

White Cell Cystine EQA

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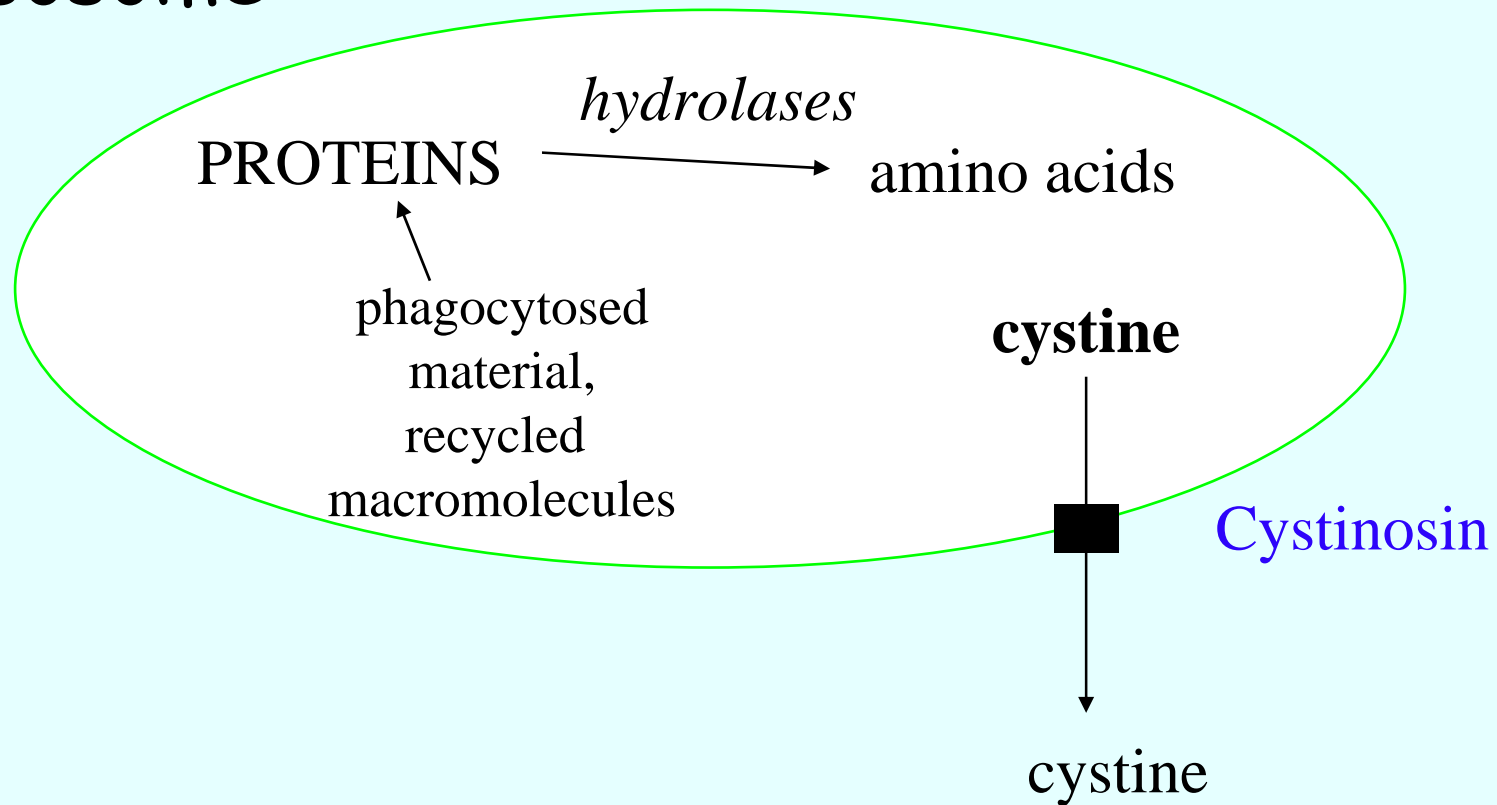
Leeds Teaching Hospitals Trust

This presentation gives a little background information about the nature of the molecular defect and the clinical presentation of the early onset form of cystinosis. It discusses the utility of measuring white cell cystine for the diagnosis and therapeutic monitoring of the disease. An external quality assurance scheme for this assay has been established and is organised by the ERNDIM organisation. The scheme is described and there is discussion about the performance of participants in one complete year.

Further information about ERNDIM and its schemes can be found on the website, www.erndim.unibas.ch/ .

The basic defect

Lysosome



Cystinosis

Clinical features of early onset type

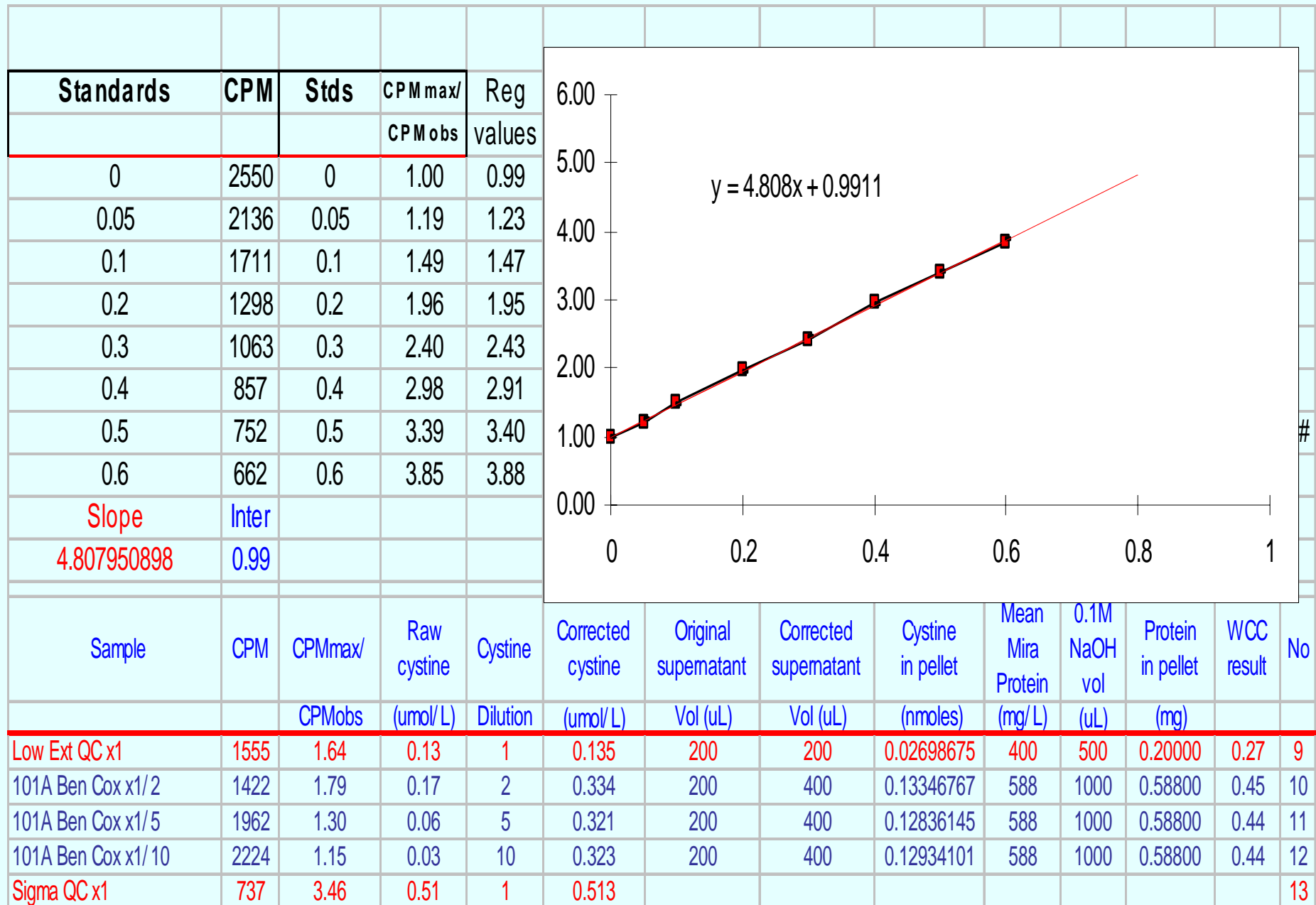
Failure to thrive age 3-6 months

- Vomiting
- Anorexia
- Polydipsia
- Dehydration with episodes of fever
- Rickets
- Growth retardation
- Cystine crystals in optic lens

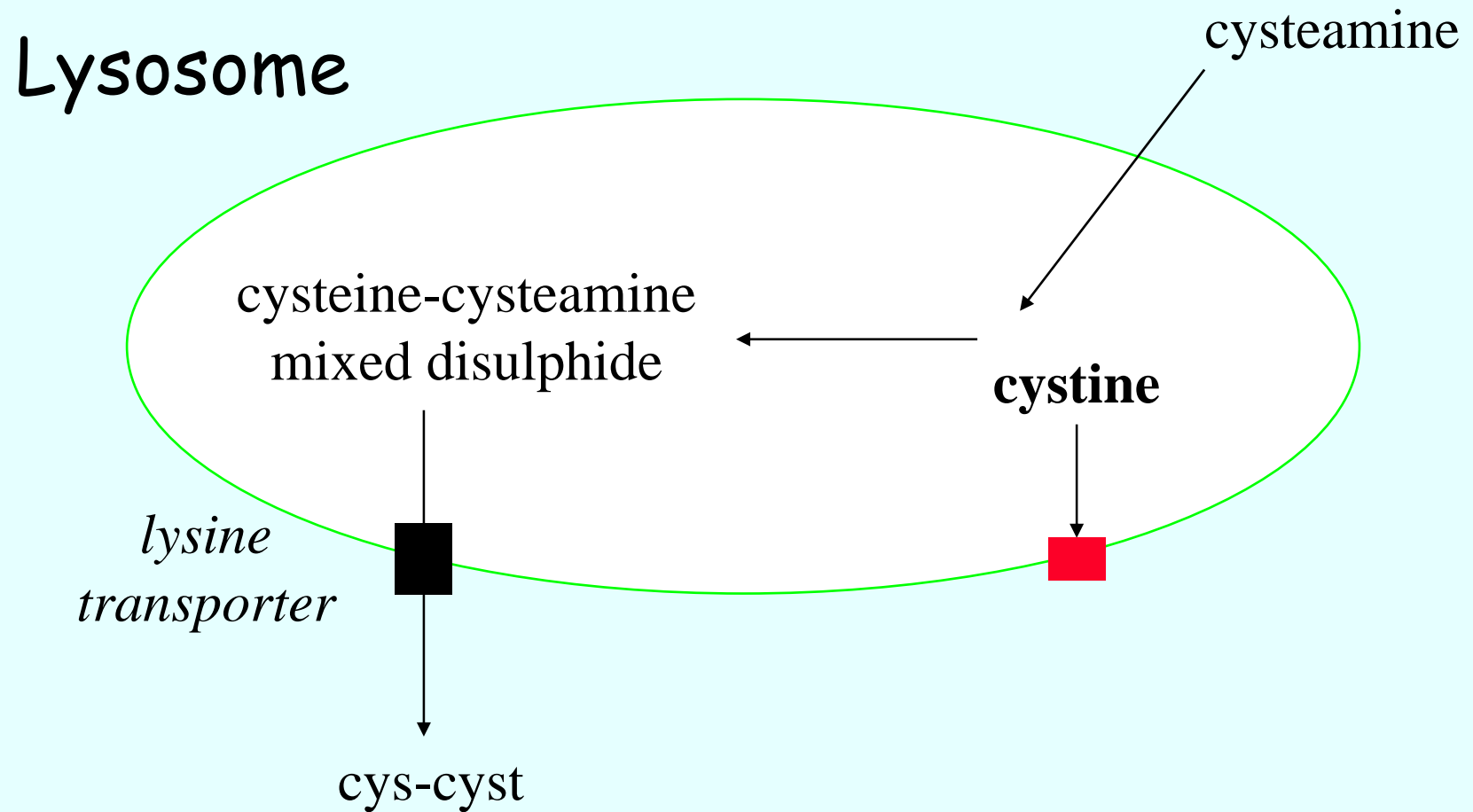
Leukocyte cystine

- Most readily available cell type to assess cystine accumulation.
- Critical for
 - Diagnosis
 - Therapeutic monitoring
- Assays involve isolating total leukocytes, or polymorphs, assaying cystine and reporting relative to protein content

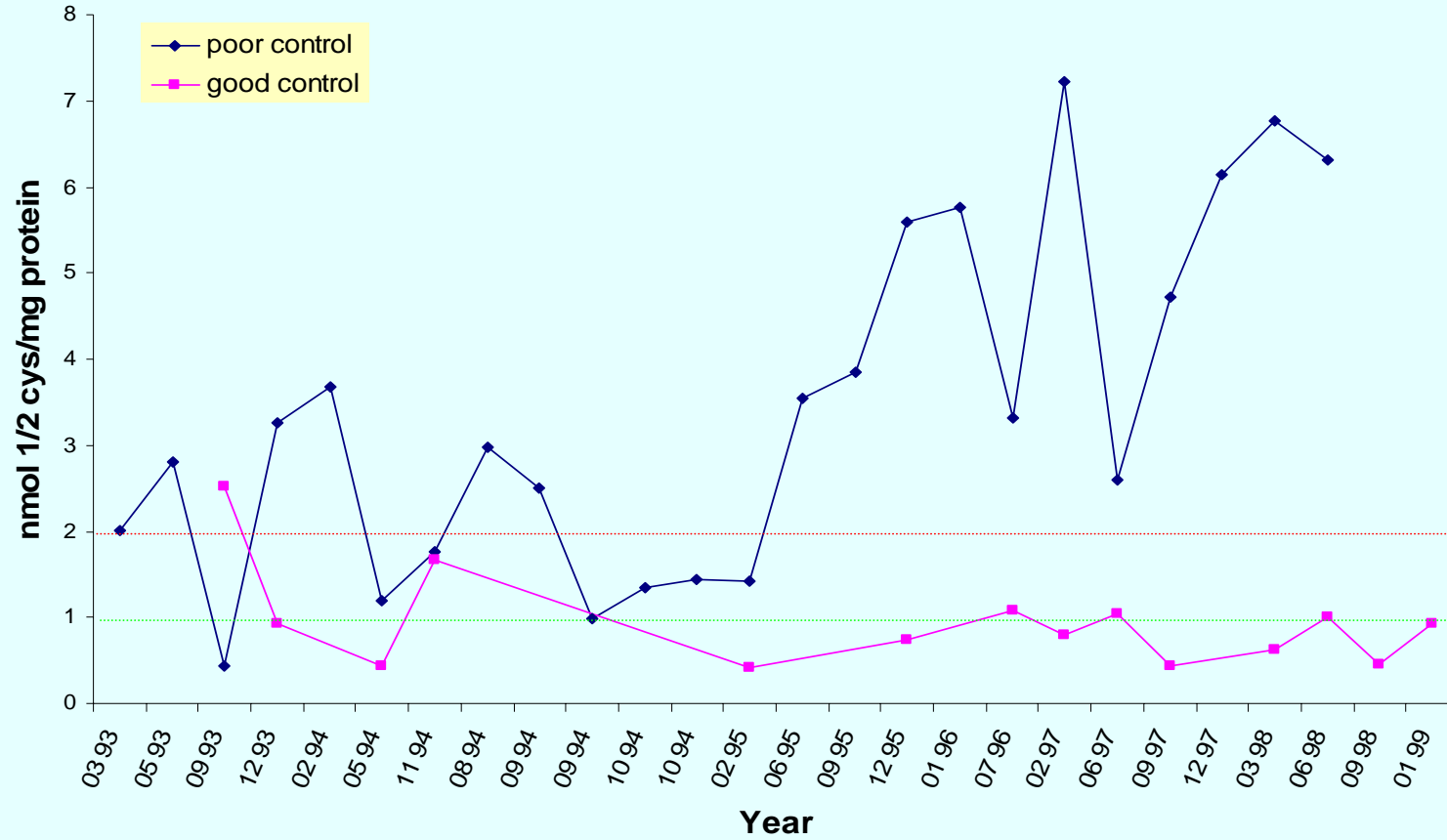
Example of spreadsheet used to calculate results



Cysteamine therapy



Two cystinotic patients



EQA Scheme Outline

Material distributed:

- Supernatant fluids extracted from leukocytes isolated from donor blood
- Freeze dried protein, BSA, pellet

Participants requested to report:

- Cystine concentration in SNT
- Protein concentration
- $\frac{1}{2}$ cys/mg prot, assume aliquots from same sample

ERNDIM EQA

- 8 samples per year
 - 4 pairs
- Scheme in 5th year
- 27 participants, three main method groups
 - CPB assay
 - HPLC
 - Amino acid analyser

Example of analyte report

ERNDIM Quantitative External Quality Assessment Schemes - Windows Internet Explorer

http://www.erndimga.nl/Subscription.php

File Edit View Favorites Tools Help

ERNDIM Quantitative External Quality Assessment Sc...

Current Report Analyte in Detail Annual Report


Cycle: 2007 Sample Nr: SNT and PP 41 Analyte: Cystine (nmol 1/2 cys/mg protein) (SNT sample) Change

Hospital Name	St. James's University Hospital	Parameter		Your Lab		Method		All Labs	
Department	Dept of Clinical Biochemistry	n		1		2		22	
Contact Person	Colin Evans / Mick Henderson	Mean		8.34		7.28		7.51	
Deadline	21-09-2007 23:59	Median		8.34		7.28		7.48	
Unit	nmol 1/2 cys /mg protein	SD				1.57		1.42	

Scale Standard Deviations	Scale nmol 1/2 cys /mg protein	
>3SD	> 11.75	
2-3SD	10.34 - 11.75	
1.5 - 2.0SD	9.63 - 10.33	
1.0 - 1.5SD	8.92 - 9.62	
0.5 - 1.0SD	8.21 - 8.91	
0.0 - 0.5SD	7.51 - 8.20	
-0.5 - 0.0SD	6.80 - 7.50	
-1.0 - -0.5SD	6.09 - 6.79	
-1.5 - -1.0SD	5.38 - 6.08	
-2 - -1.5SD	4.67 - 5.37	
-3 - -2SD	3.26 - 4.66	
<-3SD	< 3.26	

Your lab AA Analysis
 HPLC Tandem Mass Spectrophotometry
 Cystine Binding

Cystine in White Blood Cells



What is performance like overall?

Recovery of Protein

Samples	Added	Mean Measured	Recovery
21/26	0.75	0.71	95%
23/27	1.00	0.97	97%
22/25	1.25	1.20	96%
24/28	1.55	1.48	95%

Recovery of Added Cystine

Sample	Added	Measured	Recovery	Recovery %
SNT 23/27	0.00	0.04	0.00	n.a.
SNT 24/28	0.20	0.26	0.22	110%
SNT 22/25	0.50	0.56	0.52	104%
SNT 21/26	2.20	2.24	2.20	100%

However.....Overall Interlab CV

Analyte

Interlab CV

Protein

12%

Cystine, $\mu\text{mol/L}$

115%

Cystine, $\text{nmol}^{1/2}\text{cys /mg prot}$

104%

Results 2005: Cystine in nmol ½ cys/mg protein

Method	SNT and PP 21	SNT and PP 26	SNT and PP 22	SNT and PP 25	SNT and PP 23	SNT and PP 27	SNT and PP 24	SNT and PP 28
AA	9.60	2.93		0.190	0			0.160
AA	4.51	4.85	0.750		0.350	0	0.0950	0.370
AA	6.30	6.40	0.860	0.906	0	0	0.280	0.440
AA	6.70	6.25	0.990	1.04	0.0800	0.0500	0.380	0.450
AA	5.83	6.60	0.940	1.37	0.0600	0.0800	4.56	0.405
AA	5.80	6.10	0.910	1.11	0	0	0	0
AA	6.72	4.36			0	0	0.260	
AA		5.13	0.910	0.760	0	0.0600	0.270	0.290
AA	7.05	6.87	1.50	1.14	0	0.0100	0.300	0.410
AA	6.34	2.83	0.820	0.730	0.0100	0.0210	0.230	
AA	12.7	9.41	1.00	1.09	0.110	0.410	0.540	0.840
AA	8.44	6.60	2.28	0.740	0.616	0.765	0.462	0.290
Cystine	6.80	6.34	0.980	1.03	0.104	0.130	0.340	0.300
Cystine	5.97	6.58	0.920	0.930		0.140	0.380	0.340
Cystine	2.80		0.820	0.910			0.290	0.240
Cystine	4.20		0.700	0.570	0.140		0.180	
Cystine	29.7		2.91	1.33			0.570	
HPLC	3.65	5.43	1.76	1.00	0.262	0.0900	0.350	0.255
HPLC	12.1		3.45	1.83	0		0.190	
HPLC	5.00		2.70					
HPLC		6.66	0.690	1.23	0.171	0.316	0.399	0.386
HPLC	5.60	7.33	0.730	1.15		0.167	0.320	0.331
HPLC		5.25	2.22	0.429	0.291	0.930	0.800	0.410
HPLC	11.3		2.22					
HPLC	6.11	5.94	1.04	0.740	0.184	0.190	0.365	0.340
Other	7.34	6.00	0.100	0.970	0.230	0.0300	0.310	

20% data is Missing

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Cystine	6.80	6.34	0.980	1.03	0.104	0.130	0.340	0.300
Cystine	5.97	6.58	0.920	0.930		0.140	0.380	0.340
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Likely transcription errors

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Result reported as zero when should be “not measured”

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AA	5.80	6.10	0.910	1.11	0 ★	0 ★	0 ★	0 ★
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Other	7.34	6.00	0.100	0.970	0.230	0.0300	0.310	

Outliers results with no clear explanation

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AA	9.60	2.93		0.190	0			0.160
AA	4.51	4.85	0.750		0.350	0	0.0950	0.370
AA	6.30	6.40	0.860	0.906	0	0	0.280	0.440
AA	6.70	6.25	0.990	1.04	0.0800	0.0500	0.380	0.450
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AA	5.80	6.10	0.910	1.11	0	0	0	0
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Other	7.34	6.00	0.100	0.970	0.230	0.0300	0.310	

Removing all outliers leaves 10 consistent Labs.....

Method	SNT and PP 21	SNT and PP 26	SNT and PP 22	SNT and PP 25	SNT and PP 23	SNT and PP 27	SNT and PP 24	SNT and PP 28
AA	6,30	6,40	0,86	0,91	0,00	0,00	0,28	0,44
AA	6,70	6,25	0,99	1,04	0,08	0,05	0,38	0,45
AA	5,83	6,60	0,94	1,37	0,06	0,08	0,46	0,41
AA		5,13	0,91	0,76	0,00	0,06	0,27	0,29
AA	7,05	6,87	1,50	1,14	0,00	0,01	0,30	0,41
Cystine	6,80	6,34	0,98	1,03	0,10	0,13	0,34	0,30
Cystine	5,97	6,58	0,92	0,93		0,14	0,38	0,34
HPLC	3,65	5,43	1,76	1,00	0,26	0,09	0,35	0,26
HPLC	6,11	5,94	1,04	0,74	0,18	0,19	0,37	0,34
Other	7,34	6,00	1,00	0,97	0,23	0,03	0,31	

Summary Reproducibility

Cyst(nmol $\frac{1}{2}$ cys/mg protein)

Within Lab CV	12%
Between Lab CV (all data)	104%
Between Lab CV (10 best labs)	17%

Poor Performance

ERNDIM is preparing to highlight poor performance in all its schemes

Likely to be generous in interpretation initially

The hope will be to improve performance by education

Conclusions

- A viable EQA scheme to assess laboratory analytical performance has been successfully established
- It has demonstrated
 - Good agreement between at least 10 of the labs!
 - No significant differences between methods
 - Persistent poor performance by some labs, highlighting problems of standardisation, calculation or comprehension
- It does not address the major variable of sample preparation

Acknowledgements

- Cas Weykamp for preparing and distributing the QA material
- Colin Evans for running the Leeds service