95%	213	13.9%
Alanine	416	4.3%	0.999	93%	266	8.65%
Alloisoleucine	71.2	5.7%	0.997	99%	188	11.2%
Arginine	249	4.3%	0.999	96%	264	8.29%
Asparagine	92.9	8.6%	0.994	100%	246	19.5%
Aspartic acid	57.1	7.5%	0.997	81%	255	18.0%
Citrulline	299	4.7%	1.000	96%	258	11.7%
Cystine	41.5	7.2%	0.996	64%	237	13.7%
Glutamic acid	126	8.4%	0.991	111%	264	13.2%
Glutamine	623	5.7%	0.995	94%	250	10.8%
Glycine	346	4.4%	0.999	96%	265	9.02%
Histidine	180	4.9%	0.999	94%	261	11.4%
Homocitrulline	78.4	6.2%	0.993	96%	99	17.3%
Hydroxyproline	47.4	8.4%	0.991	96%	222	14.4%
Isoleucine	141	4.0%	1.000	96%	267	10.3%
Leucine	321	4.2%	0.999	95%	268	8.56%
Lysine	260	4.0%	0.999	91%	264	8.00%
Methionine	198	7.0%	0.995	90%	262	28.7%
Ornithine	380	4.4%	0.999	96%	264	11.4%
Phenylalanine	307	3.9%	0.999	93%	270	8.14%
Phospho ethanolamine	11.6	14.2%	0.888	30%	160	32.8%

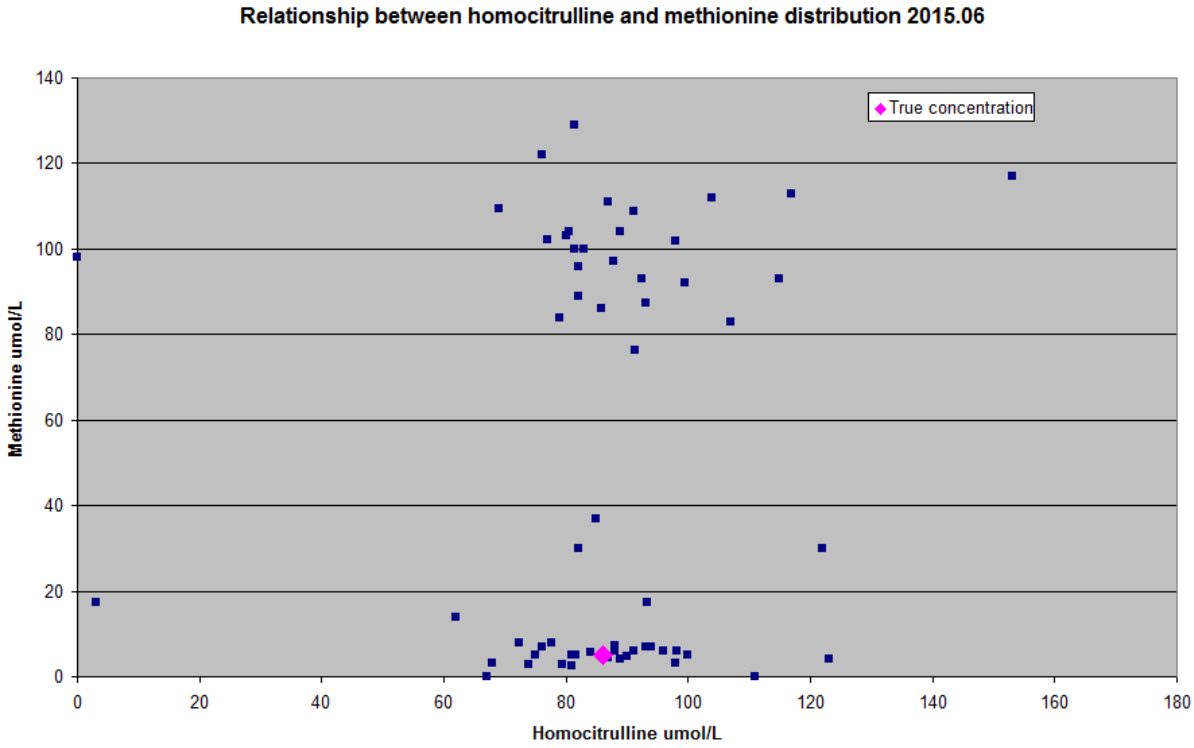
Proline	358	5.1%	0.997	97%	249	9.65%
Saccharopine	102	5.3%	0.998	97%	99	14.3%
Serine	174	4.6%	0.998	97%	266	8.32%
Taurine	171	4.8%	0.999	97%	251	8.79%
Threonine	191	4.3%	0.998	93%	262	7.39%
Tyrosine	250	4.7%	0.999	92%	270	9.17%
Valine	394	4.2%	0.999	97%	269	8.30%
Overall	211	5.8%	0.993	92%	240	12.7%

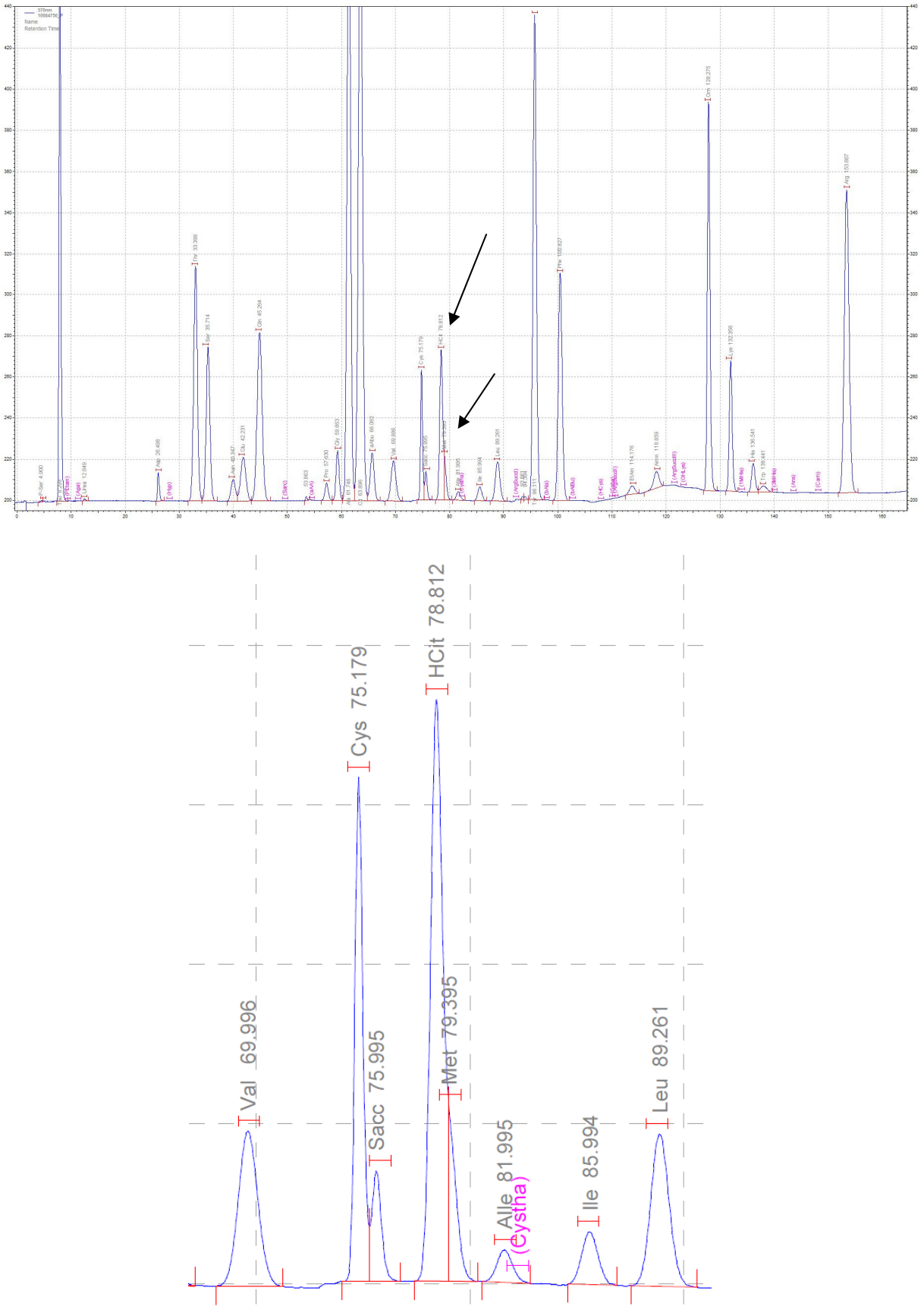
Interference homocitrulline on Methionine

We want to remark that a substantial number of labs use a method where homocitrulline interferes with methionine. This is illustrated by figure of 2015.06 and can also been seen in the analyte in detail report for methionine for sample 2015.08 on the website. Results all into 2 distinct groups:

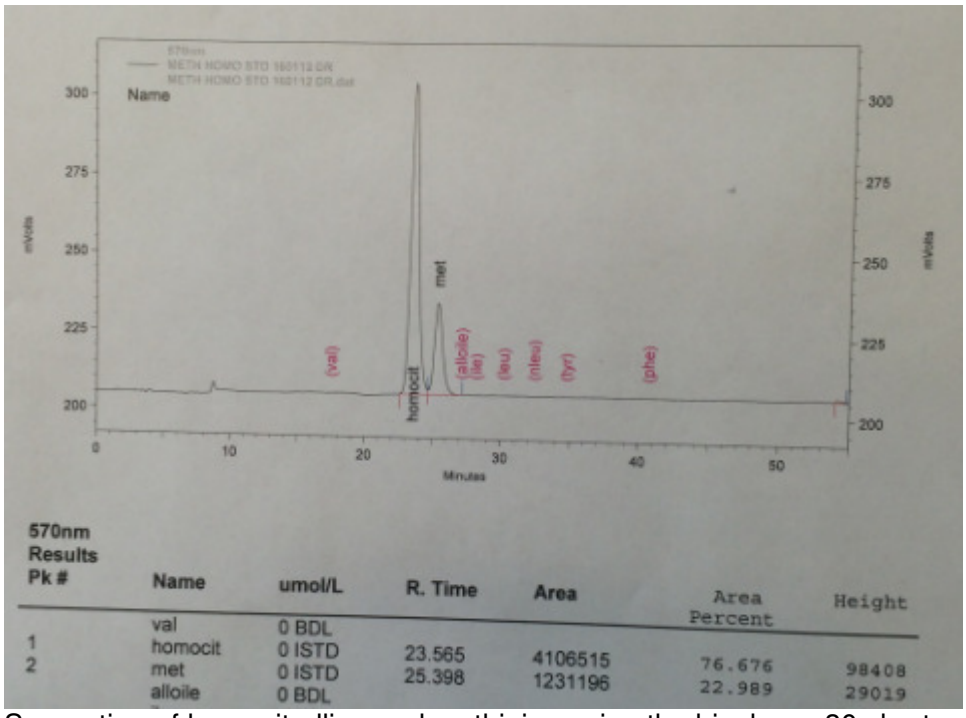
- those with methods that separate homocitrulline and methionine (low methionine reported = correct)
- those methods where the 2 analytes col-elute (high methionine reported = not correct)

Due to the statistics used the reported ALTM (All laboratories Trimmed Mean) is correct.





Full run from biochrom 30 with analysis time of 180 minutes. Note the clear peak of homocitrulline with methionine as shoulder



570nm Results

Pk #	Name	umol/L	R. Time	Area	Area Percent	Height
	val	0 BDL				
1	homocit	0 ISTD	23.565	4106515	76.676	98408
2	met	0 ISTD	25.398	1231196	22.989	29019
	alloile	0 BDL				

Separation of homocitrulline and methioine using the biochrom 30 short programme. Further details available on request.

Educational Effect of ERNDIM

Greater experience of amino acid analysis as reflected by longer participation in ERNDIM schemes clearly seems to contribute to improved performance. Beyond this the learning/educational effect of EQA as provided by ERNDIM is undoubtedly a major factor in improving performance.

6. *Preview of the Scheme for 2016*

Our continuing policy is to include the same common amino acids in each year's samples as well as a few unusual ones which are selected year to year.
Thus for 2016 the common amino acids remain although for some the range of concentrations has been modified compared with those in the 2015 scheme and four special amino acids are included. We are very pleased to announce that Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) has continued in the role of deputy scientific advisor for this scheme.

We are pleased to announce that from 2016 we will introduce a modified reporting system that will refine the red versus green flag delineation to allow assessment of performance.
In the Monthly Review Report you will already see a small modification (z-score added).
Major modifications are in the Annual Report – we will send you the explanation in December 2016. By then also the text in the General Information of the website will be modified.

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@ukbb.ch), Dr.Rachel Carling (Rachel.Carling@viapath.co.uk) and/or the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).