White cell cystine assay: Calculation of final results using an Excel spreadsheet	
Site/Area of application	Biochemical Genetics
Index code	SLF2BGM026 Ver 3.0
Superseded documents	BIPABGA1 v1
Implementation date of this version	01/03/2012
Approver of content of SOP	Daniel Herrera
Reason for change	Modification of EXCEL spreadsheet and change in protein cut-off for sample rejection
Keywords for search on EQMS	White cell cystine, cystinosis

Signature

1.

CLINICAL RELEVANCE/PURPOSE OF PROCEDURE

White cell cystine is used to diagnose and monitor the treatment of the inborn error cystinosis.

One function of lysosomes is to break down proteins into amino acids for re-use by the cell. These amino acids are then exported from lysosomes via specific transport proteins. Patients with Cystinosis have an inactive lysosomal transport protein for cystine. This causes an accumulation of cystine which disrupts the integrity of the lysosomal membrane leading to cell damage by release of proteolytic enzymes. Tissues and organs of low cell turnover/regenerative ability are the most vulnerable to this damage. Proximal renal tubule cells are particularly vulnerable with significant renal impairment being seen by 1 to 2 years of age. This will cause polyuria, polydypsia, failure to thrive and rickets. A generalised aminoaciduria, glycosuria and phosphaturia is also observed (Fanconi syndrome).

White cells contain lysosomes and are therefore used as a source of cells for diagnosis or monitoring. Cystagon (cysteamine bitartrate) binds with cysteine which is in equilibrium with cystine. The resulting disulphide has a similar size and shape to lysine and is exported from lysosomes via the lysine transport protein. This systemic treatment is for all affected organs, although eye drops are used to avoid cystine crystal formation in the eye. The lower the white cell cystine, the better the treatment, although there have been some reports of side effects in over-treated individuals.

2. PRINCIPLE OF PROCEDURE

The white cell cystine assay has many separate parameters. These are all entered into a Microsoft Excel spereadsheet which performs a series of calculations to achieve a final white cell cystine result in nmoles of ½ cystine per mg of protein.

3. PERSONNEL / TRAINING REQUIREMENTS

Senior BMS, Clinical Scientist

4. SPECIMEN REQUIREMENTS

N/A

5. EQUIPMENT

N/A

6. HEALTH AND SAFETY/RISK ASSESSMENT

Further guidance relating to laboratory accommodation, personal protective equipment and other general safety considerations is available in the Pathology Safety Manual [PHS039].

7. REAGENTS

N/A

8. CALIBRATION

N/A

9. QUALITY CONTROL

N/A

10. COMPUTER / TELEPATH CODES

CYSW1J

11. PROCEDURE OR METHODOLOGY

Use the EXCEL spreadsheet located in G:Specialist laboratory medicine folder/Biochemical genetics folder/White cell cystine folder/WCC QCs to enter the QC values.
Use the EXCEL spreadsheet [SLF2BGM027] located in G:Specialist laboratory medicine folder/Biochemical genetics folder/White cell cystine folder/WCC template waters if the Waters Premier tandem mass spectrometer has been used for analysis or the EXCEL spreadsheet [SLF2BGM028] located in G:Specialist laboratory medicine folder/Biochemical genetics folder/White cell cystine folder/WCC template API 4000 if the API-4000 tandem mass spectrometer has been used for analysis.
The spreadsheet performs automatic calculations of the white cell cystine result in nmol ½ cystine per mg protein.

12. UNCERTAINTY OF MEASUREMENT

The limit of quantification of the cystine assay is 0.2 µmol/L. Any result below this is entered as 0.2 µmol/L a final result (X) is reported on telepath less than this final result (<X).If the protein result is less than 100 mg the white cell yield is too low and should be reported as Na,COM on telepath with the free text comment: “Insufficient yield of white cells for reliable results”.
For such samples, review the volume of blood sent and comment as required (5mL is the recommended volume, 2mL is the absolute minimum volume for which the rejection rate increases markedly).

13. REFERENCE RANGE / ACTION LIMITS

Reference Ranges (Diagnosis):

Normal: up to 0.5 nmol ½ cystine per mg protein.

Heterozygotes: up to 1.0 nmol ½ cystine per mg protein.

Cystinosis patients: usually greater than 2.0 nmol ½ cystine per mg protein.

NB there is overlap between the normal and heterozygote ranges.

These are entered as free text comments during the NPCL stage of authorisation for ? diagnosis samples (first delete the auto-comment below about therapeutic ranges).

Therapeutic ranges for Cystagon therapy.
These are automatically added by telepath. The comment is:
“Therapeutic target: less than 1.0 (ideal) or 2.0 (adequate). Monitoring samples should be taken as trough levels, i.e. pre-dose, whilst maintaining the normal dosage pattern”.

14. REFERENCES

N/A

15. APPENDICES

N/A

16. TRAINING

Training in this procedure is recorded in the staff members training competency assessment [SLB9BG001] within the Trust Training Record File which is held centrally at each site and remains the property of the Trust