Total Leukocyte Cystine Measurement, European Quality Assurance Initiative

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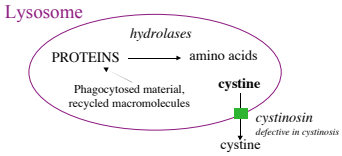
Introduction



Cystinosis is an inborn error of metabolism which usually presents in infancy. Children have symptoms of renal tubular disease, polyuria and Fanconi syndrome. It is characterised biochemically by an intracellular accumulation of cystine and is caused by a failure to transport cystine out of lysosomes. The measurement of total leukocyte cystine content is critical in establishing the diagnosis. Leukocytes being the most readily available nucleated cell type. This parameter also plays a crucial role in monitoring the effectiveness of disease control by disulphide exchange drugs. Clinical prognosis has improved dramatically since their introduction.

However the measurement of leukocyte cystine is problematic. It depends on isolating a leukocyte pellet from whole blood then measuring relatively low concentrations of cystine in small volumes of lysate supernatant before relating these to the total protein content. There has been concern that the results may be method dependant and be subject to varying interferences. Establishing quality assurance for the whole procedure would necessitate large volumes of abnormal, whole blood and having to distribute aliquots rapidly. This would be extremely difficult to organise on an international basis. It was therefore proposed to circulate material that emulated the isolated leukocyte pellets.

Description of the Scheme

A base material of leukocyte supernatant is prepared from donated blood. Aliquots are spiked with added cystine and distributed to participants together with freeze-dried pellets of protein, bovine serum albumin. Samples are distributed in a single batch early in the year and participants are requested to assay and report in a prescribed order. Duplicate samples are prepared and assigned lot numbers that should mean they are analysed in separate batches across the year. A spread of concentrations allows the scheme organisers to assess both linearity and precision.

The participating laboratories are asked to report measured cystine concentrations *per se* as well as nmol ½ cystine per mg protein, the unit accepted internationally for patient management.

The scheme has now been running for three years, the first of which was a pilot. The scheme was then incorporated into the ERNDIM programme and made available to all ERNDIM (European research network for diagnosis of inborn metabolic disease) participants.

There are currently 26 participants comprising three principal assay groups; competitive protein binding, ion exchange chromatography and high pressure liquid chromatography. There have been 28 samples distributed, below is presented a summary of the results from year three.

Results Summary Year Three

The following are trimmed results, significant outliers and erroneous results have been omitted.

Protein:

Samples	Added	Mean Measured	Recovery %
SNT21/26	0.75	0.71	95%
SNT23/27	1.00	0.97	97%
SNT22/25	1.25	1.20	96%
SNT24/28	1.55	1.48	95%

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 21/20	2.20	2.24	2.20	100%

If all the returned results are included, the following is the result:

Analyte	Interlab CV
Protein	12%
Cystine/Aliquot	115%
Cystine ½cys/mg prot	104%

Conclusions

- We have shown that limited quality assurance of this procedure is possible.
- There is clearly considerable room for improvement.
- There does not appear to be a problem with standardisation or matrix interference since laboratories that do not produce outlier results are in good agreement.
- There does not appear to be any discernable method bias in the results.
- We continue to observe simple calculation errors on a frequent basis. If this reflects clinical practice it would be of great concern. It may however be due to unfamiliarity with the scheme. The possibility remains that some laboratories have serious errors in their calculation spreadsheets.

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Participating laboratory identity remains confidential to the scheme organisers.

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