



CSF Neurotransmitters

ERNDIM Workshop, 21st & 22nd November 2017,
Manchester, UK

Overview



- Short explanation of the scheme
 - Review of last years samples
 - Discussion - questions, concerns, suggestions
-
- This is a workshop so please feel free to interrupt, ask questions and participate

CSF Scheme



- Started in 2014
- The purpose was to set up an EQA scheme so labs measuring CSF Homovanillic acid, 5-hydroxyindole acetic acid, 3-methyl dopa and 5-hydroxytryptophan could compare their results against other labs. This helps for accreditation purposes but also give greater confidence in results given to patients.
- Interpretation of metabolite patterns was added as considered as important as quantitation
- Currently ~30 labs participating - a mix of HPLC-ECD and LC-MS methods
- Simon Heales and I are scientific advisors

The samples



- 8 lyophilised samples a year - 4 samples in duplicate
- Samples a mix of pooled CSF and artificial
- Samples prepared in London and lyophilised in the Netherlands
- Tested for stability before sending to participants

Scoring



- Scoring based on 4 criteria - accuracy, precision, linearity and recovery for each metabolite
- Scoring done automatically by website
- Scores outside 3 SDs are flagged, as are no results
- A score of >25 (25% of results flagged) will get a warning letter
- More details on the ERNDIM website and in Cas's talk tomorrow

Scoring example

ERNDIMQA - ANNUAL REPORT
Quant.org.acids 2014
ERN0332 - DMC University Laboratories - Biochemical Genetics Laboratory

Methodset : GC-MS

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-OH Glutaric acid	MP	152	32.0%	20.0%	0.077	0.900	09%	90%	77	35.0%	
3-methylglutamic acid	MP	70.4	29.3%	15.0%	0.914	0.955	117%	101%	77	23.8%	
3-OH-3-methylglutaric acid	FR	90.9	FR	24.7%	FR	0.903	FR	77%	65	62.4%	
3-OH-Isobutyric acid	OR	100	OR	20.4%	OR	0.979	FR	71%	53	37.0%	
3-OH-Isovaleric acid	FR	70.1	FR	39.2%	FR	0.940	FR	112%	73	55.5%	
4-OH-Butyric acid	FR	72.8	FR	31.2%	FR	0.982	FR	70%	63	56.7%	
Adipic acid	MP	178	31.6%	12.6%	0.950	0.903	85%	101%	85	26.4%	
Creatinine		3258		3.4%		0.000		0%	67	5.25%	
D,L Glycolic acid	FR	278	FR	22.0%	FR	0.985	FR	83%	61	47.0%	
Ethylmalonic acid	ORFR	50.0	ORFR	17.1%	ORFR	0.992	ORFR	96%	89	36.3%	
Fumaric acid	MP	80.0	19.6%	14.6%	0.961	0.991	75%	100%	78	28.5%	
Glutaric acid	MP	112	44.0%	13.1%	0.976	0.994	86%	101%	90	21.8%	
Glycolic acid	OR	236	OR	20.6%	OR	0.963	OR	89%	77	38.3%	
Hexanoylglycine	MP	17.0	29.3%	29.5%	0.946	0.950	111%	100%	64	31.2%	
Keto-glutaric acid		168		21.4%		0.992		103%	73	40.1%	
Malic acid		117		19.8%		0.995		81%	53	47.4%	
Methylmalonic acid		305	330	31.2%	11.4%	0.955	0.995	95%	102%	104	27.6%
Mevalonic acid	FR	132	FR	21.7%	FR	0.992	FR	82%	56	46.1%	
Pyroglutamic acid		322	61.6%	23.0%	0.879	0.950	108%	85%	73	44.2%	
Sebacic acid	MP	55.4	15.2%	19.4%	0.987	0.987	91%	101%	83	35.9%	
Suberic acid	OR	172	11.5%	16.2%	0.952	0.990	94%	105%	64	32.4%	
Tiglylglycine	MP	30.6	17.0%	24.8%	0.870	0.907	54%	07%	57	40.0%	
Vanillic acid				19.4%		0.975		90%	38	33.0%	
Overall		260		20.1%		0.044		88%	80%	71	37.7%

Calculation Score

Total Number Relevant Boxes

-23 Analytes

-4 analytes no results (empty boxes)

-5 analytes Few Results (FR)

Relevant Analytes: 23 – (4+5) = 14

Relevant Boxes: 14 X 4 = 56

Total Number (weighed) Flags

4 Yellow boxes = 4/4 = 1

8 Purple boxes = 8/4 = 2

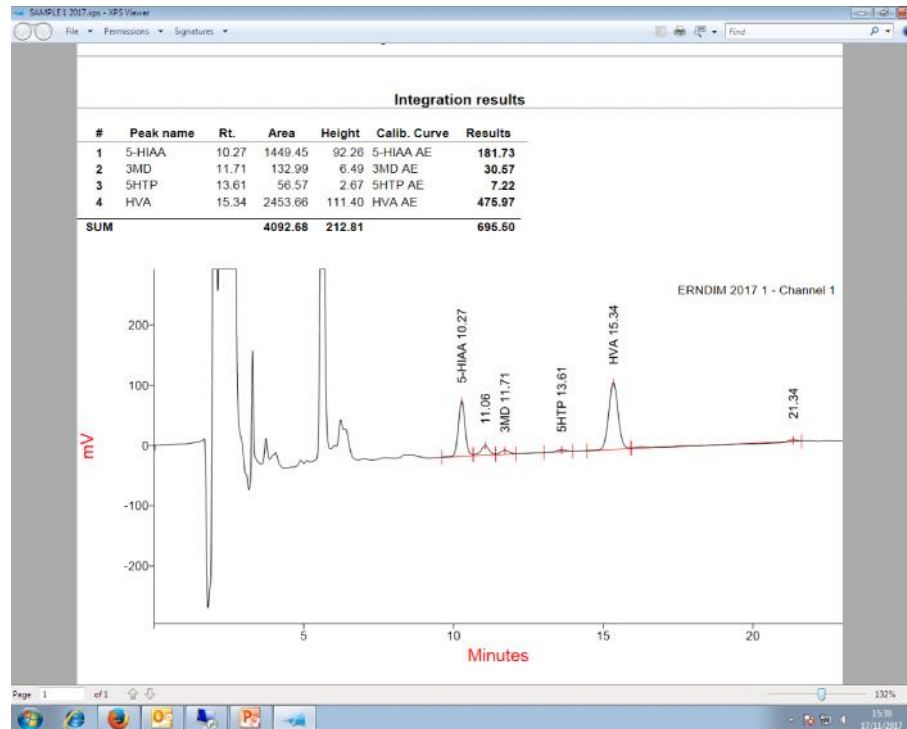
8 Amber boxes = 8/1 = 8

3 Red boxes = 3/1 = 3

Total weighed flags: 1+2+8+3 = 14

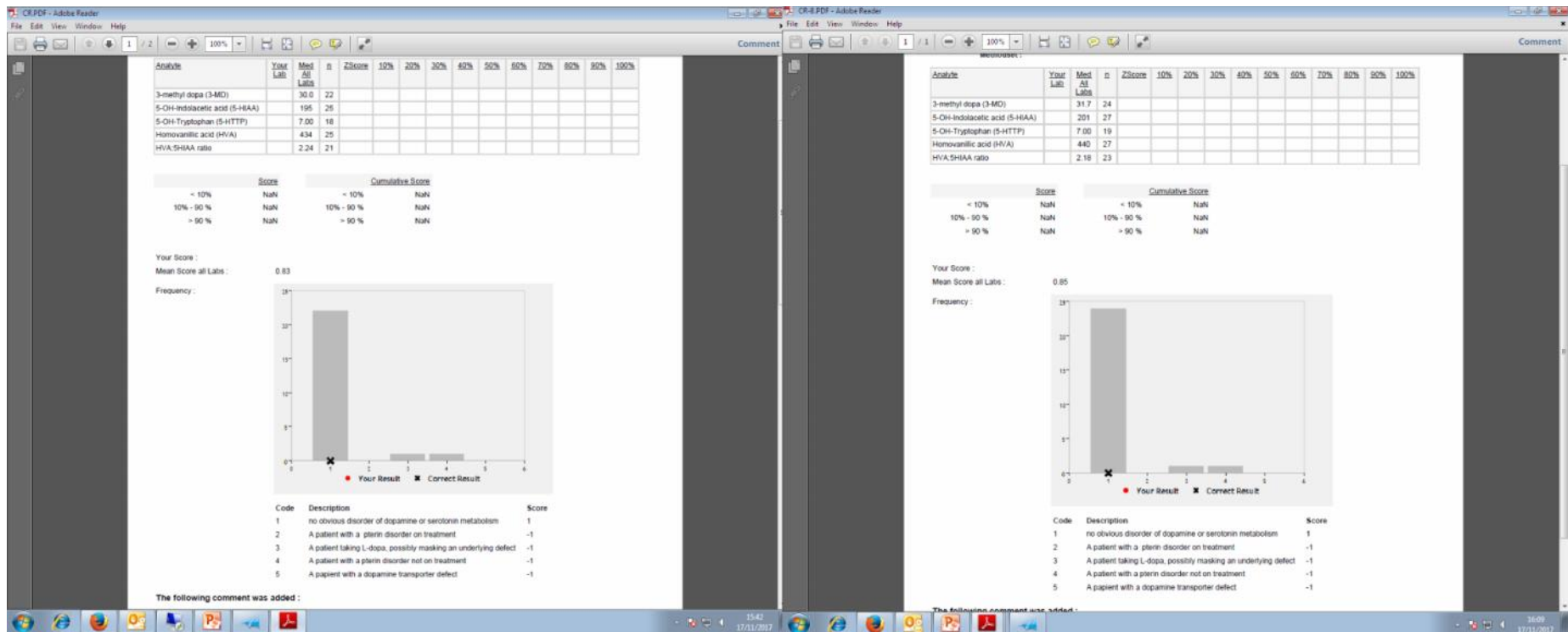
$$\text{Score} = \frac{\text{Total number flags}}{\text{Total number boxes}} = \frac{14}{56} \times 100\% = 25$$

This year's samples 1 and 8

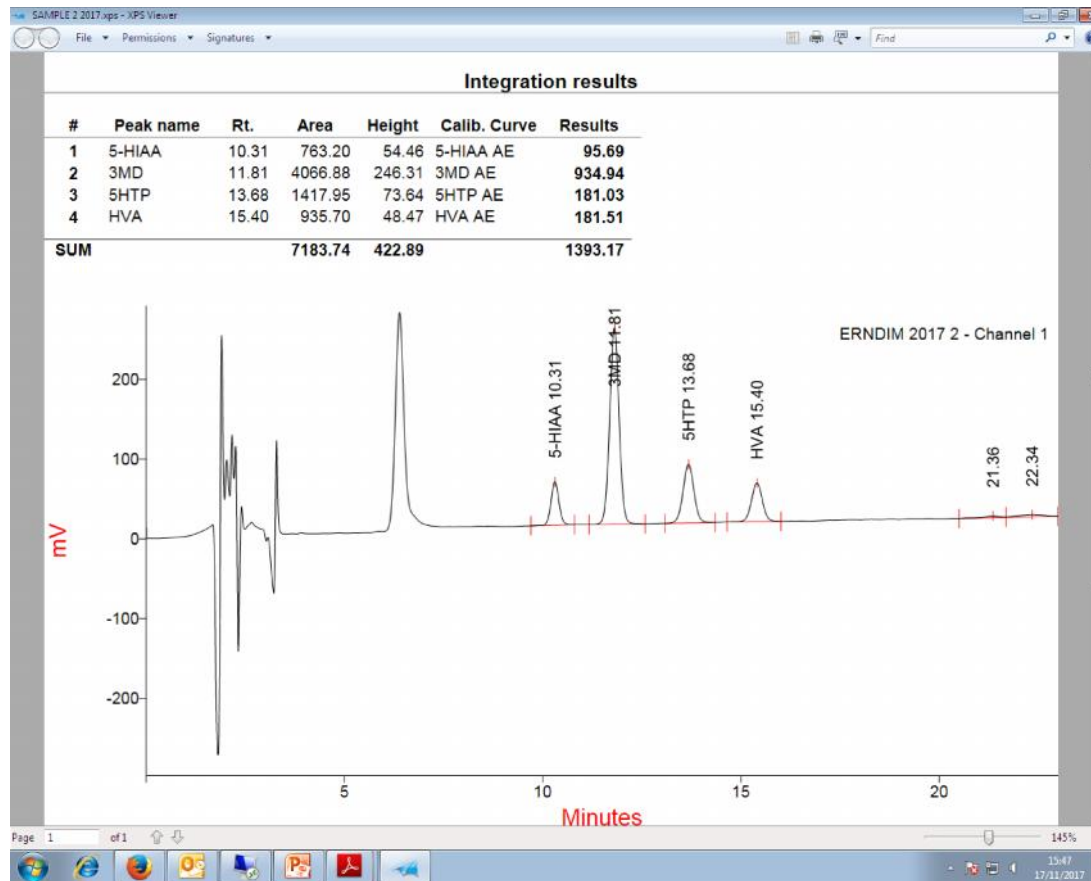


3 or 4 year old
Normal ratio and metabolites
within reference ranges

Samples 1 and 8

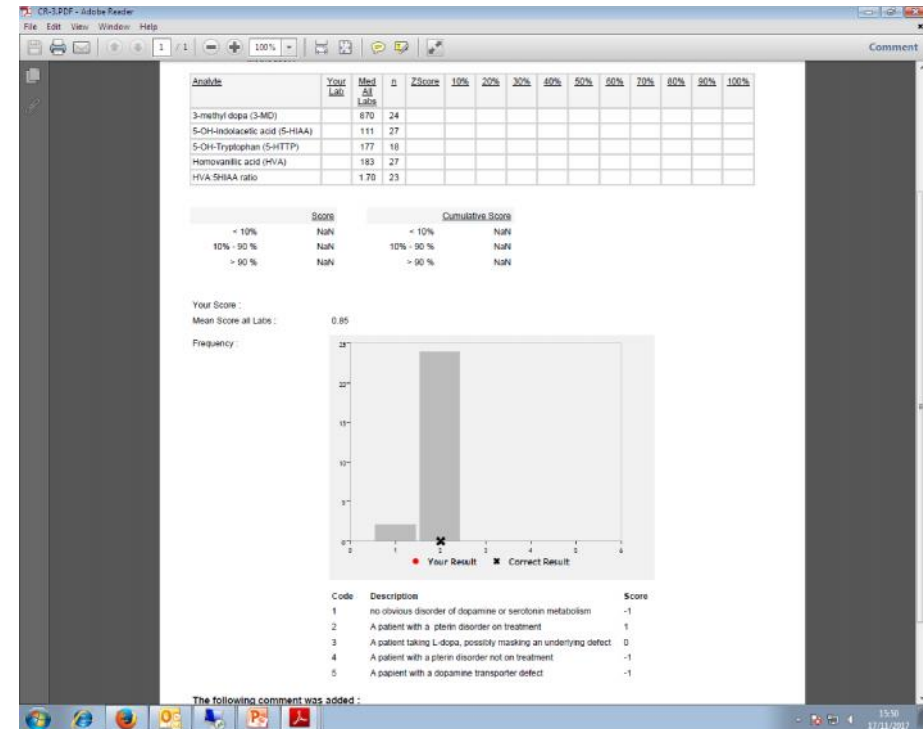
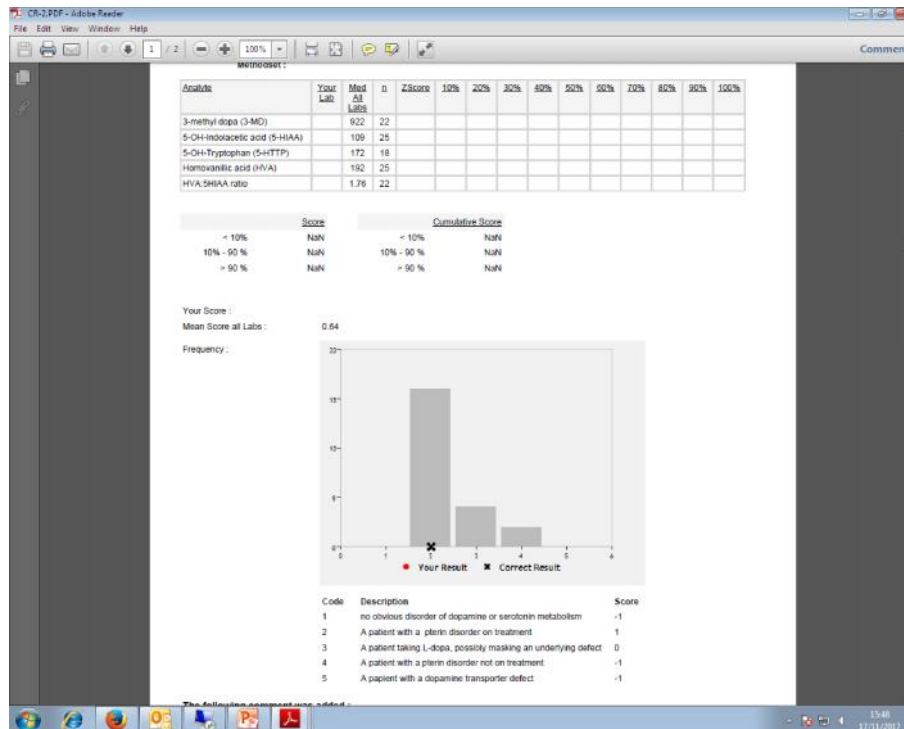


Samples 2 and 5

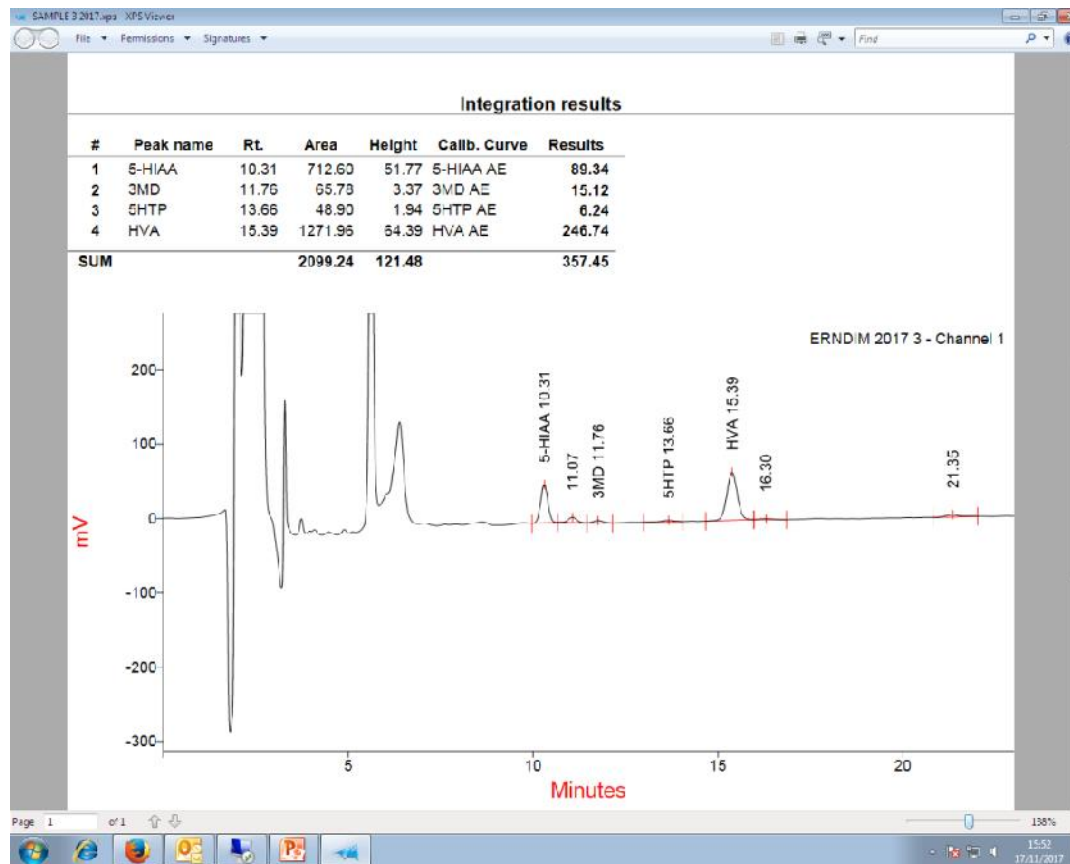


5 or 7 year old
Normal ratio but elevated
3-methyl dopa and
5-hydroxytryptophan

Samples 2 and 5

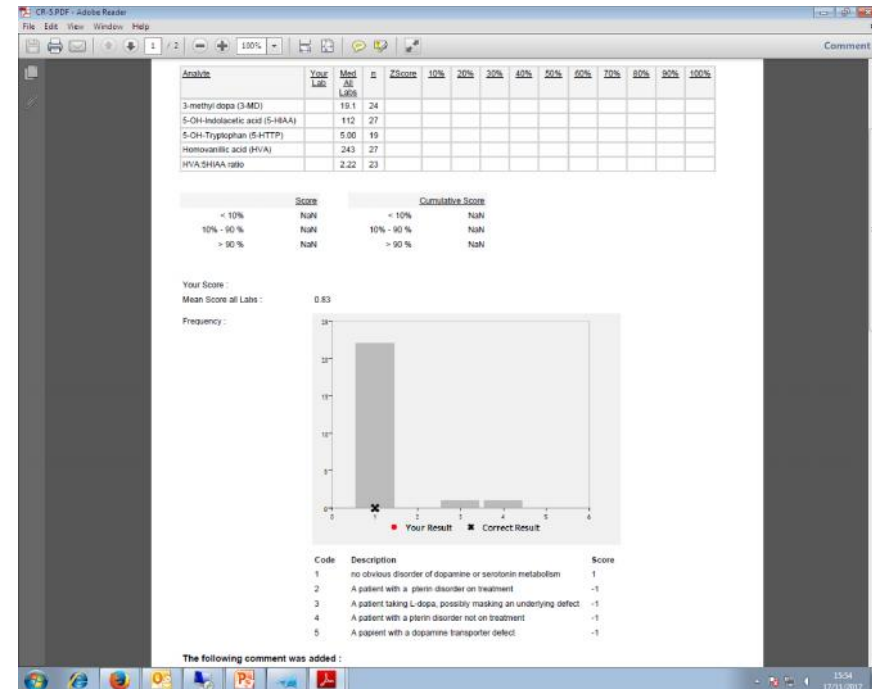
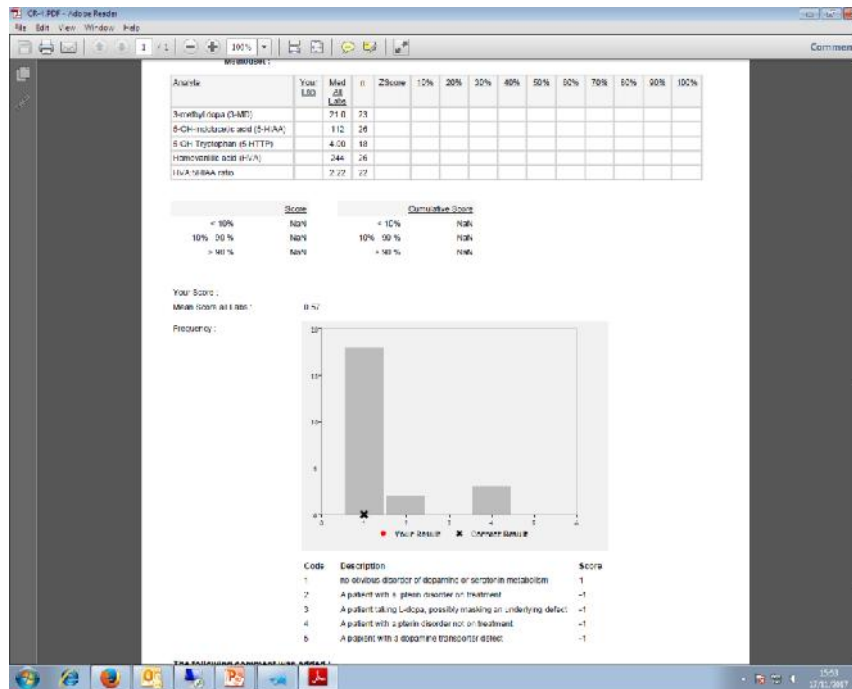


Samples 3 and 7

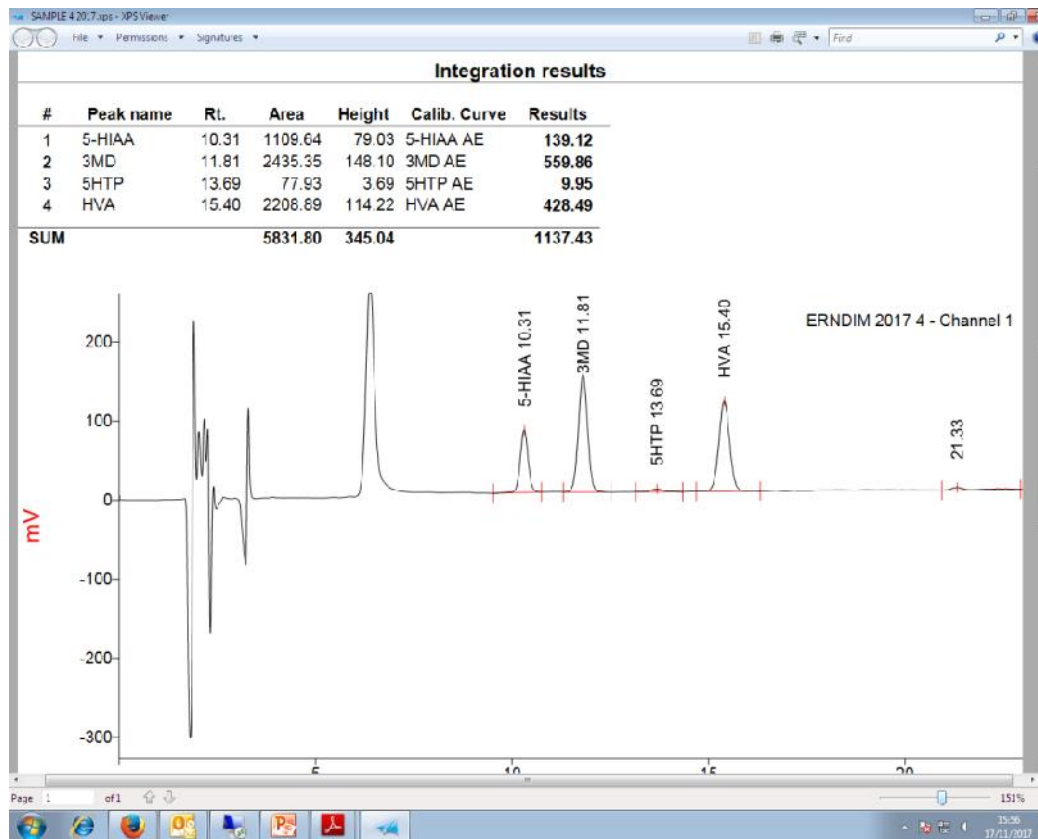


5 or 7 year old
Normal ratio
All metabolites within reference
ranges

Samples 3 and 7

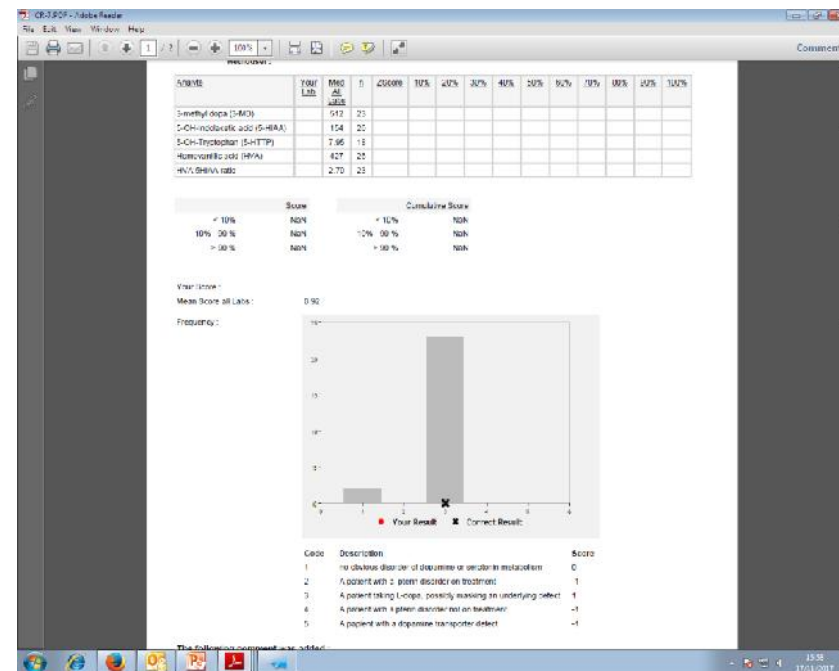
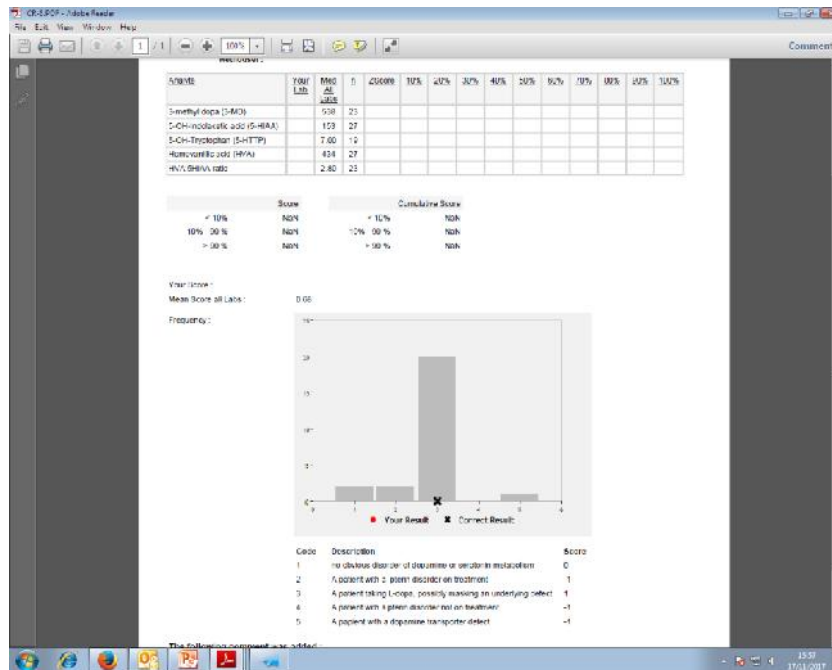


Samples 4 and 6



3 or 4 year old
Normal ratio
Elevated 3-methyl dopa

Samples 4 and 6



Overview of performance

- Results for 5HIAA, HVA and ratio generally good with CVs of ~10% between labs
- Much greater variation for 3-methyl dopa and 5-hydroxytryptophan - >30%

Methodset : hplc ecd

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
3-methyl dopa (3-MD)	372	369	8.0%	11.3%	0.998	0.995	101%	100%	24	34.0%
5-OH-Indoleacetic acid (5-HIAA)	138	145	5.3%	9.9%	0.989	0.932	104%	104%	27	13.2%
5-OH-Tryptophan (5-HTTP)	57.8	49.0	10.5%	18.4%	0.999	0.996	115%	100%	19	66.1%
Homovanillic acid (HVA)	336	317	2.2%	6.7%	0.989	0.985	106%	101%	27	11.2%
HVA/5HIAA ratio	2.38	2.28	4.5%	8.3%	0.959	0.914	95%	103%	23	12.6%
Overall	181	176	6.1%	10.9%	0.987	0.964	104%	102%	24	27.4%

Possible reasons for poor performance



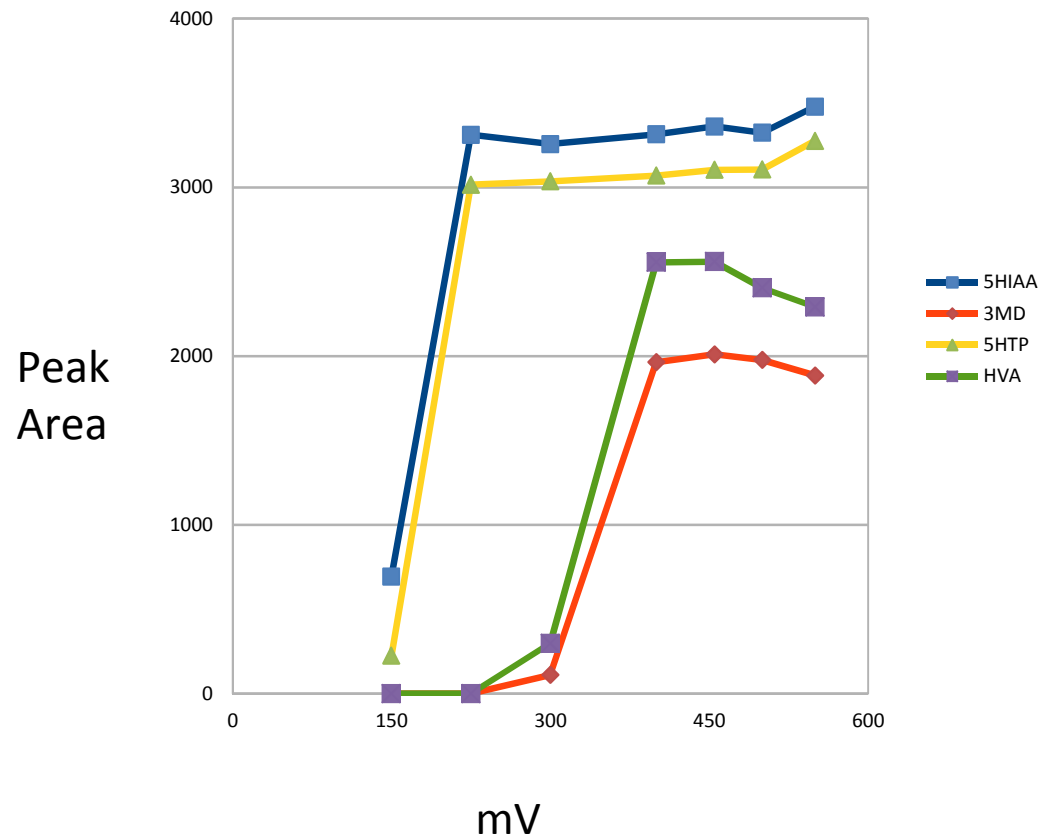
Random mistakes

- Reconstitution - too much, too little, not long enough
- Wrong sample analysed

Systematic

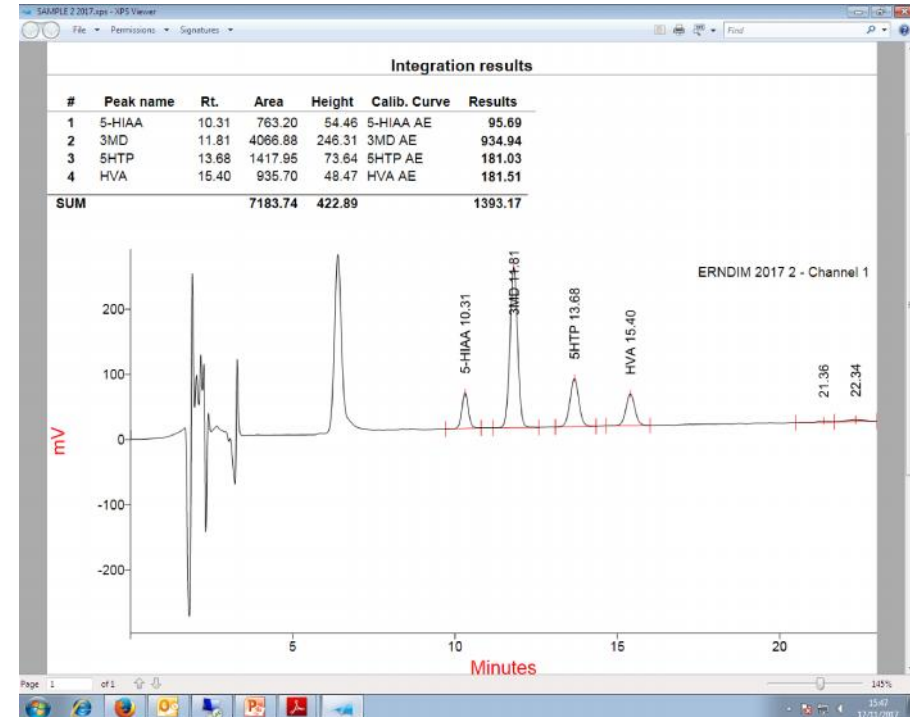
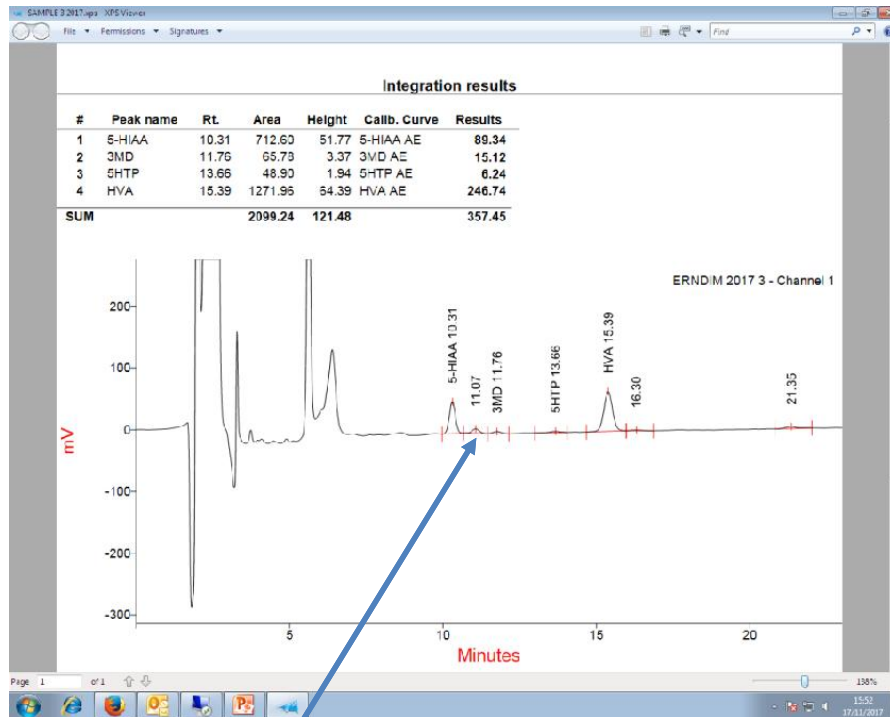
- Poor control of assay - standards, QC or voltamagram problems
- Mis-identification of metabolites - variation in pH can shift peaks
- Co-elution of peaks

Voltammogram



Very important to check voltammogram and peak areas regularly. 3-methyl dopa hardest to oxidise so changes to voltammogram most likely to affect 3-methyl dopa first. This may explain greater variation for 3-methyl dopa.

Mis-identification



Other peaks in CSF could be mis-identified

← 3MD and 5HTP if pH increased
→ 3MD and 5HTP if pH lowered

Any suggestions?



- Suggestions, complaints, improvements, discussion
- Possible changes to the scheme - greater emphasis on interpretation?