Uncertainty in diagnostic metabolite assays white cell cystine as an example

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This presentation will look at

- Clinical utility of the white cell cystine assay
- The steps involved in the process
- Utility of EQA
- Limitations of UoM

Clinical Utility

- Diagnosis and monitoring of cystinosis
  - Defect of cystine transport out of lysosomes
  - Treatment with cysteamine is effective but compliance can be difficult
- Cystine accumulation is intracellular
- Plasma and urine metabolite changes are non specific
- Mixed leukocyte preparations preferred *but* cystine accumulates predominantly in polymorphonuclear lymphocytes (PMN or Neutrophils, 60-70% WBC)

White Cell Cystine Analysis

Isolation of Leukocytes

- 5mL whole blood – add ACD-dextran to precipitate most RBC
- Brief hypotonic shock to remove remaining RBC
- Sonicate WBC pellet to break up cells
- Precipitate proteins with SSA
- Freeze ppt and SNT separately

Analtye Assays

- Colorimetric protein assay
- TMS cystine assay

Final result

- Complex calculation embedded in a spreadsheet

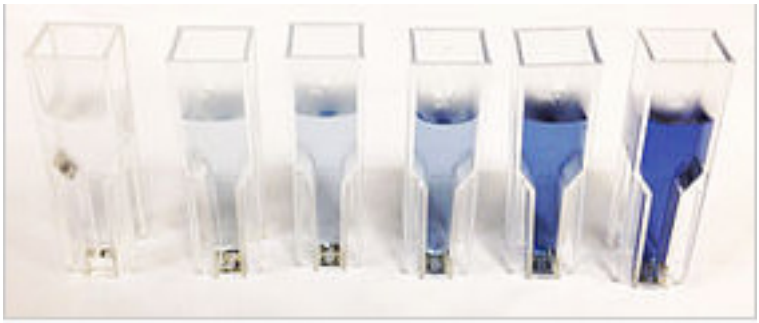
Cystine analysis

Variety of methods

- Competitive binding protein assay
- Automated ion exchange chromatography
- HPCL
- Tandem mass spectrometry

Protein analysis

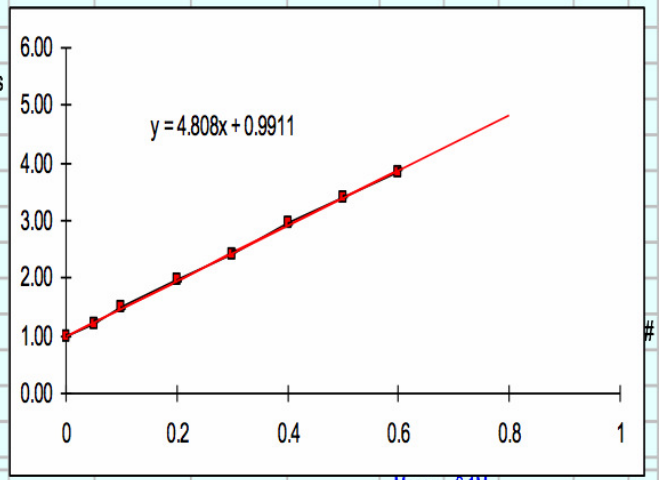
- Two main methods, both similar, colorimetric



Calculation of final result

- Complex algorithm using formulae embedded in a spreadsheet
- Reporting units: nmol ½ cystine / mg protein

Standards	CPM	Stds	CPMmax/ CPMobs	Reg values
0	2550	0	1.00	0.99
0.05	2136	0.05	1.19	1.23
0.1	1711	0.1	1.49	1.47
0.2	1298	0.2	1.96	1.95
0.3	1063	0.3	2.40	2.43
0.4	857	0.4	2.98	2.91
0.5	752	0.5	3.39	3.40
0.6	662	0.6	3.85	3.88
Slope	Inter			
4.807950898	0.99			



Sample	CPM	CPMmax/ CPMobs	Raw cystine (umol/L)	Cystine Dilution	Corrected cystine (umol/L)	Original supernatant Vol (uL)	Corrected supernatant Vol (uL)	Cystine in pellet (nmoles)	Mean Mira Protein (mg/L)	0.1M NaOH vol (uL)	Protein in pellet (mg)	WCC result	No
Low Ext QC x1	1555	1.64	0.13	1	0.135	200	200	0.02698675	400	500	0.20000	0.27	9
101A Ben Cox x1/2	1422	1.79	0.17	2	0.334	200	400	0.13346767	588	1000	0.58800	0.45	10
101A Ben Cox x1/5	1962	1.30	0.06	5	0.321	200	400	0.12836145	588	1000	0.58800	0.44	11
101A Ben Cox x1/10	2224	1.15	0.03	10	0.323	200	400	0.12934101	588	1000	0.58800	0.44	12
Sigma QC x1	737	3.46	0.51	1	0.513								13

ERNDIM EQA Scheme

- ERNDIM distributes protein pellets and leukocyte SNTs spiked with cystine
- 36 participants from around the world
- Return results through a website
- Get feedback about performance monthly
- Annual report and feedback

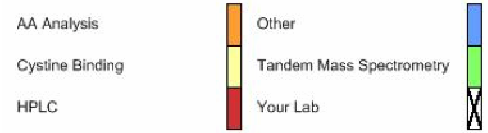
ERNDIM experience

- Participating labs submit data separately for cystine and protein analysis together with calculated combined result.
- So ERNDIM can see if there is an identifiable problem with any of these (cys, prot, calc)
  - Precision
  - Accuracy
  - Blunder

ERNDIM results

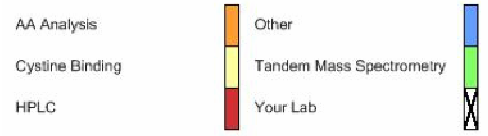
All Labs results	
n:	29
Mean:	7.17
Median:	7.23
SD:	0.890

Scale Standard Deviations	Scale nmol 1/2 cys /mg protein
>3SD	>9.84
2-3SD	8.95 - 9.84
1.5 - 2.0SD	8.50 - 8.95
1.0 - 1.5SD	8.06 - 8.50
0.5 - 1.0SD	7.61 - 8.06
0.0 - 0.5SD	7.17 - 7.61
-0.5 - 0.0SD	6.72 - 7.17
-1.0 - -0.5SD	6.28 - 6.72
-1.5 - -1.0SD	5.84 - 6.28
-2 - -1.5SD	5.39 - 5.84
-3 - -2SD	4.50 - 5.39
<-3SD	<4.50



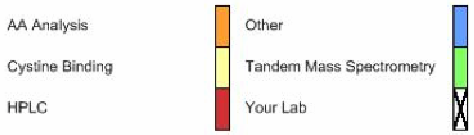
All Labs results	
n:	29
Mean:	2.15
Median:	2.17
SD:	0.383

Scale Standard Deviations	Scale nmol 1/2 cys /mg protein
>3SD	>3.30
2-3SD	2.92 - 3.30
1.5 - 2.0SD	2.73 - 2.92
1.0 - 1.5SD	2.53 - 2.73
0.5 - 1.0SD	2.34 - 2.53
0.0 - 0.5SD	2.15 - 2.34
-0.5 - 0.0SD	1.96 - 2.15
-1.0 - -0.5SD	1.77 - 1.96
-1.5 - -1.0SD	1.58 - 1.77
-2 - -1.5SD	1.39 - 1.58
-3 - -2SD	1.00 - 1.39
<-3SD	<1.00



All Labs results	
n:	31
Mean:	0.514
Median:	0.485
SD:	0.170

Scale Standard Deviations	Scale nmol 1/2 cys /mg protein
>3SD	>1.03
2-3SD	0.855 - 1.03
1.5 - 2.0SD	0.770 - 0.855
1.0 - 1.5SD	0.685 - 0.770
0.5 - 1.0SD	0.599 - 0.685
0.0 - 0.5SD	0.514 - 0.599
-0.5 - 0.0SD	0.429 - 0.514
-1.0 - -0.5SD	0.344 - 0.429
-1.5 - -1.0SD	0.258 - 0.344
-2 - -1.5SD	0.173 - 0.258
-3 - -2SD	0.0027 - 0.173
<-3SD	<0.0027



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UoM from Leeds lab

Cystine

cv = 7%, 95% confidence limits @ 0.96 µmol/L = 0.8 – 1.01

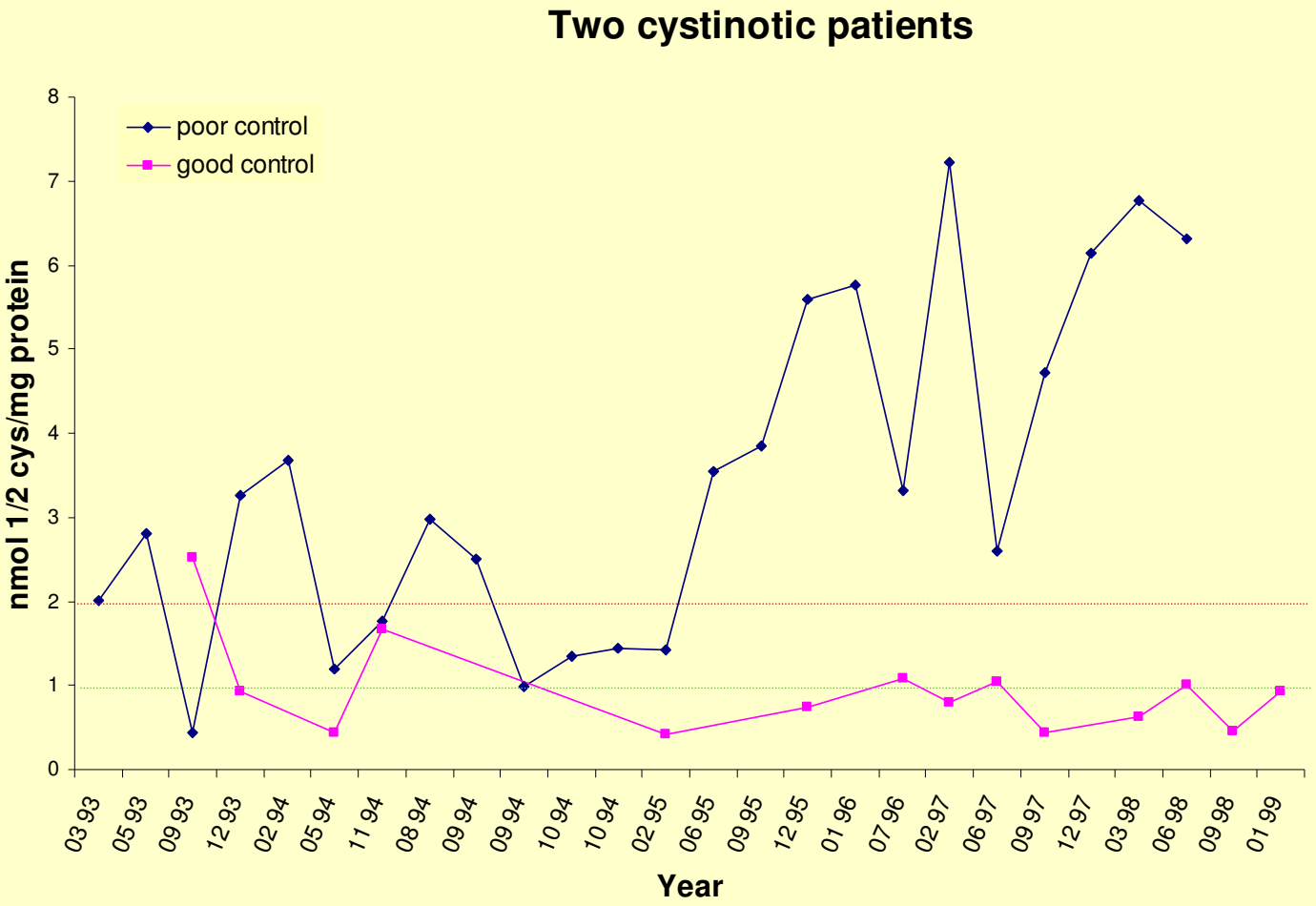
Protein

cv = 9%, 95% confidence limits @ 335 mg/L = 268 – 401

Final Result

cv = 7.3%,

95% confidence limits @ 1.15 nmol½cys/mgpr = 0.98 – 1.32


Two cystinotic patients
poor control
good control
nmol 1/2 cys/mg protein
0
1
2
3
4
5
6
7
8
03 93
05 93
09 93
12 93
02 94
05 94
11 94
08 94
09 94
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10 94
02 95
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01 99
Year

Summary

- Difficult to give clear guidance on UoM for this assay because main variable, the isolation of the pellet, is very hard to assess or control for
- Implication is that consistency of lab practice is important
- ***But***, we can assess analytical variation in the analyte assays and through EQA we can identify analytical problems and help rectify them