

## White cell cystine assay: White Cell Protein Analysis

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<i>Signature</i>
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## 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 protein pellet from tandem MS cystine analysis is dissolved in 0.1M sodium hydroxide. These are assayed on the Cobas Mira analyser using a modified Lowry method.

Initially, the Mira is put through a hypochlorite decontamination cycle to remove any traces of serum protein from the sample probe (many white cell protein concentrations will be around 1g/L rather than around 70 g/L for serum protein).

There are two steps to the Lowry protein assay:-

### **1) Incubation of sample with alkaline copper sulphate solution.**

Sodium dodecyl sulphate unravels polypeptide chains.  
Alkaline copper (II) ions form copper-peptide bond-protein complexes.

### **2) Addition of Folin-Ciocalteu reagent.**

The phosphotungstic-phosphomolybdic acid in the reagent reacts with the copper complexes as well as tryptophan and tyrosine residues in the *unraveled* polypeptide chain producing a blue colour.

The absorbance is monitored at 600nm using a Cobas Mira absorbance method.

## 3. PERSONNEL / TRAINING REQUIREMENTS

Senior BMS or Clinical Scientist

#### 4. SPECIMEN REQUIREMENTS

Sample type: White cell protein dissolved in 1000µL of 0.1M sodium hydroxide.

#### 5. EQUIPMENT

##### **Cobas Mira S.**

Originally supplied by Roche.

This instrument is covered by a service contract with AS Diagnostics Ltd, Blackpool.

#### 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 [\[PHS039\]](#).

See COSHH and procedure risk assessments [\[SLC5CBG014\]](#) and [\[SLC5RBG005\]](#) and [\[SLC5RBG006\]](#)

Unless otherwise specified all laboratory work must be performed at containment level 2 [\[PHS007\]](#)

Hand and eye protection must be worn when handling blood and serum/plasma.

Laboratory procedures that may give rise to infectious aerosols must be conducted in a microbiological safety cabinet [\[PHS006\]](#).



High risk samples – Laboratory work must be performed at containment level 2+.




Additional precautions as described in [\[PHS007\]](#)

- Gloves and disposable apron must be worn
- Eye protection must be worn where splashing is assessed as a risk
- Analysis must only be undertaken by experienced BMS staff
- Analysis must be undertaken as a discrete task
- Access by other staff to the area should be restricted

All reagents are disposed of in accordance with the Waste Management procedures outlined in section 15 of the Health & Safety Manual [\[PHS011\]](#)

Any spillages should be dealt with according to the spillage procedures outlined in section 13 of the Pathology health & safety manual [\[PHS009\]](#)

Material	Source	Main Hazard Information	Precautions	Hazard Symbols
Sodium Hydroxide Pellets and solutions.	Merck Cat No: 10252 (500g)	Causes severe burns. In case of contact with body, particularly the eyes, rinse immediately with plenty of water and seek medical advice.	Wear gloves and eye protection.	 Corrosive
Copper (II) sulphate	Merck Cat No: 27849	Harmful by ingestion or if inhaled as dust. Causes burns to eyes, and is irritating to skin.	Wear gloves, goggles.  when weighing solid wear a dust mask.	 Harmful Irritant

Sodium Dodecyl sulphate	Merck Cat No: 44244	Harmful by ingestion. Irritating to eyes and respiratory system as dust.	Wear gloves, goggles.  when weighing solid wear a dust mask.	 Harmful Irritant
Folin and Ciocalteu Reagent	Merck Cat No: 1.09001.0 500	Causes burns. Harmful by inhalation, skin contact, and if swallowed. <b>Possible teratogen.</b> danger of cumulative effects: target tissue: nervous system.	Wear gloves and goggles when pipetting this reagent. Dispose of small quantities (off Mira) down the sink with lots of water.	 Harmful Possible teratogen
Sodium hypochlorite	VWR Cat No: 27900.36 5	Causes burns to eyes and skin. Bleaching agent. Toxic by ingestion: causes internal irritation and damage. Toxic vapour. Evidence of mutagenic effects.	Wear gloves and eye protection. use fume cupboard where possible.	 Harmful Possible mutagen

## 7. REAGENTS

### 2% Cupric Sulphate Pentahydrate Solution.

Dissolve 2.0 g of cupric sulphate pentahydrate (Merck Cat No: 27849) in deionised water to give a final volume of 100ml .

Store at room temperature.

### Sodium Dodecyl Sulphate (SDS)

Dissolve 0.5g of sodium dodecyl sulphate (Merck Cat No: 44244) in deionised water to give a final volume of 10mL. Mix and stand for 10 minutes + centrifuge to get a clear suspension.

Prepare freshly as required.

### 3% Anhydrous Sodium Carbonate in 0.1M Sodium Hydroxide.

Dissolve 6.0 g of anhydrous sodium carbonate (Merck Cat No: 10240) in 0.1M sodium hydroxide to give a final volume of 200ml. Also add 100 µl of SDS solution.

Prepare freshly every 12 months. And store at room temperature

### 4% Sodium Tartrate dihydrate Solution.

Dissolve 4.0 g of D (+) sodium tartrate dihydrate (Hopkin and Williams Cat No: 32460 ) in deionised water to give a final volume of 100ml .

Store at room temperature.

### 2M Folin Ciocalteu Phenol Reagent.

Ready to use reagent (Merck Cat No: 1.09001.0500).

Store at room temperature.

### **1000mg/L Albumin Solution.**

Supplied in packs of 10 sealed ampoules (Sigma Cat No: P 0914).

Store at 2-8°C.

Ready to use, store at 2-8°C.

### **Bovine serum albumin (solid).**

Sigma Cat No: A-7030.

Stored at 2-8°C in a sealed desiccated container.

Silica gel desiccator crystals must be blue (see below for regeneration).

### **Silica Gel Desiccant (Self indicating)**

Merck Cat No: 30062. Avoid breathing the silica dust when in use.

When free of moisture, the crystals are blue. If they are turning pink they should be regenerated in the oven by placing in a glass beaker overnight at 80°C. Allow silica gel to cool to room temperature before use.

### **Sodium Hypochlorite Stock Solution (Approx 14% active chlorine).**

VWR catalogue no: 27.900.365.

### **25% Sodium Hypochlorite solution.**

In a 25mL plastic universal, mix 4mL of stock sodium hypochlorite solution (VWR catalogue no: 27 900 365) with 16 ml of deionised water.

## **8. CALIBRATION**

The method is calibrated using a 500 mg/L bovine serum albumin solution prepared by dilution of a standard supplied by Sigma (Cat No: P 0914). The calibration curve is blanked using deionised water. Calibration is checked by running 250, 500 and 1000mg/L standards at the beginning of each batch.

## **9. QUALITY CONTROL**

### **Internal QC.**

Low Protein QC, High Protein QC

### **External QA.**

ERNDIN white cell cystine QA scheme.

Samples are stored at -20 °C before analysis.

## **10. COMPUTER / TELEPATH CODES**

Set name: CYSW1J

## **11. PROCEDURE OR METHODOLOGY**

The protein assay has a number of steps:

- Preparing samples for use.
- Cleaning the probes with hypochlorite and rinsing afterwards with water.
- Preparing standards for use.
- Preparing reagents for use.
- Running a blank to prime reagents.
- Calibrating, running samples and precision checking a batch.

- Disposal of samples and reagents and clearing old files.

### Preparing samples for use.

See short instructions for sample preparation [\[SLF2BGM024\]](#)

### Cleaning the probes with hypochlorite and rinsing afterwards with water.

Top up the deionised water reservoir on the Mira.

Wearing gloves, empty the Mira waste container down a laboratory sink with a copious quantity of tap water. **Do not use a hand washing sink.**

Sodium hypochlorite is used to remove protein from the Mira probes.

Prepare 20mL of sodium hypochlorite solution as detailed above.

Place in the Mira wash boat and run the decontamination procedure as follows:-

Press **INFO**, Press **6**, Press **9**, Press **F1** to start the needle cleaning.

Re-run this procedure until at least 15mL of the hypochlorite has been used up (top up as required).

Replace the sodium hypochlorite with deionised water and repeat until 10mL of water has been used.

*Note that without a thorough rinse the assay will not work.*

### Running a blank to prime reagents.

Place a blue mira sample tube containing deionised water in a sample rack and perform the assay *WITHOUT CALIBRATION*:

Press **ROUTINE**, Type in cup number and press **ENTER**, Press **TEST LEVEL (F4)**, Press **2**, Press **WC Prot** key (L), Press **ENTER**

Repeat this to analyse samples in duplicate.

**MAKE SURE THE REAGENTS ARE IN PLACE!**

When ready press **START**. The results obtained are not used.

### Calibrating, Running Samples and Precision Checking a Batch.

One at a time using a plastic pastette, at least 200µL of standard, test or QC is pipetted into appropriate blue mira sample tubes **\*\*Do not to introduce an air bubble - the needle may short sample by aspirating air\*\***

The blue tubes are placed in sample racks noting the cup position and identity on the worksheet.

At regular intervals throughout the batch place a 1000 mg/L standard, making sure that the one in position 4 has extra standard for precision checks.

Place tubes in the following positions in a Mira CAL rack positions:-

0 standard - position 8 in the CAL rack

500 standard - position 7 in the CAL rack

**A calibration request is programmed as follows (type carefully – see note):-**

Press **ROUTINE**, Type **C** then **A**, Press **TEST LEVEL (F4)**, Press **2**, Press the **WC Prot** key,

Press **ENTER** (**note**: after pressing **ENTER** there is no on-screen confirmation of the calibration request).

**Check standards (0,250, 500 and 1000µmol/L, patient sample, and QC are programmed as follows:-**

Press **ROUTINE**, Type in the first vial number, then **F2**, then the final vial number.

Press **TEST LEVEL**, Press **2**, Press the **WC Prot** key, Press **ENTER**

Repeat this to do each vial in duplicate.

**A precision check is also performed as follows:-**

Extra 1000µmol/L standard was put in position 4 to allow this.

Press **ROUTINE**, Type in the vial number (**4**), Press **TEST LEVEL**, Press **2**, Press the **WC Prot** key, Press **ENTER**

Repeat this 9 times (10 in total)

When ready press **RUN**

**\*\*\*Danger of injury: Once run has been pressed do not put your hands into the movement area!\*\*\***

A typical batch takes about 45 minutes to calibrate and analyse all samples.

Any samples with results greater than 2600 mg/L (linearity limit) are re-run as a 1 in 2 dilution (i.e. 200µL sample + 200µL water) and the results for the sample multiplied by 2 to give the final result.

### **Disposal of samples and reagents.**

Wearing gloves, empty the Mira waste and any waste reagents down a laboratory sink with a copious quantity of tap water. **Do not use a hand washing sink.**

Used cuvettes, plastic tips, and sample cups are disposed of in a sharps bin.

## **12. UNCERTAINTY OF MEASUREMENT**

<b>Source of Error</b>	<b>Effect on Protein</b>	<b>Effect on Final White cell cystine result</b>
Air bubbles in blue Mira sample tubes	May give falsely low	Falsely High
Failure to clean probes with hypochlorite	May give falsely high protein or poor precision – may not even calibrate.	Falsely low or very imprecise
Failure to clean hypochlorite off with deionised water	Assay does not work	No results
Not running a reagent blank prior to calibration	Assay does not work for first few samples resulting in poor calibration.	No useful results

Results are linear up to 2600 mg/L. All results above this must be re-run on dilution (see above). A protein result of less than 100 mg/L indicates a very low white cell yield. The final result for such samples should be reported as insufficient white cell yield for reliable analysis.

### 13. REFERENCE RANGE / ACTION LIMITS

N/A

### 14. REFERENCES

Methodology References:

This method was introduced at SJUH in 1993, and is a direct copy of the above method as used by the Well Child Lab at St Thomas' Hospital, London (formerly the Renal Research laboratory at Guy's Hospital). This unit set up the method after close co-operation with the International Cystinosis Reference laboratory run by Dr Gerry Schneider in San Diego, California. The San Diego lab performed the main research work from which the diagnosis and treatment data are derived.

### 15. APPENDICES

[\[SLF2BGM024\]](#)-Short instructions for white cell protein preparation

[\[SLFOBHM025\]](#)- White cell cystine worksheet

### 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.