

Ensuring Quality in the Absence of EQA

HealeS@gosh.nhs.uk



Accredited Medical Laboratory
Reference No:
0250/1370



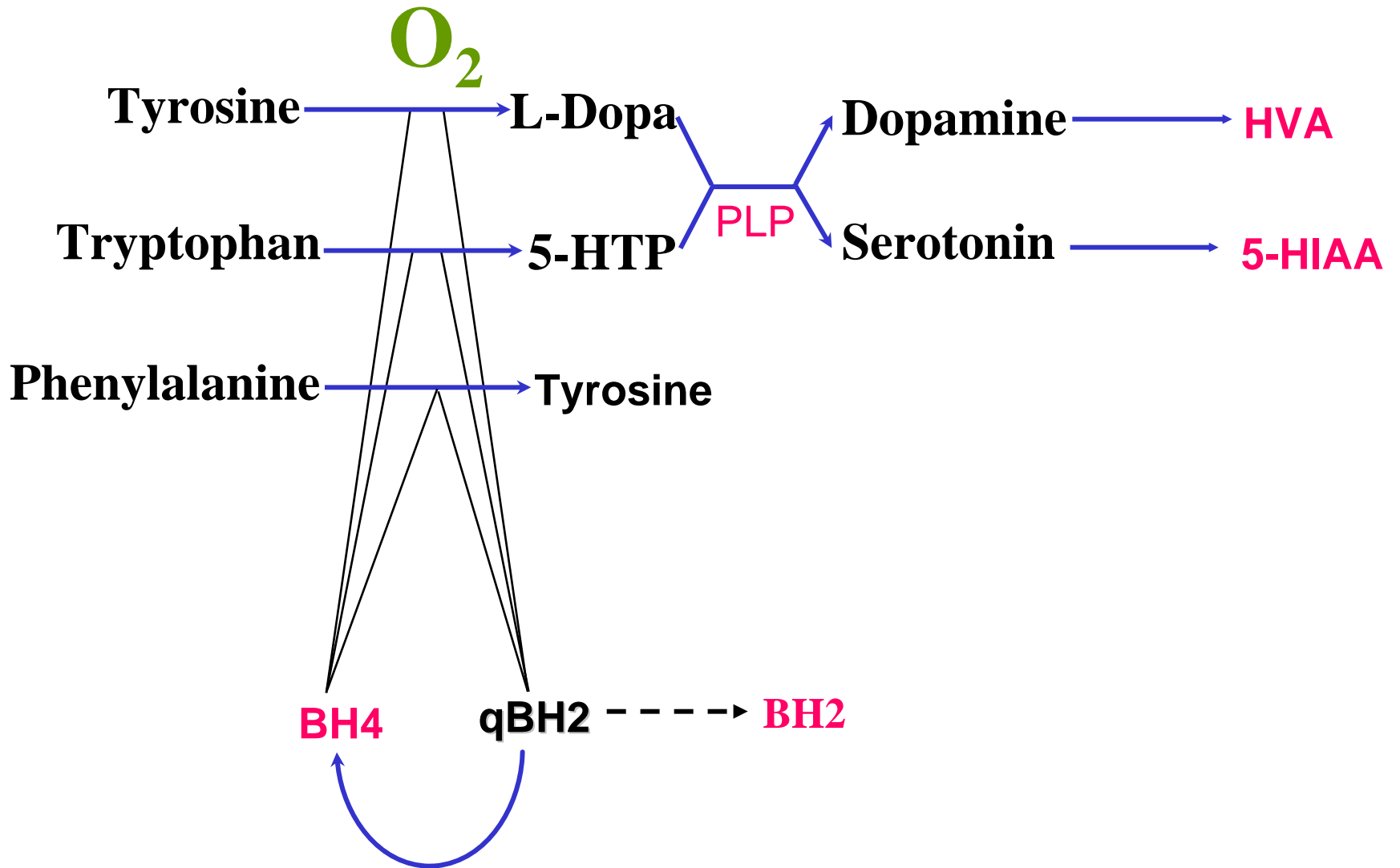
Outline

- “Neurotransmitters”
- Mitochondria
- Lysosomal - DBS



“Neurotransmitters”





CSF “Neurotransmitters”

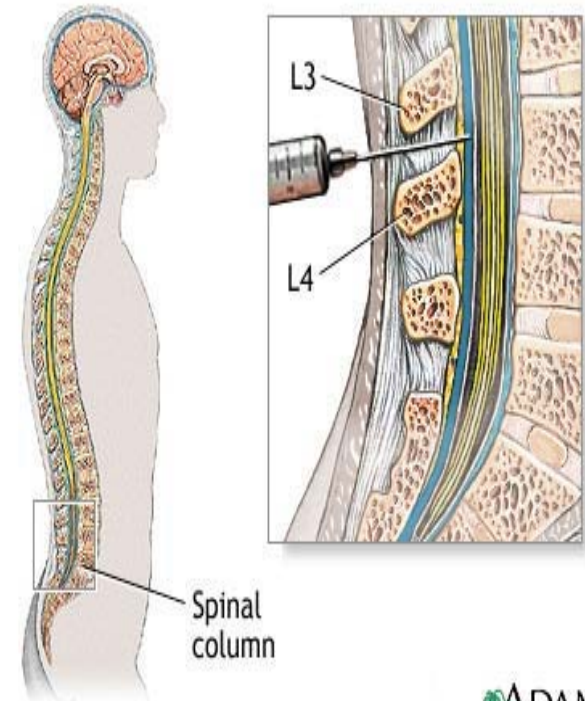
- Disorders of BH4 Metabolism
- Tyrosine Hydroxylase Deficiency
- Aromatic Amino Acid Decarboxylase Deficiency
- Dopamine Transporter Defect
- Cerebral Folate Deficiency
- Disorders of Pyridoxal Phosphate Metabolism

CSF – Sample Requirements

- *Tube 1* 0.5ml **HVA & 5-HIAA**
- *Tube 2* 0.5ml **5-MTHF & PLP**
- *Tube 3* 1.0ml **Pterins**

(DTE/DETAPAC)

Strong Rostro-caudal Gradient



Collect at bedside and freeze immediately (not the form !)

CSF “Neurotransmitters”

- Approximately 75 samples per month received & processed
- HPLC with electrochemical/fluorescent detection
- No existing external QC scheme
- Necessary to create an in house system
- “Interpretative scheme”
 - Monitor and respond to queries/concerns arising as a result of comments issued !

In House QC



Pooled “processed” CSF



Aliquots

Analyse 5x (separate analytical run)

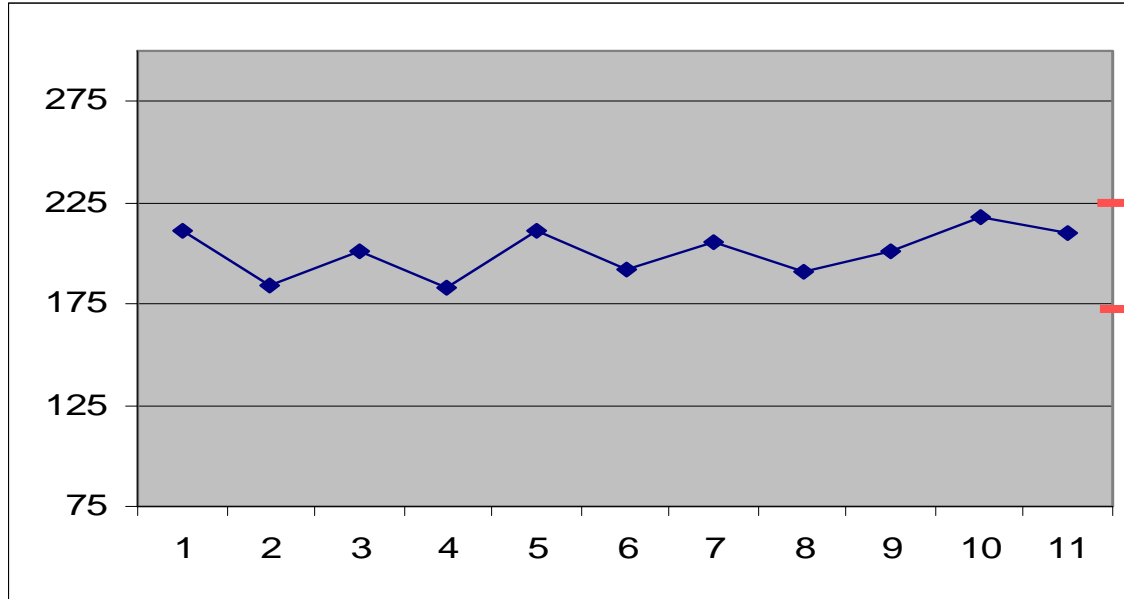
Overlap with previous “QC”

Mean \pm 2SD

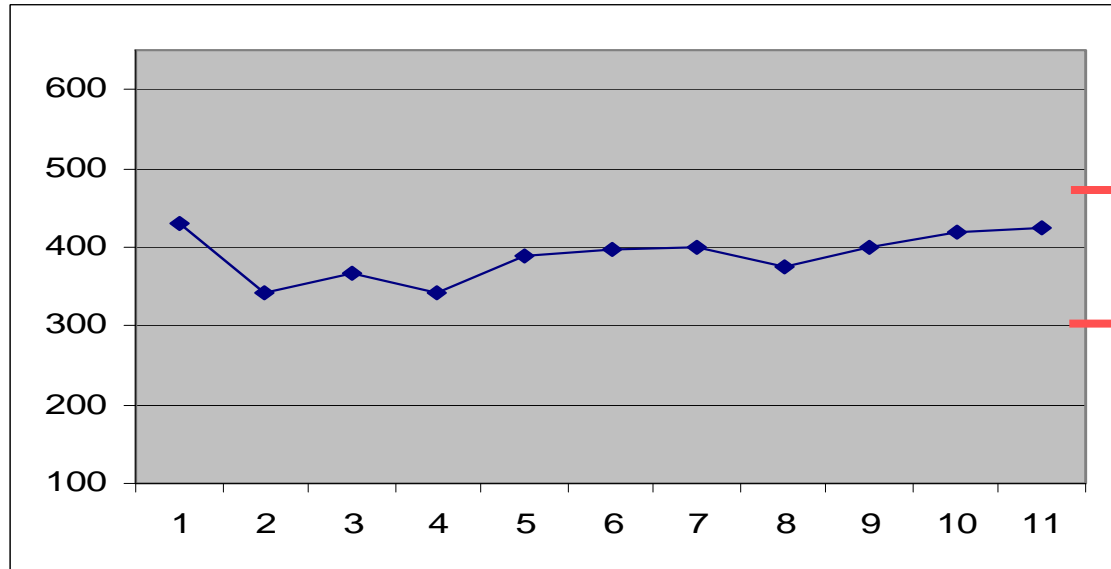
Pterins, HVA, 5-HIAA & 5MTHF

CSF "in house" QC scheme

5-HIAA nmol/L



HVA nmol/L



Neurotransmitter metabolites in CSF: An external quality control scheme

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²*Queen Beatrix Hospital, SKZL, Winterswijk;* ³*University Medical Centre
Nijmegen, Laboratory of Pediatrics and Neurology, Nijmegen, The Netherlands*

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Pediatrics and Neurology, Reinier Postlaan 4, 6525 GC Nijmegen, The Netherlands.
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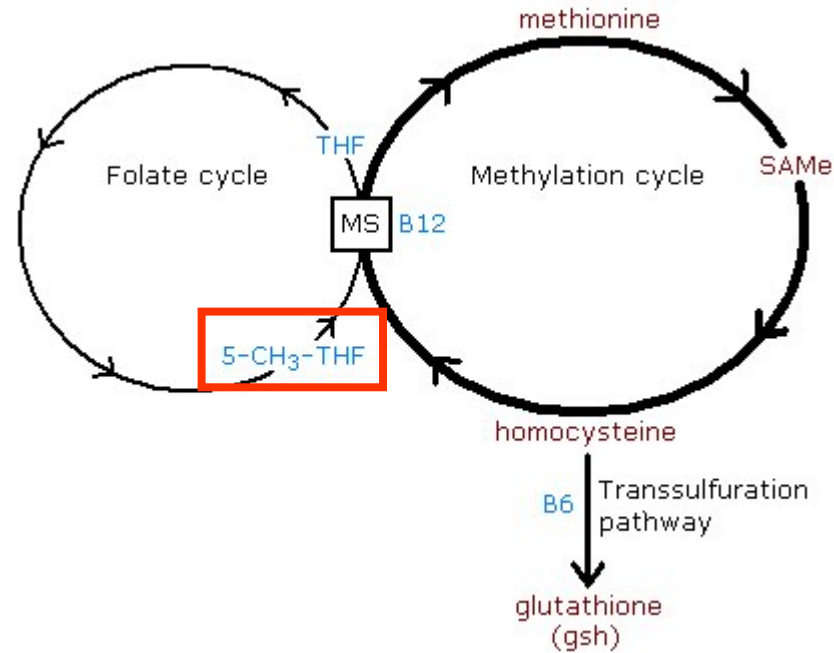
MS received 22.01.02 Accepted 12.03.02

- 12 Labs recruited worldwide
- Pooled CSF - spiked with different amounts of HVA and 5-HIAA
- Sent to labs by courier on dry ice
- 3 labs rejected at first stage due to unacceptable CVs
- CVs, linearity, recoveries were acceptable for remaining 9
- Lyophilisation of CSF gave comparable results – Ease of shipment
- Marked differences in quoted reference ranges - Interpretation Implications
- Plan to continue with scheme x2 per year (2002)

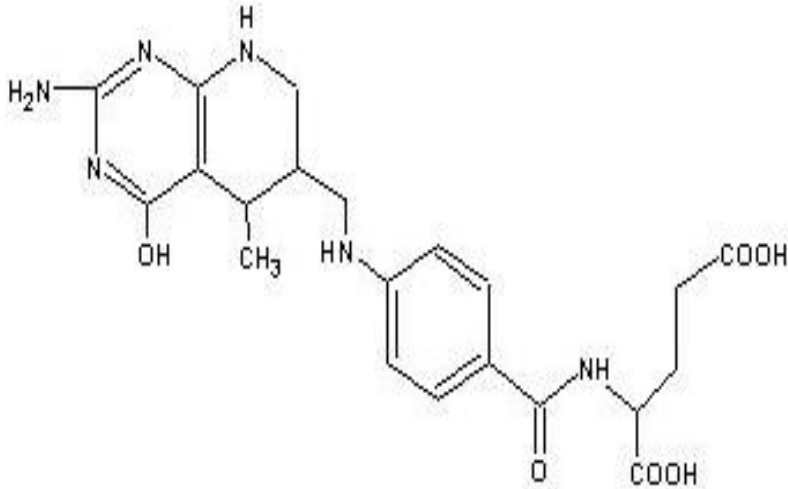
Metabolite	Age (years)	nmol/L	
		Mean	Range
HVA	0 - 0.33	714	324-1098
	0.34 - 0.66	587	362-955
	0.67 - 1.00	508	176-851
	1.10 - 5.00	465	154-867
	5.1- Adult	281	71-565
5-HIAA	0 - 0.33	417	199-608
	0.34 - 0.66	271	63-503
	0.67 - 1.00	250	68-451
	1.10 - 5.00	185	89-367
	5.1- Adult	98	58-220

Pediatr Res (1993) 34, 10-14

5-Methyltetrahydrofolate



CSF 5-MTHF Deficiency

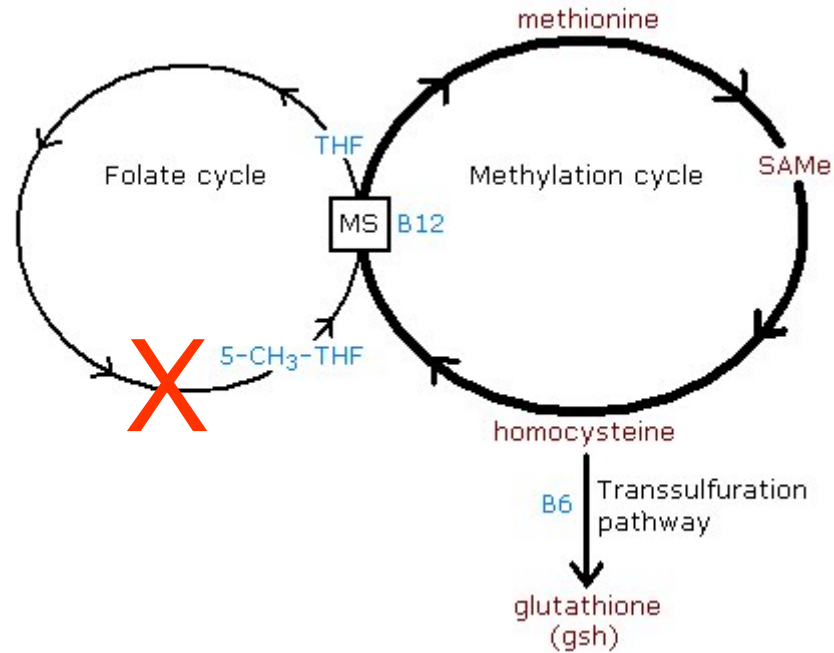


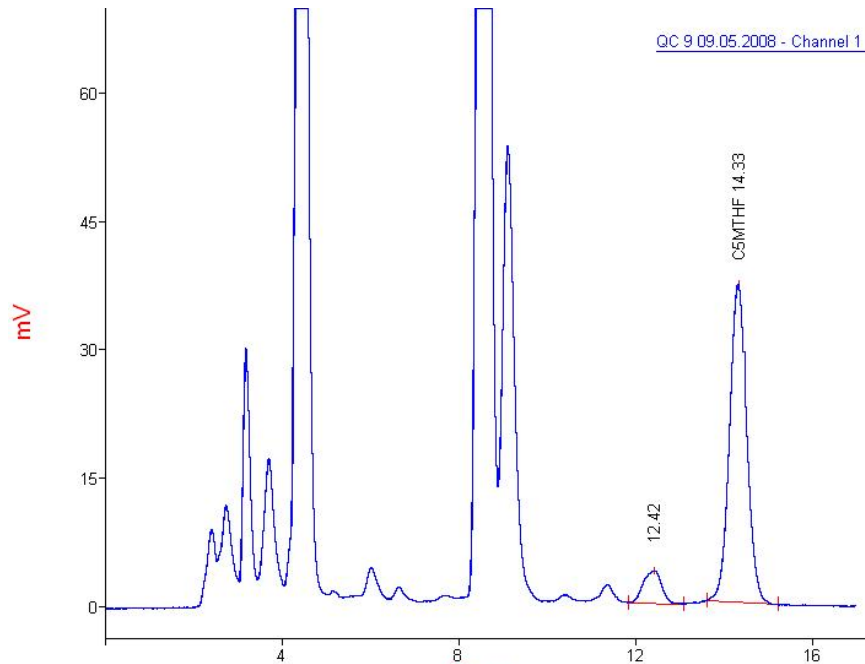
5-methyl tetrahydrofolate

- DHPR deficiency
- MTHFR deficiency
- Folate transporter mutations
- AADC deficiency
- 3-Phosphoglycerate dehydrogenase def
- Rett syndrome
- Aicardi Goutieres
- Mitochondrial disorders
- L-dopa treatment
- Methotrexate
- Anticonvulsants
- Steroids
- Co-trimoxazole

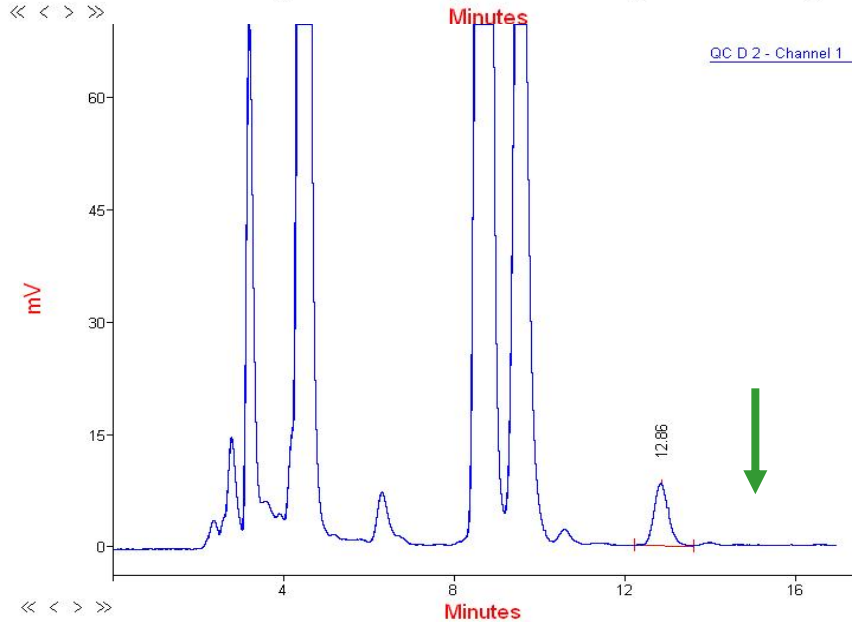
Cerebral Folate Deficiency - Neurological syndrome associated with low CSF 5-MTHF and normal peripheral folate.

5-Methyltetrahydrofolate – QCd





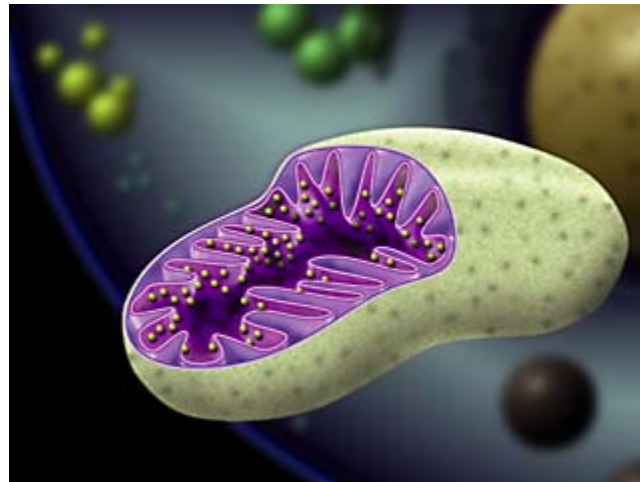
Control CSF



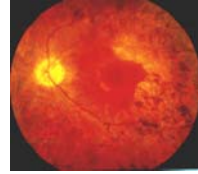
MTHFR Def CSF

“QCd”
Sufficient CSF for 15 years

Disorders of Mitochondrial Electron Transport Chain & Complex V



Respiratory Failure



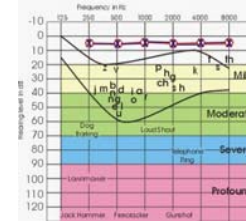
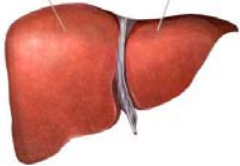
Optic Atrophy / Retinitis Pigmentosa

Cardiomyopathy



Seizures /
Developmental delay

Liver Failure

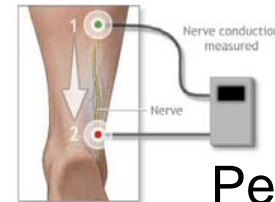


Deafness

Short Stature

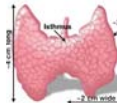


Marrow Failure

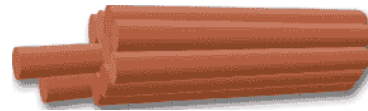


Peripheral Neuropathy

Diabetes

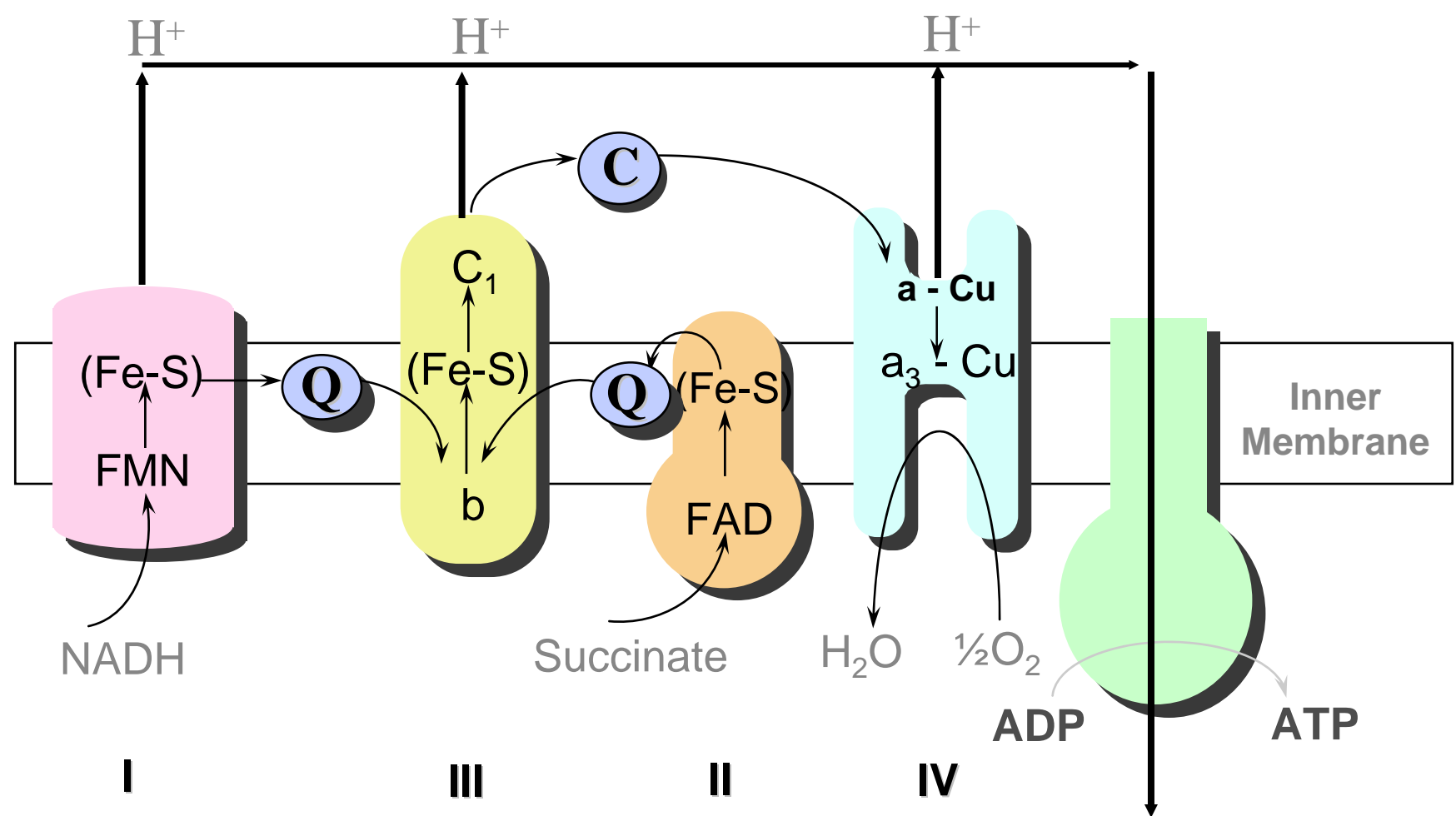


Thyroid Disease



Myopathy

Outer Membrane



Inner Membrane

I

III

II

IV

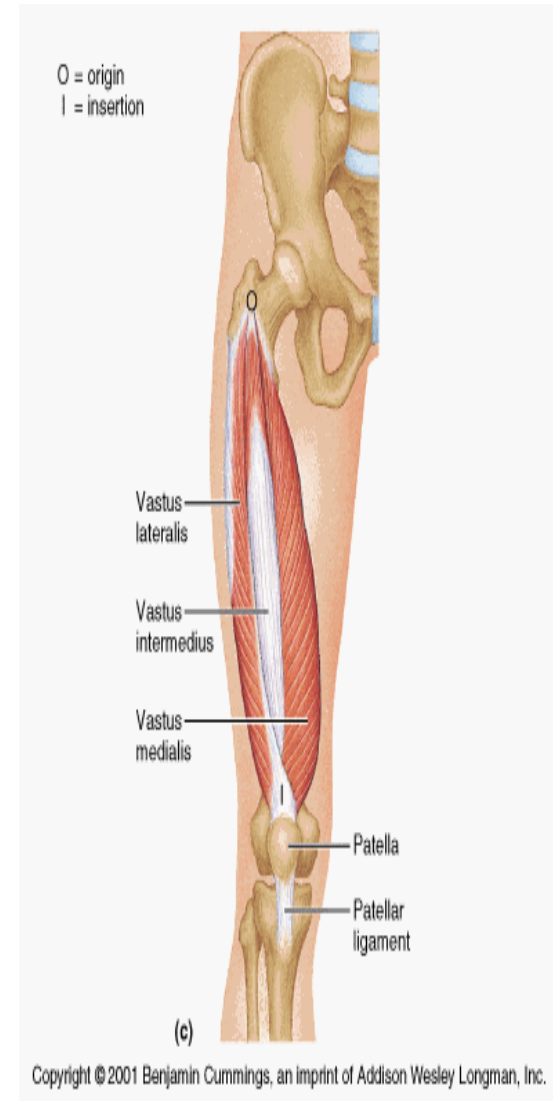
ADP

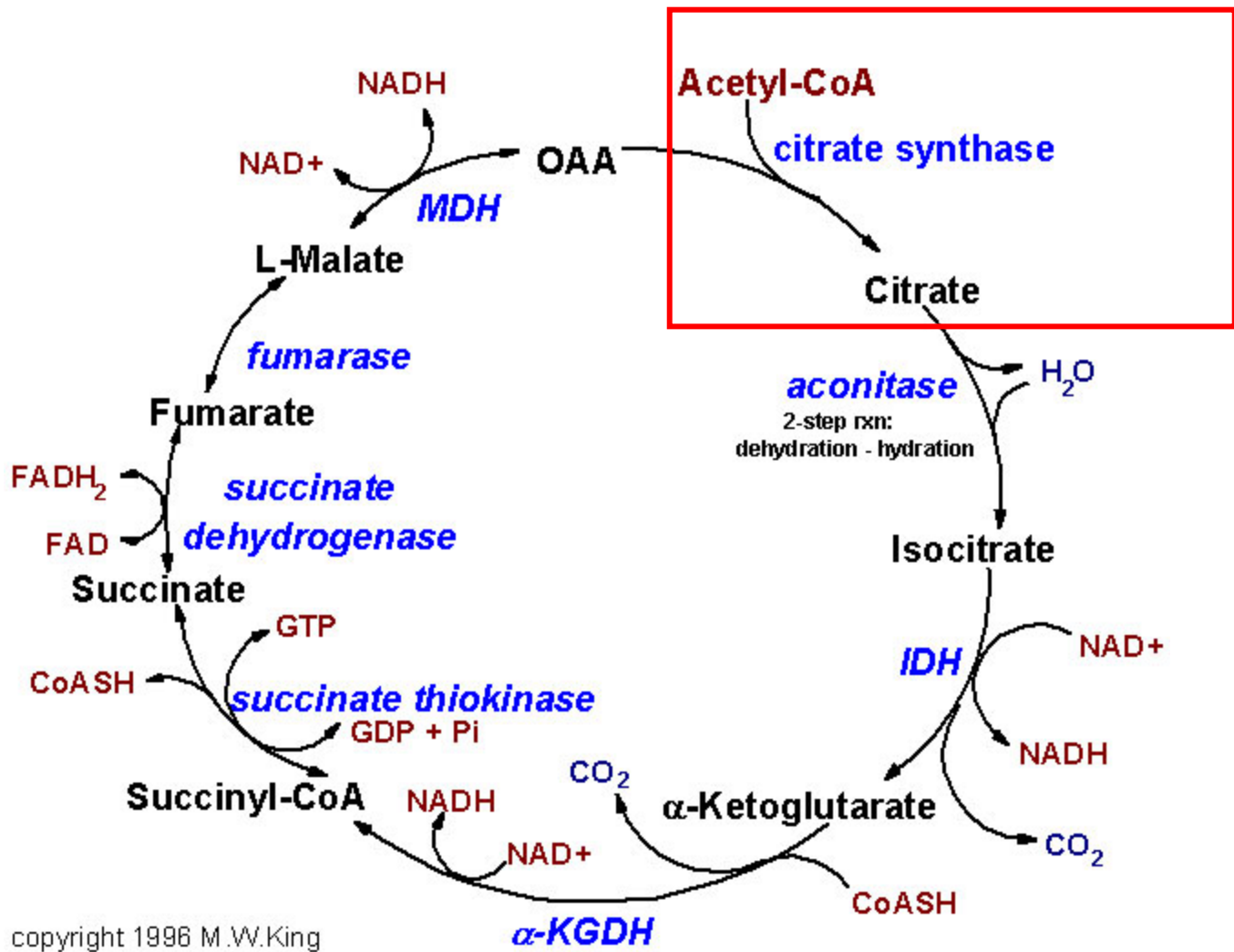
ATP

Sample Handling

- Skeletal muscle (50 - 100mg*).
- Flash frozen at bedside.
- Store at -70°C.
- Transported on dry ice.
- Store at -70°C.
- Homogenise (1:9 w/v)
- Freeze Thaw (x3).
- Assay.

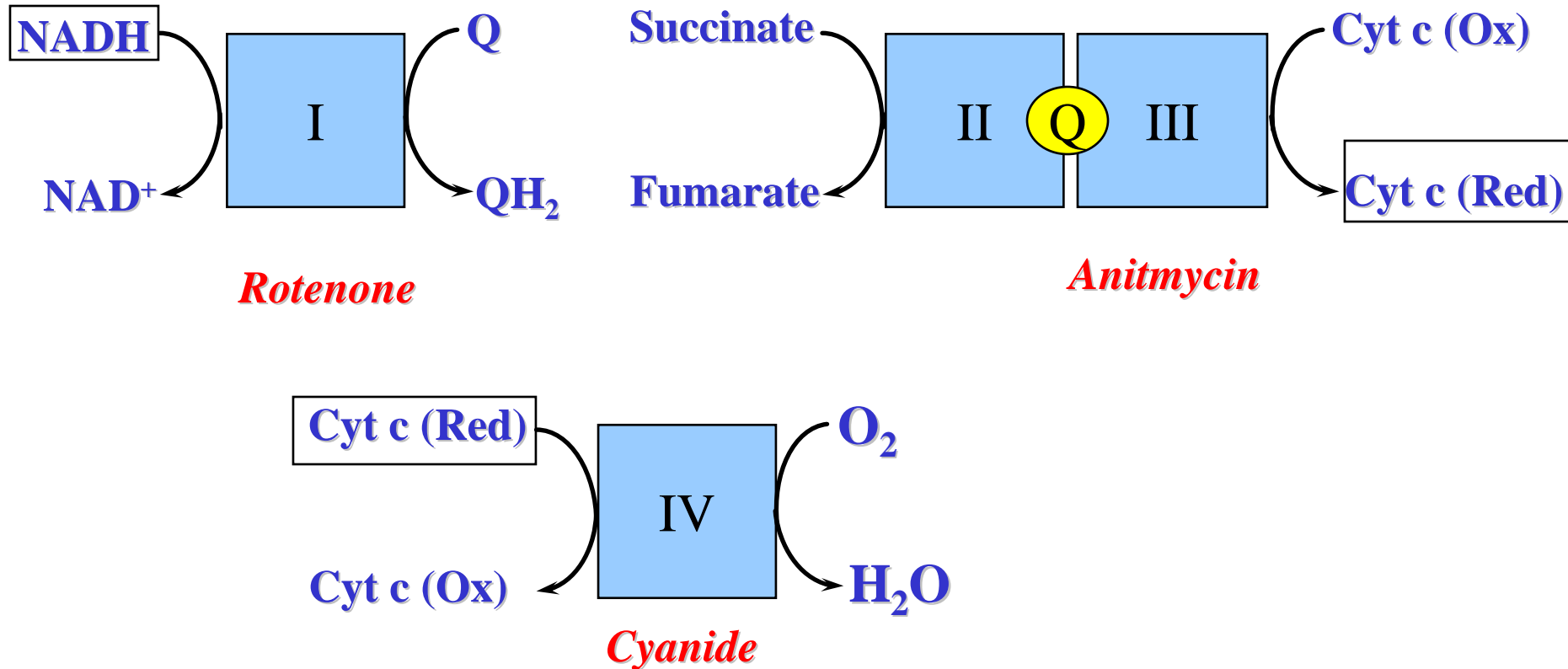
*Think Orange Pip





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Mitochondrial Complex Assays



In House QC



Pooled "processed" Muscle Homogenate



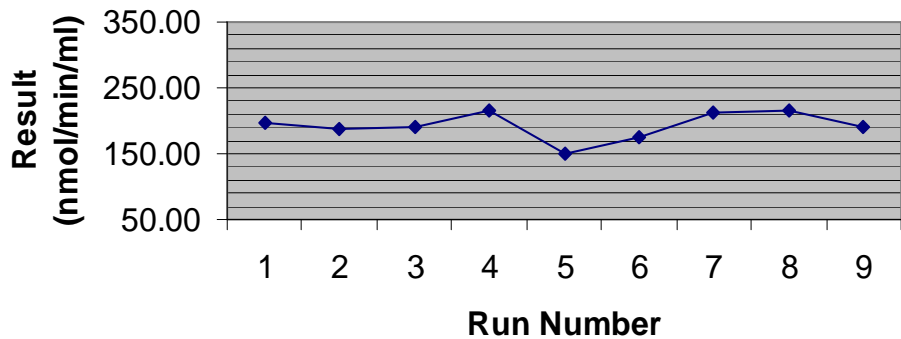
Aliquots

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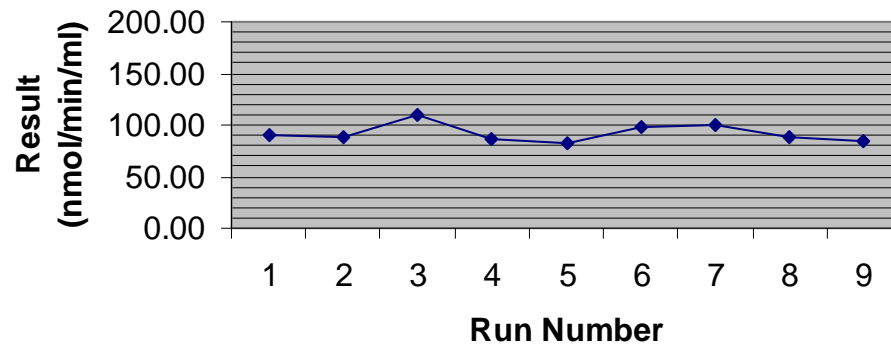
Overlap with previous "QC"

Mean \pm 2SD

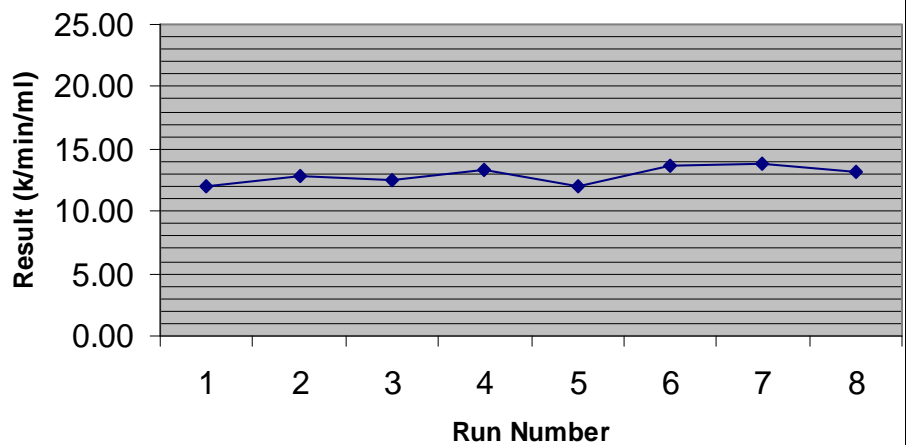
QC9 Complex I



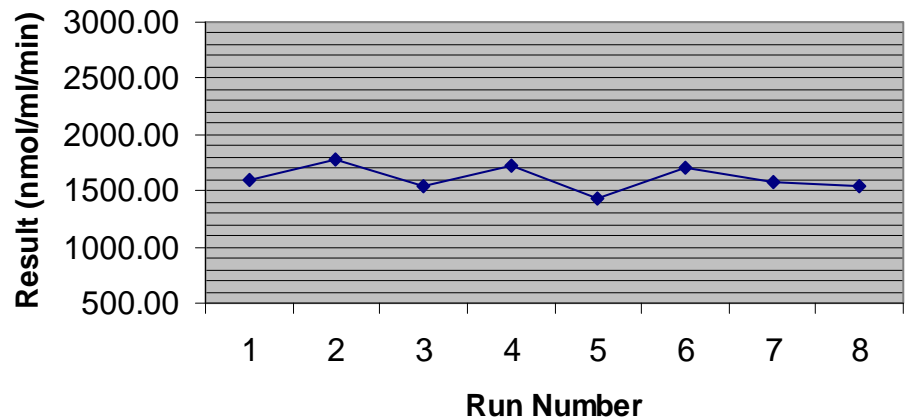
QC9 Complex II/III



QC9 Complex IV



QC9 Citrate Synthase



Mitochondrial NCG Service



- Labs use comparable but not identical methods
- Different reference ranges
- Opportunity to pilot a “QC scheme”

www.mitochondrialncg.nhs.uk

Pilot QC Scheme for Mitochondrial Enzymes

- 3 pooled muscle homogenates (1:10) - A, B & C
- C = control
- A & B had deficiencies of I, II+III and IV - judged by London – *independent assay*
- Sent on dry ice to Oxford and Newcastle
- Instructions provided.

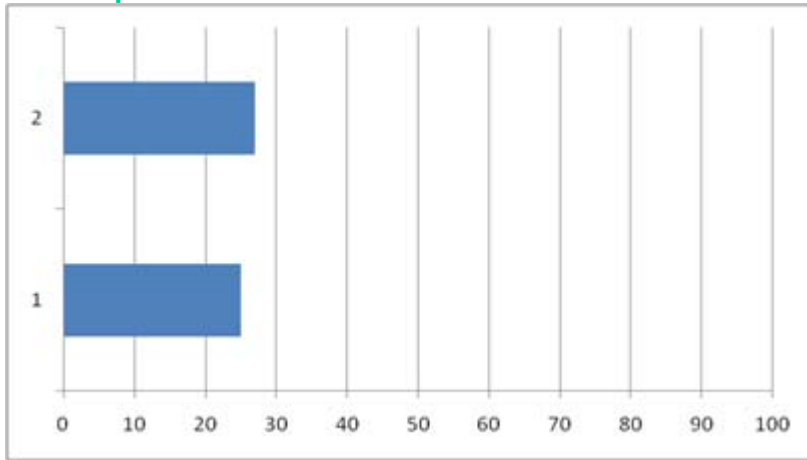
Methods

Each centre has slightly different methods – So:-

- Calculate activity of respiratory chain enzymes
- Calculate citrate synthase activity
- Calculate ratio to citrate synthase
- Control (C) ratio = 100%
- Express ratio results for A and B as % of C

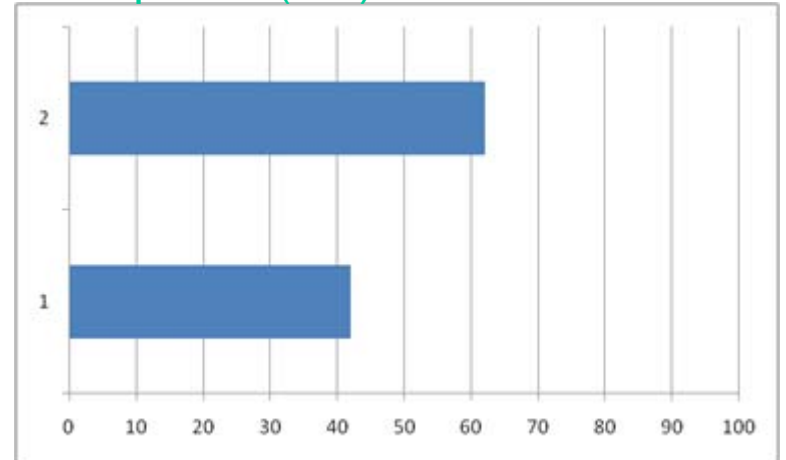
A

Complex I



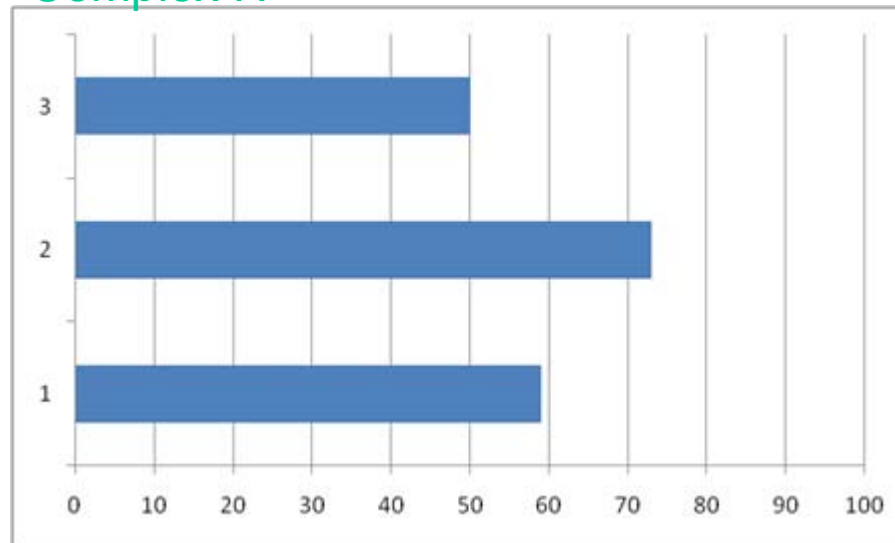
% of Control C

Complex II (+III)



% of Control C

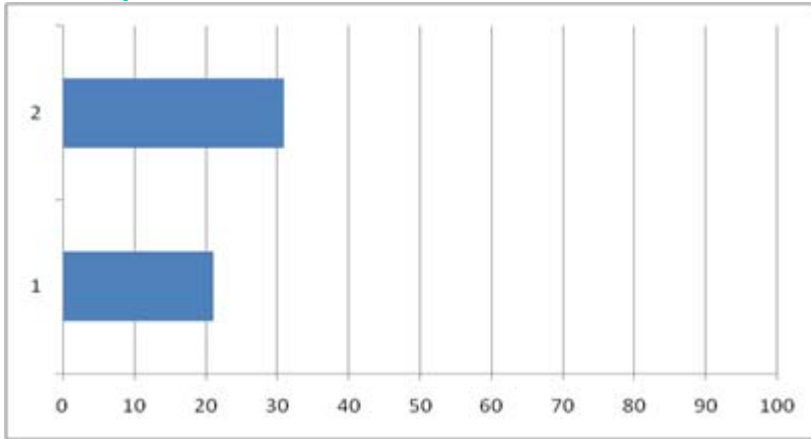
Complex IV



% of Control C

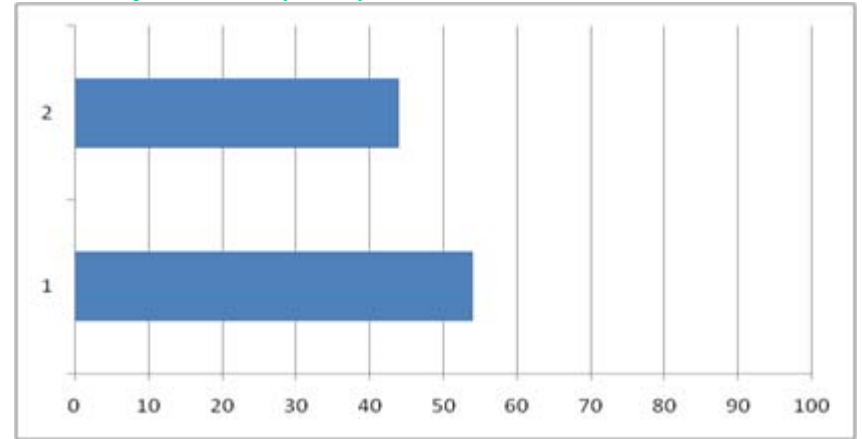
B

Complex I



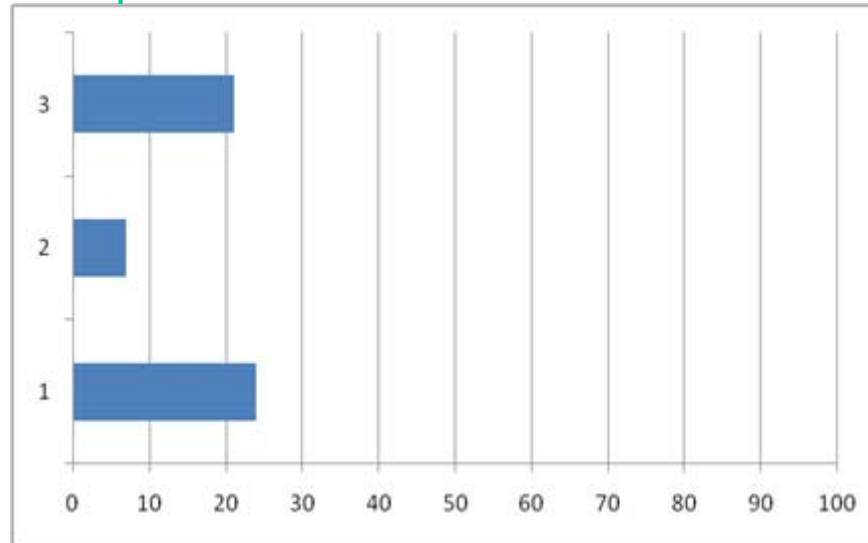
% of Control C

Complex II (+III)



% of Control C

Complex IV

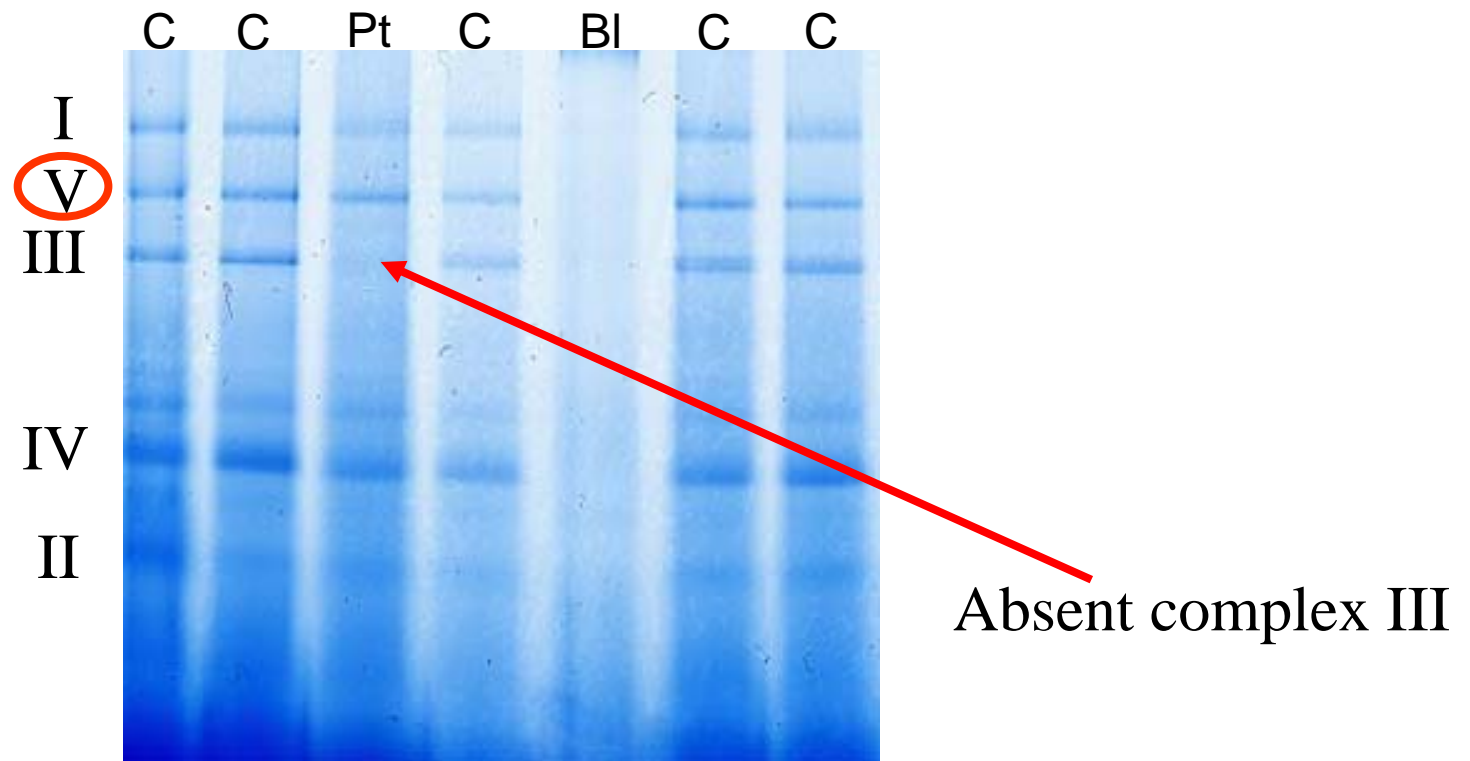


% of Control C

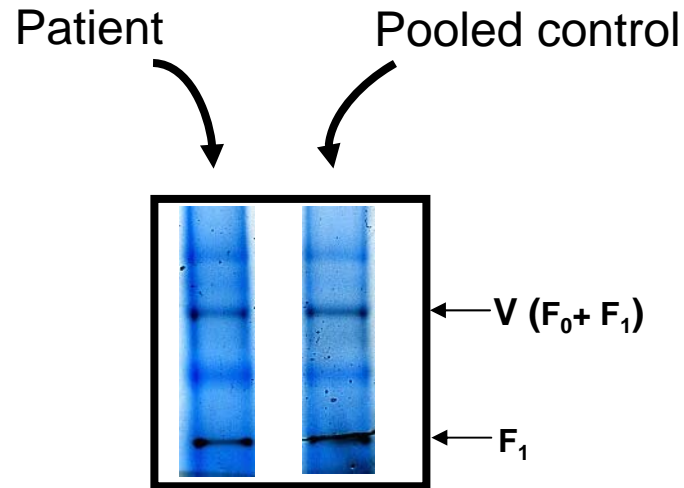
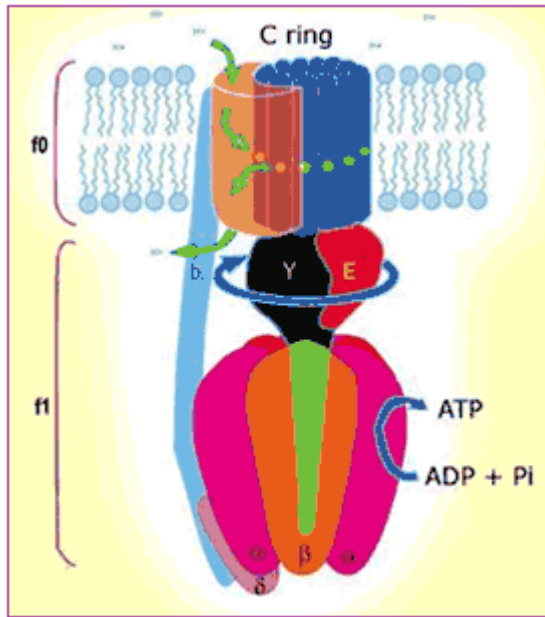
Pilot QC Scheme for Mitochondrial Enzymes

- A step in the right direction
- Generally agreement good
- Deficiencies identified by all groups
- Include date for return of results
- Plan to continue with scheme x2 per year (2009)

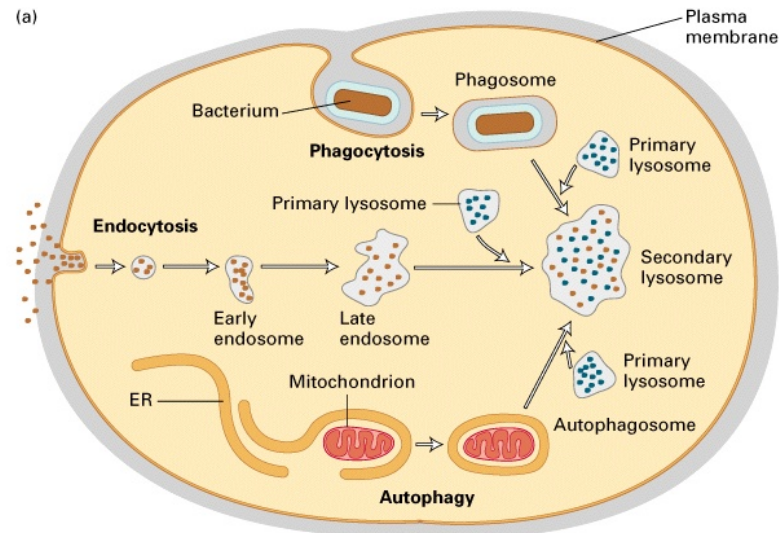
Gel Electrophoresis



Blue Native Gel Electrophoresis Complex V



Lysosomal Disorders



Lysosomal Disorders -EQA Schemes Available

- Lysosomal Enzymes
- White Cell Cystine
- Urinary GAGs
- Sialic Acid

Chitotriosidase - ?Pilot sample exchange scheme

Pompe Diagnosis – Dried Blood Spots

NEWBORN SCREEN		NHS	
Baby's NHS No		NHS No: 047 863 3118	
SURNAME	NHS No: 01/03/2009 Female		
FORENAMES	19 Scaepost Road Berwick TD15 1BW		
HOME ADDRESS	3600 (g)		
Rank	1/1	40+2 weeks	A
Dr. AS Smith	25/03/1967	Printed 01/03/2009	
Berwick Birthing Centre	Berwick Hospital, Ward 09		
GP PRACTICE NAME	MOTHER'S FULL NAME	BIRTH WEIGHT (g)	
UKNSPC Clinic	Christina Johnson	Berwick Hospital, Ward 09	
GP ADDRESS	MOTHER'S DOB	MOTHER'S NHS NUMBER	
130 Soldiers Road	25/03/67	1 2 3 4 5 6 7 8 9 0	
Berwick	PARENT TELEPHONE NUMBER		
GP PRACTICE CODE	012 8599 4234		
1 A A 2 2 2	ALTERNATIVE SURNAME		
GP PRACTICE PCT	N/A		
HOSPITAL OF BIRTH	TEL NO OF PERSON TAKING SAMPLE	NAME OF PERSON TAKING SAMPLE (PRINT)	
Berwick	075 5432 2345	Sr. Lancet	



- Reject if blood spots are over 8 weeks old
- Very Clear information required, including request for Pompe !
- Good Quality blood spots – essential – otherwise rejection !

Dried Blood Spots - LSDs

- Currently not participating in an external QC scheme
- Pompe – currently always run +ve in batch
- DBS amenable to sample exchange
- Expanding to Fabry, Gaucher & other LSDs (multiplex assays)



Conclusions

- Absence of EQA not a blessing !
- Good to have external evaluation
- When not available, critical to develop rigorous “in house” schemes
- Approach will depend on analyte and availability of spare material
- Pilot CSF scheme – lyophilisation – stability
- In absence of official EQA - sample exchange between centres should be explored
- Expansion of DBS assays – need for EQA schemes ?

Acknowledgements

- Katie Bainbridge
 - Garry Brown
 - Derek Burke
- Iain Hargreaves
 - Amanda Lam
 - John Land
- Marcus Oppenheim
 - Rob Taylor

GLYCOGEN – BIOCHEMICAL, CLINICAL & DIAGNOSTIC ASPECTS

**Wednesday 2nd February
UCL Institute of Child Health
30 Guilford Street
London WC1N 1EH**

£20

6 CPD Points

Lunch