Treatment Monitoring and Multicentre Studies in Inherited Metabolic Disorders (IEM)

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ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Introduction

Can you trust your amino acid results for treatment modifying decisions? ***Can they be used in multi-centre studies?***

The ERNDIM amino acid EQA scheme assesses accuracy, recovery, precision, linearity and inter laboratory variance (CV) of quantitative measurements for 24 common and selected special amino acids relevant to IEMs and provides a means to answer these questions ***(answer below)***

Scheme Details

- 8 samples of lyophilised human serum spiked with physiologically relevant levels of 28 amino acids
- Results submitted via ERNDIM interactive website
- Certificate issued once a year by ERNDIM Board
- Scheme operated since 1993
- 8 monthly reports plus annual report
- 243 participants from 48 Countries worldwide (2013)
- Cost (2013): 227 EURO

Methods

	Participants (%) 2002	2012
Ion-exchange chromatography + ninhydrin	82 %	74%
Ion-exchange chromatography + other deriv.	2 %	1%
Reverse phase HPLC	12 %	16%
GC (MS)	2.%	1%
Other	2%	2%
Tandem MS	0	6%

Participants' Annual Report

- Performance based on 4 parameters:
  - ♦ Accuracy ♦ Precision
  - ♦ Linearity ♦ Recovery
- The parameters are scored for each analyte; those falling outside the 95th percentile for all laboratories are indicated with red shading
- Two or more parameters with red shading or insufficient submissions are equated to unsatisfactory performance for that particular analyte
- Green shading indicates satisfactory performance for that analyte

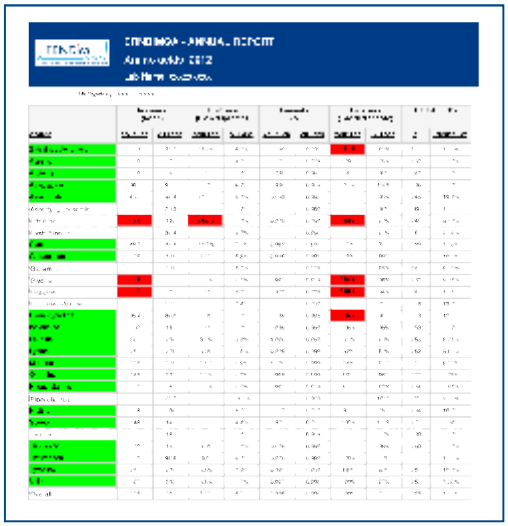

Figure 1: Participants' Annual Report (anonymised example)

Examples of Detailed Reports

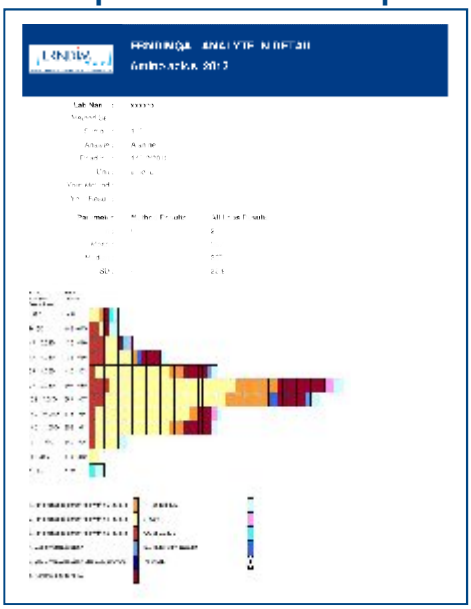

Figure 2: Detailed Analyte Report - alanine

1. Alanine [Figure 2]

- Consensus alanine value of 397umol/L
- 8 laboratories reported values of >3 S.D. above or below the median (<319 and >475)

2. Pipecolic acid [Figure 3]

- Only a few labs reported this amino acid
- Even with the quite high level of 39umol/L several labs reported very low values
- Current methods used in Amino Acid analysis are less suitable for detection of pipecolic acid than other methods e.g. GC-MS with stable isotope dilution

Variation within and between labs

1. Usual Amino Acids: (2002 vs 2012) [Table 1]

- **2012**: most amino acids have good performance with precision <5% & interlab variation <10%; worst performance is for asparagine and aspartic acid
- **2002 vs 2012**: both precision and interlab variation show clear improvement for nearly all amino acids

Table 1: Usual Amino Acids (2002 vs 2012)

	2002			2012		
	Precision (CV% duplicates)	Interlab variation		Precision (CV% duplicates)	Interlab variation	
Amino Acids	All labs	n	CV%	All labs	n	CV%
2-Aminobutyric acid	11.40%	138	20.30%	6.80%	204	11.50%
Alanine	4.00%	158	8.45%	4.20%	252	8.13%
Arginine	6.00%	153	10.70%	4.40%	247	9.50%
Asparagine	19.70%	137	36.60%	6.70%	236	24.20%
Aspartic Acid	10.00%	151	18.80%	6.80%	245	19.10%
Citrulline	6.90%	150	12.10%	4.70%	245	9.44%
Cystine	9.50%	145	13.70%	7.40%	229	12.60%
Glutamic acid	8.50%	154	15.60%	5.80%	251	10.50%
Glutamine	8.10%	153	12.00%	5.60%	241	9.40%
Glycine	4.30%	158	8.10%	4.10%	252	9.81%
Histidine	7.30%	155	11.70%	5.60%	247	11.10%
Hydroxyproline	17.10%	133	26.80%	7.60%	213	12.50%
Isoleucine	5.20%	156	9.10%	4.00%	253	9.17%
Leucine	4.90%	157	8.32%	3.90%	253	8.71%
Lysine	5.30%	157	8.71%	4.50%	252	9.81%
Methionine	6.70%	156	10.60%	4.90%	253	9.86%
Ornithine	5.40%	155	9.83%	4.70%	252	9.78%
Phenylalanine	4.50%	158	8.04%	4.50%	254	8.91%
Proline	8.30%	140	13.40%	5.60%	234	10.50%
Serine	4.60%	156	8.64%	4.40%	251	9.64%
Taurine	5.70%	153	11.20%	4.40%	230	8.32%
Threonine	3.90%	155	7.61%	4.10%	250	7.30%
Tyrosine	5.20%	158	9.09%	3.90%	254	10.40%
Valine	4.50%	157	8.48%	3.70%	253	7.30%

2. Special Amino Acids [Table 2]

- Performance less satisfactory than for usual amino acids
- Submission of results for some amino acids low: 19% of labs for aspartyl glucosamine and 24% for pipecolic acid compared to 89% for α-amino adipic acid
- Repeat circulation: improvement for argininosuccinic acid, hydroxylysine and sarcosine

Table 2: Special Amino Acids (various years)

Amino Acids	Scheme year	Precision (CV% duplicates) All labs	Interlab variation n	CV%
α-amino adipic acid	2005	6.7%	162/182	18.1%
ß-alanine	2005	12.2%	157/182	20.9%
δ-aminolaevulinic acid	2011	7.6%	76/245	19.3%
Allo-isoleucine	2011	5.9%	181/245	12.6%
Argininosuccinic acid	2002	15.2%	121/158	32.3%
	2010	11.4%	142/233	26.9%
Aspartyl glucosamine	2012	7.7%	49/254	17.1%
Cystathionine	2002	9.3%	138/158	18.9%
	2012	8.7%	181/254	21.9%
Homocystine	2007	11.7%	163/205	25.0%
Hydroxylysine	2002	13.9%	124/158	24.2%
	2008	4.7%	155/205	9.3%
Phosphoethanolamine	2010	9.6%	166/233	24.4%
Pipecolic acid	2012	13.1%	62/254	27.8%
Saccharopine	2010	5.4%	112/233	13.5%
Sarcosine	2002	9.3%	127/158	14.9%
	2008	11.3%	162/205	17.9%
Sulpho-cysteine	2008	13.9%	101/205	33.4%
Tryptophan	2012	6.4%	191/254	11.9%

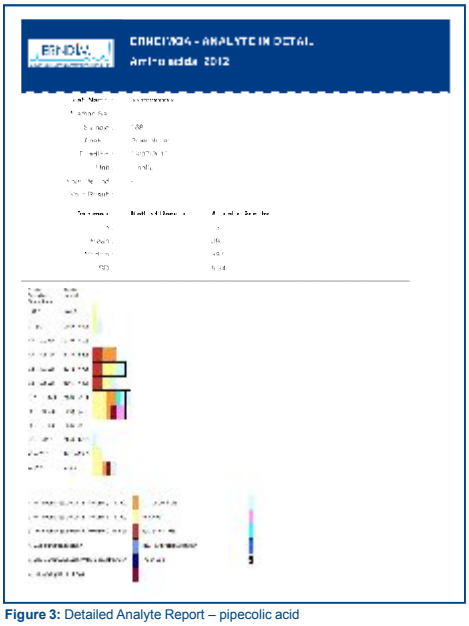

Figure 3: Detailed Analyte Report – pipecolic acid

Conclusions

- Performance assessment in an EQA scheme provides a valuable tool to test **a)** application of agreed treatment decisions; and **b)** the validity of metabolite values in concentrations multicentre studies of treatment outcome.
- Inclusion of special amino acids can reveal methodological inadequacies in separation, standardisation or insufficient sensitivity e.g. arginino succinic acid, pipecolic acid.
- Laboratory experience and educational aspects of ERNDIM appear to have led to improvement in performance of amino acid analysis

Ask your lab how they perform in ERNDIM EQA schemes!

For further information please contact: admin@erndim.org
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