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 interupt, 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

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
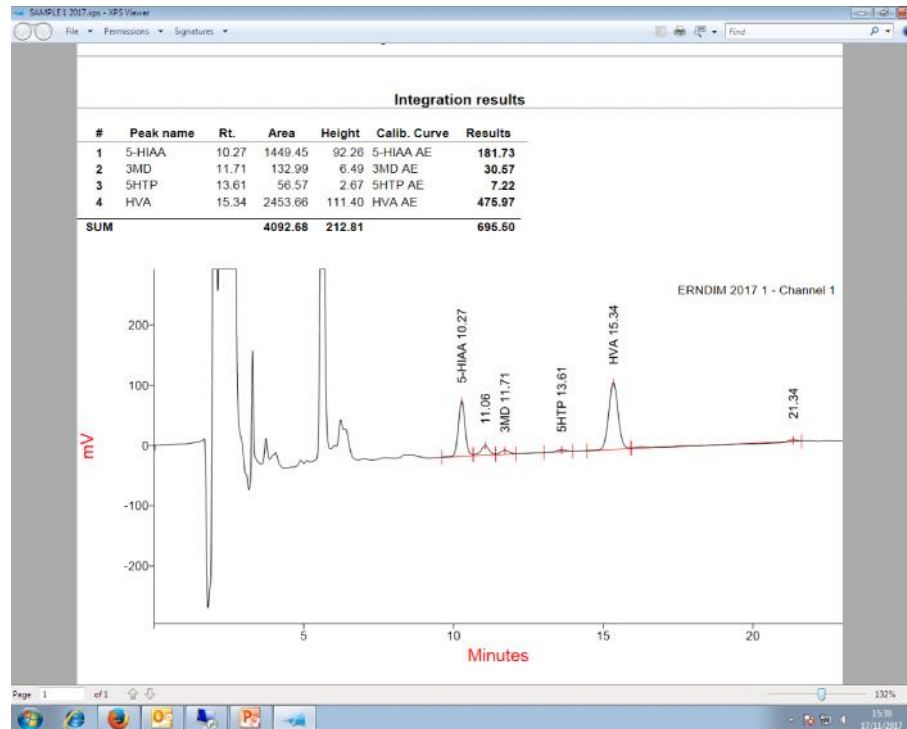8 Purple boxes = 8/4 = 2
8 Amber boxes = 8/1 = 8
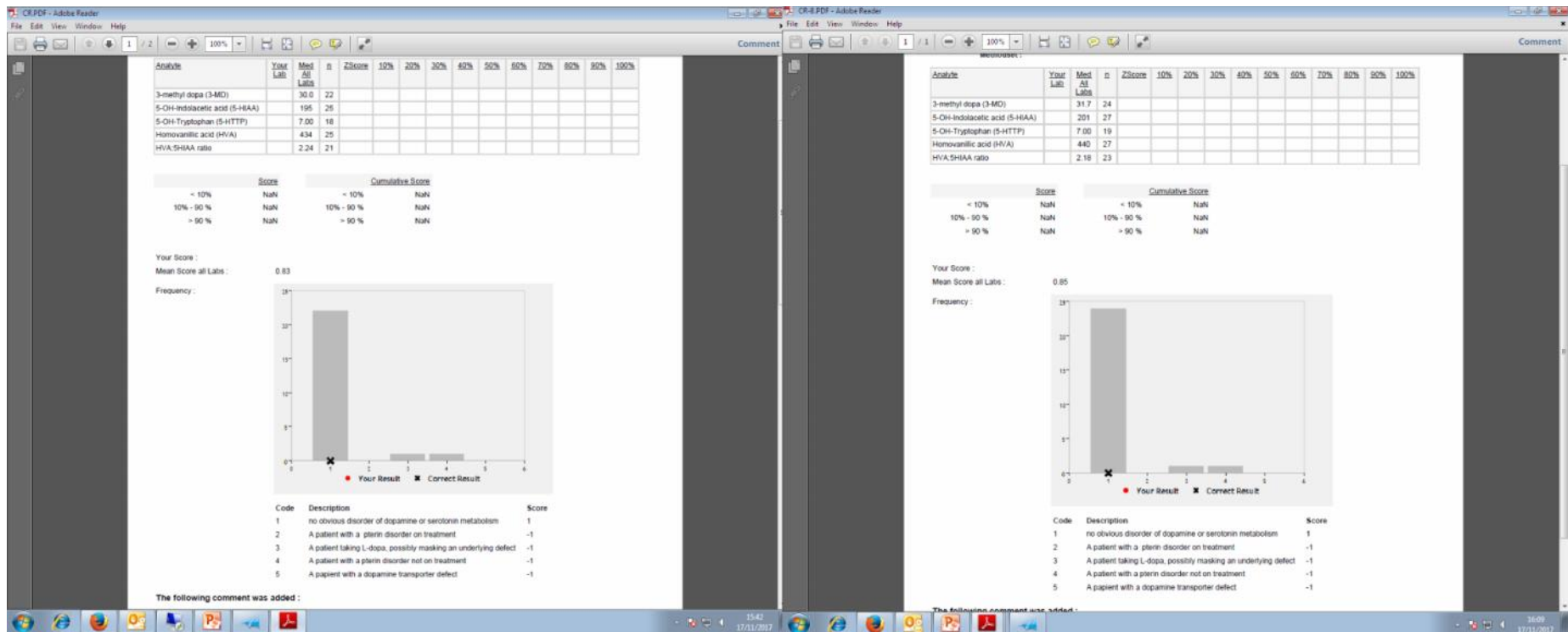3 Red boxes   = 3/1 = 3
Total weighed flags: 1+2+8+3 = 14

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

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MP - missing pair; OR - outlying result; ORFR - outlying/few results; FR - few results (<6/8); Red flag - poor performance
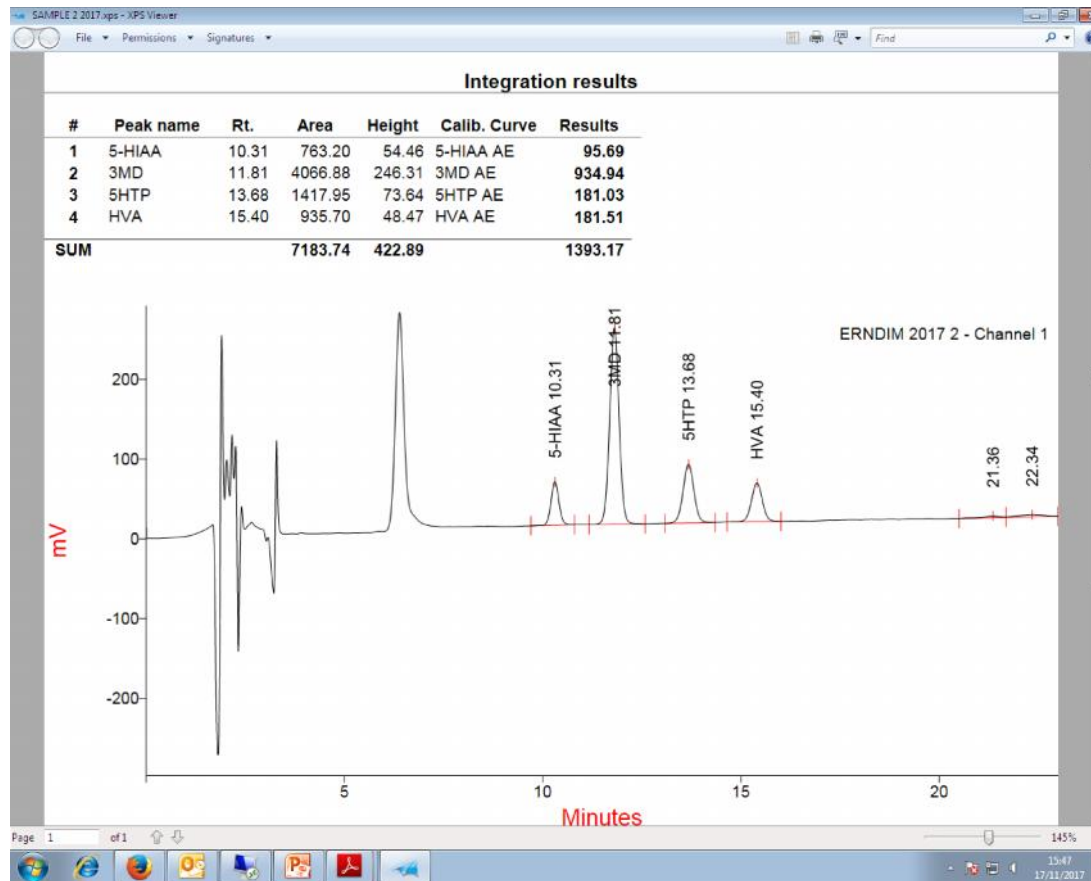
This year's samples
1 and 8

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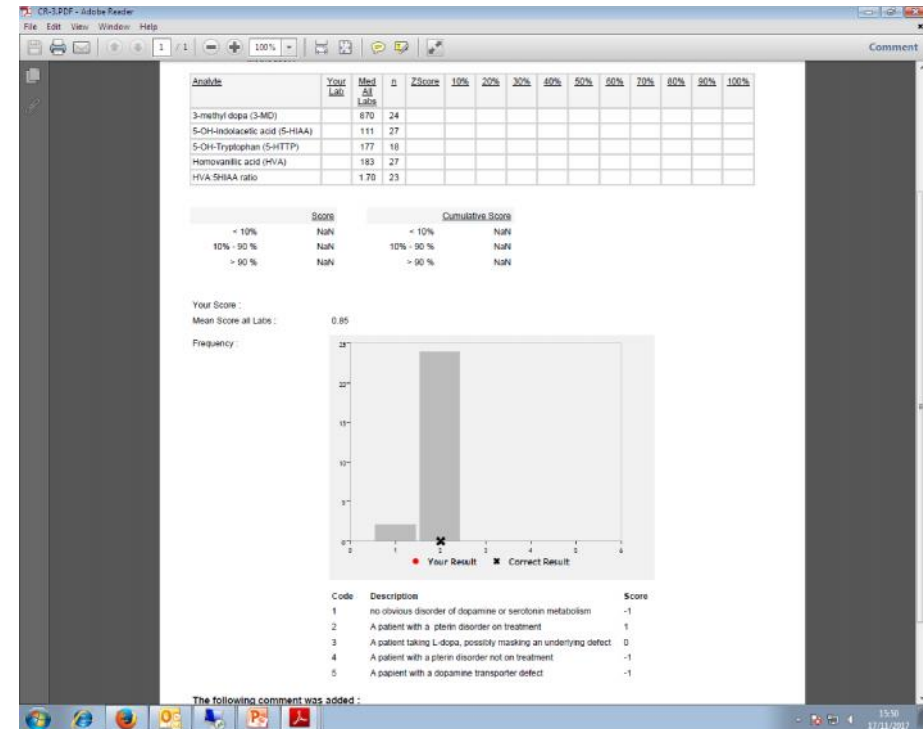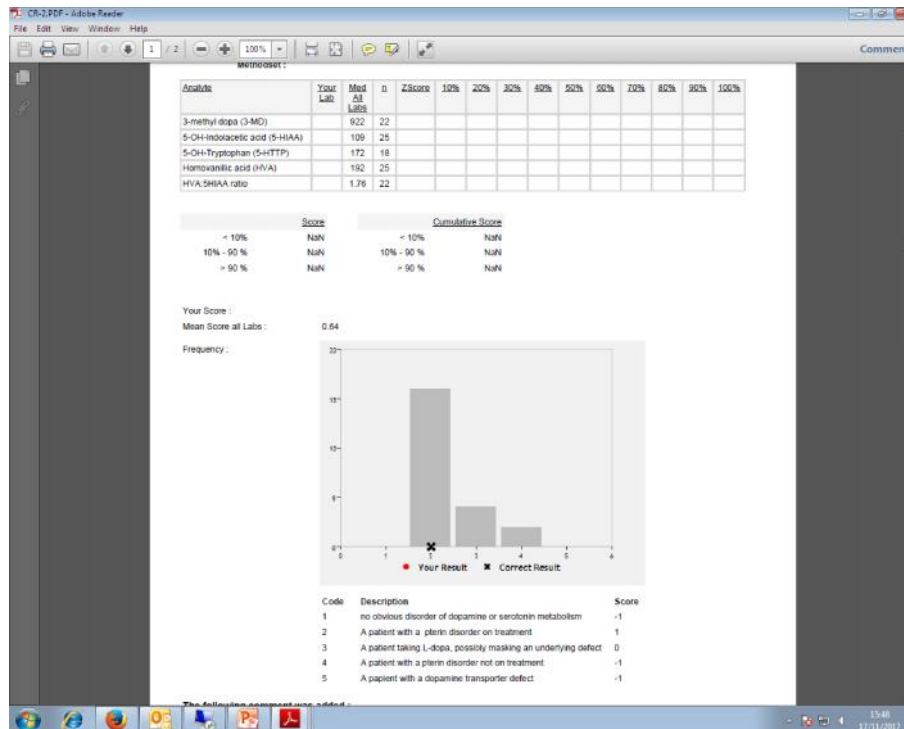within reference ranges

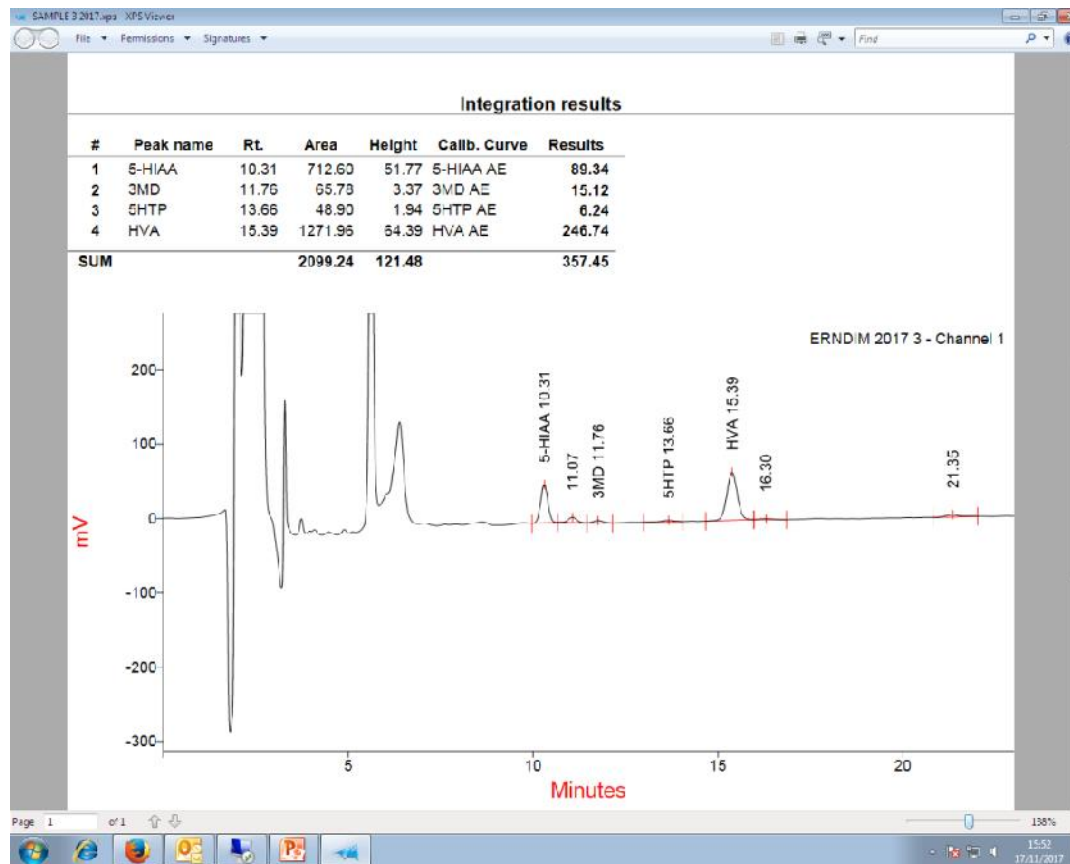# Samples 1 and 8

Samples 2 and 5

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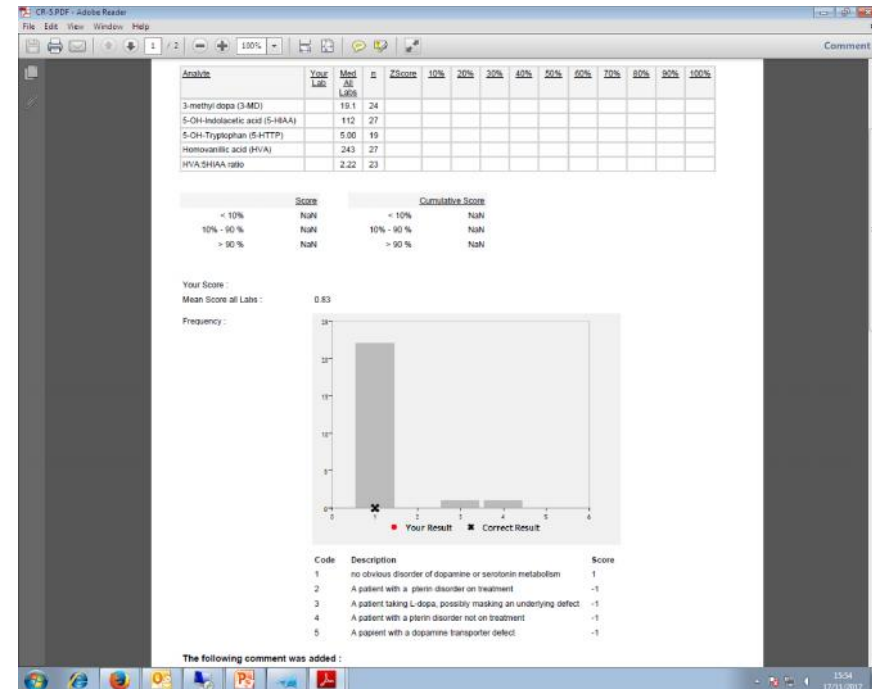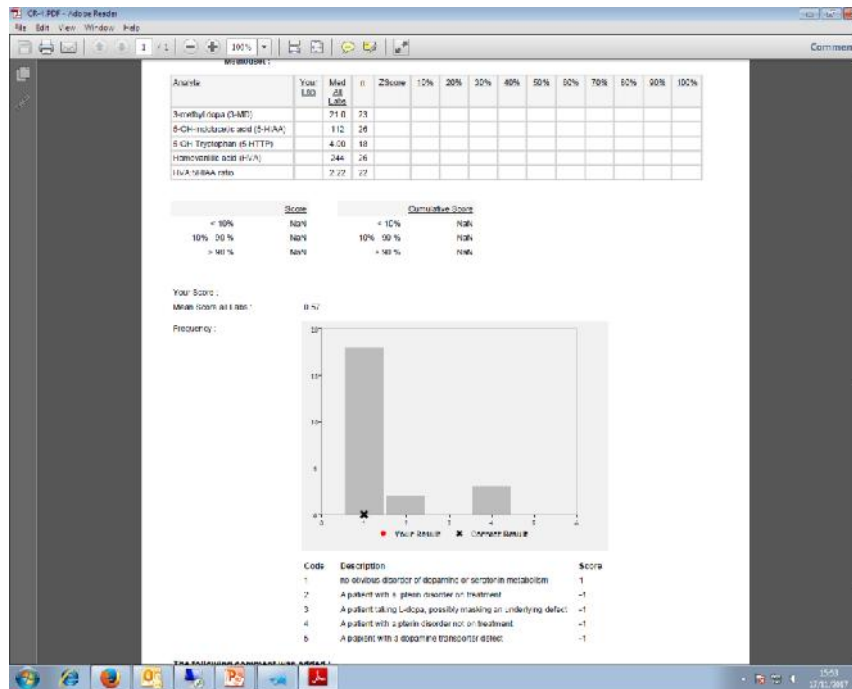5-hydroxtryptophan

Samples 2 and 5
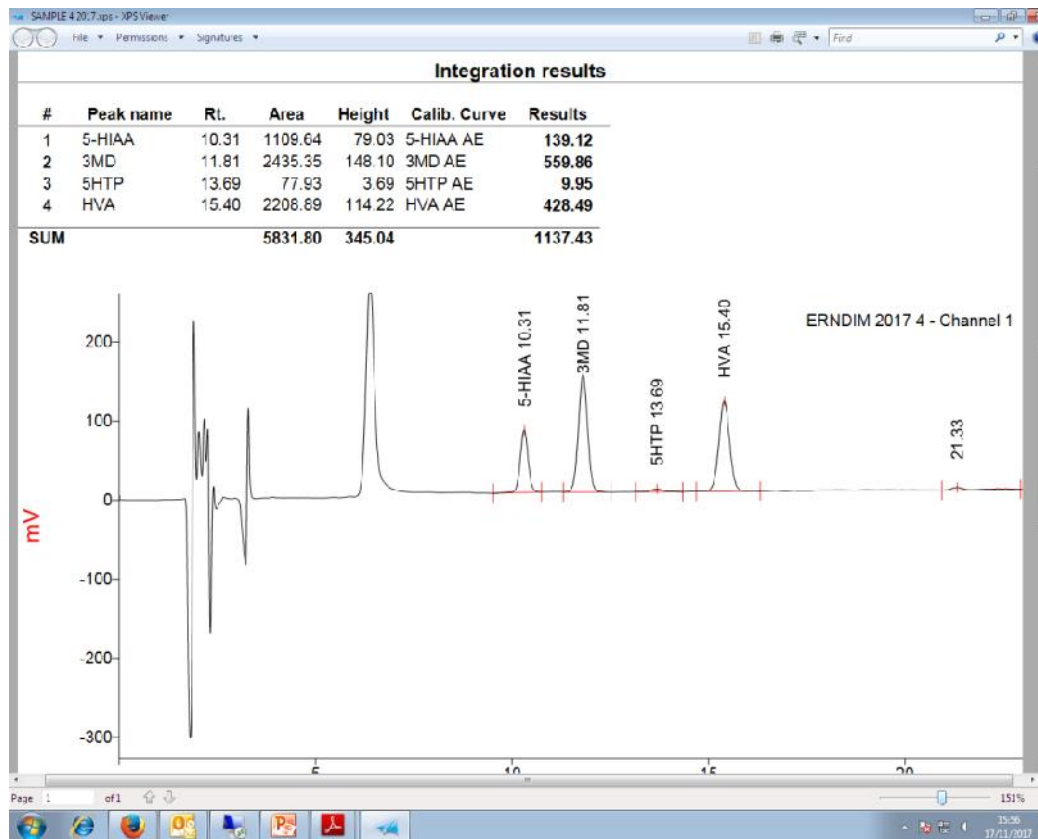
Samples 3 and 7

5 or 7 year old
Normal ratio
All metabolites within reference ranges
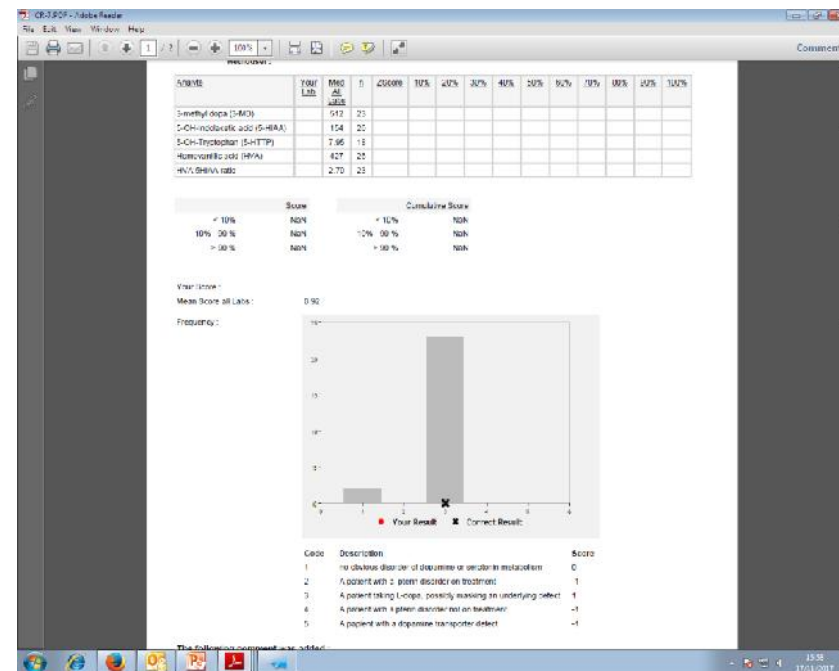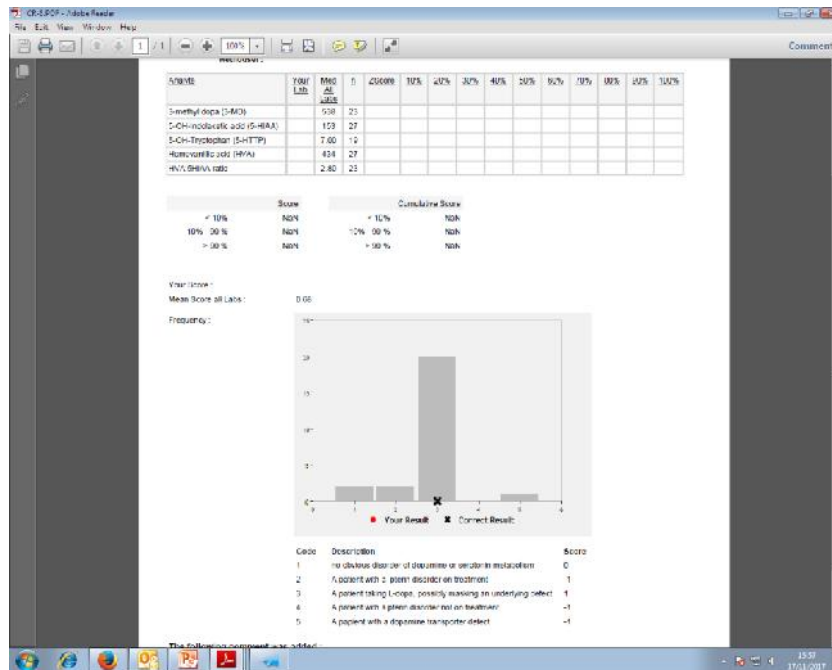
Samples 3 and 7

Samples 4 and 6

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

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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%
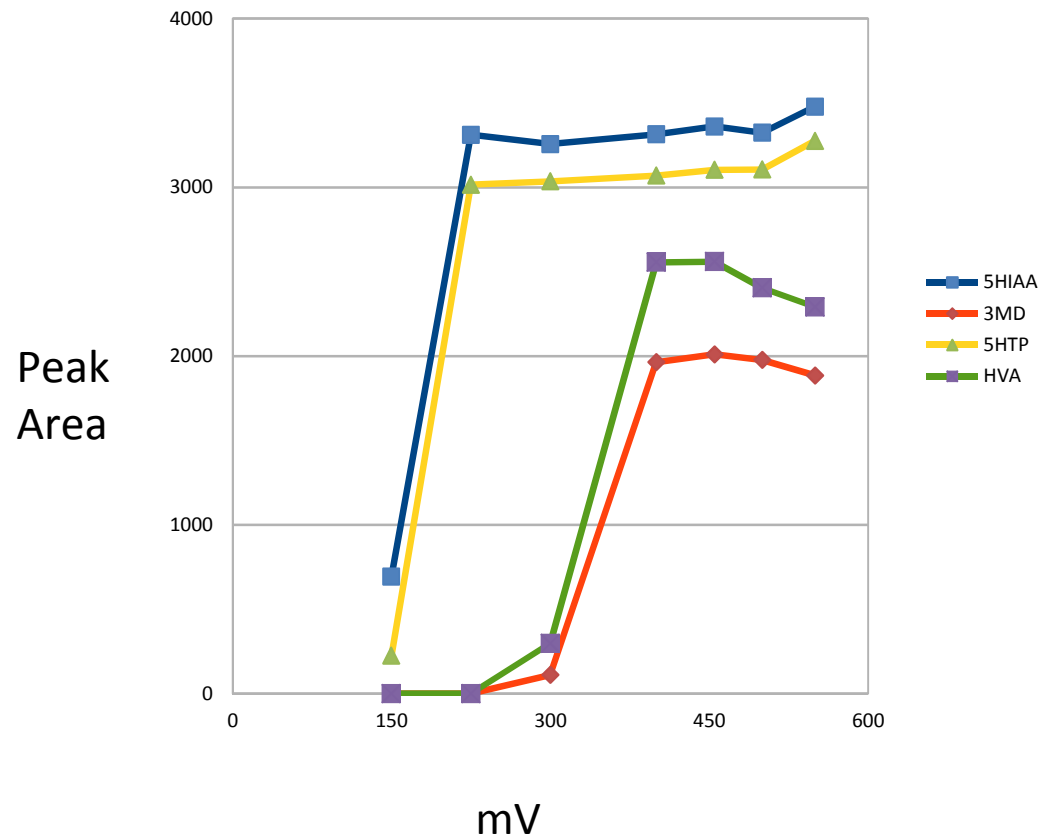
Possible reasons for poor performance

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

Voltammagram

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

Mis-identification
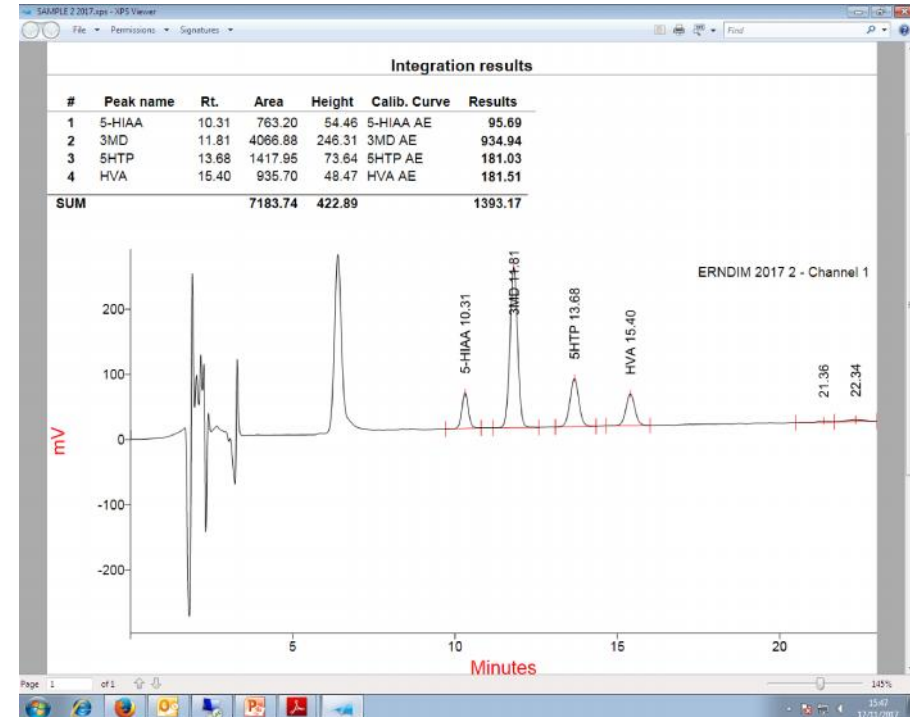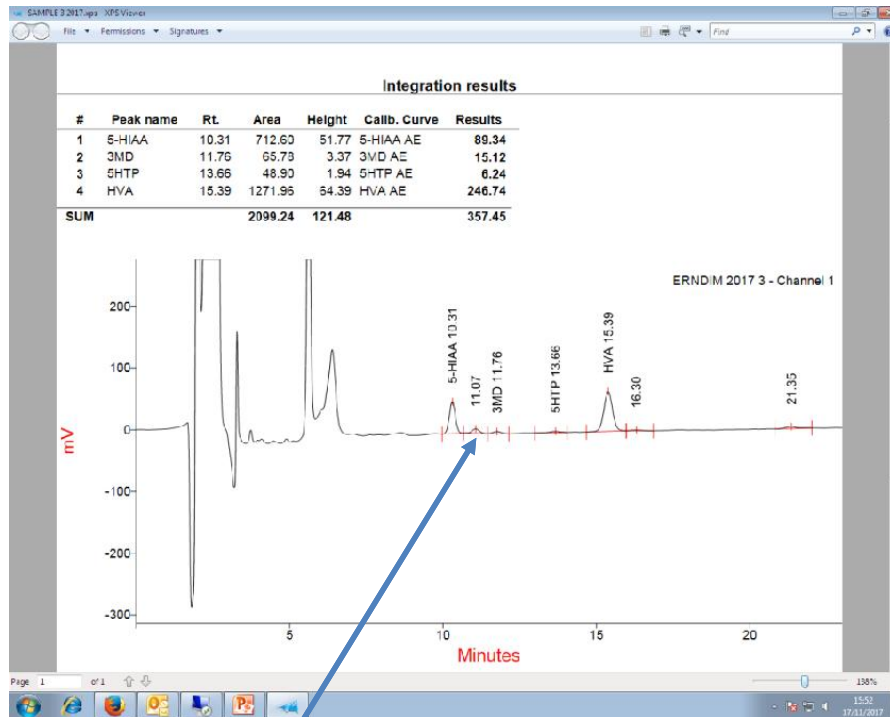
Other peaks in CSF could be mis-identified

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?