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Work Instruction

Title: AU 680 Total Protein - CSF & Urine		**WI Number** SFOWI-1305 **Revision:** 5
Department: Chemistry **Area:** 2425 Geary Blvd SFO Hospital Lab	***Approved & Released Work Instruction***	**Implementation Date:** 08/13/2019
Type of Document: Work Instruction		**Review Period -** 365 **Days**

I. Purpose

Instructions for the quantitative determination of Total Protein in human urine and cerebrospinal fluid (CSF) on Beckman Coulter AU Chemistry analyzers.

A	Principle
	Many methods are available for the determination of urinary/CSF protein. These are based on colorimetric, turbidimetric, electrophoretic or immunological principles. Of the colorimetric methods the Biuret method lacks sensitivity, the Coomassie Brilliant Blue method has a limited linear range and has the disadvantage of staining glassware and cuvettes. Results with the turbidimetric methodologies may vary depending on the type of precipitant and type of protein. The Urinary/CSF Protein reagent is a colorimetric method. Pyrogallol red is combined with molybdate to form a red complex with a maximum absorbance at 470nm. The assay is based on the shift in absorbance that occurs when the pyrogallol red-molybdate complex binds basic amino groups of protein molecules. Under the conditions of the test in the presence of protein, a blue-purple complex is formed with a maximum absorbance at 600nm. The absorbance of this complex is directly proportional to the protein concentration in the sample. For random urine protein:creatinine ratio, urine protein is quantitated based on the above pyrogallo red-molybdate colorimetric principle. The urine creatinine is quantitated using the Jaffe method.

B	Clinical Indication
	Measurement of total protein in urine is important in the diagnosis and treatment of diseases associated with renal,cardiac and thyroid function. These diseases are often characterised by proteinuria of which there are four main types: (a) increased glomerular permeability (glomerular proteinuria) (b) defective tubular reabsorption (tubular proteinuria) (c) increased concentration of low molecular weight protein (overload proteinuria) (d) abnormal secretion of protein into the urinary tract (postrenal proteinuria). Increased levels of urinary protein may also be present following strenuous exercise or in the following conditions: monoclonal gammopathies, nephritis, diabetic nephropathy or urinary tract infections. The random urine protein:creatinine ratio is used to screen for clinically significant proteinuria. If the ratio is <0.2, then no significant proteinuria is present. A ratio that is >3.5 is in the nephrotic range. This test is also useful when it is not possible to obtain a 24 hour urine collection. The measurement of total protein in CSF is important in detecting increased permeability of the blood/brain barrier to plasma proteins or to detect increased intrathecal production of immunoglobulins. Increased permeability of the blood brain barrier may result from conditions such as brain tumor, intracerebral hemorrhage or by inflammation caused by bacterial or viral meningitis, encephalitis or poliomyelitis. Determination of increased intrathecal synthesis of immunoglobulins is important in the diagnosis of demyelinating diseases such as multiple sclerosis.

II. Scope

This work instruction covers patient testing of Urine- and CSF- Total Protein on the AU680 Chemistry analyzer.

III. Safety Precautions

Testing personnel must take normal infectious disease precautions, including but not limited to PPE.

IV. Specimen

All blood should be handled as though potentially infectious. Follow laboratory bloodborne pathogen policy and guidelines when handling body fluid specimens.

A	Specimen Requirements

	1. Type: a. Urine i. Sent to REGIONAL LAB: 24 hour urine collected without preservatives 1. Keep refrigerated during collection. 2. pH is in the range of 4.6 to 8.0 ii. IN-HOUSE TESTING: Random urine collected without preservatives for protein/creatinine ratio. b. CSF i. CSF samples collected in plain collection devices. Avoid blood contamination during collection. ii. CSF Tube #2 is the preferred tube for chemistry tests
	2. Volume: a. Minimum - Urine & CSF: 0.5 mL b. Sample Size (dead space excluded) - Urine & CSF: 1.6 uL
	3. Stability: a. Urine can be stored up to 48 hours at 2-8°C prior to analysis. b. (If urine sample is stored at 2-8°C for < 7 days and pH is in the range of 4.6 to 8.0, the urine sample is acceptable for testing)

V. Equipment Calibration and Maintenance

A	**Calibration**
	1. One-point calibration (AB) using a water blank and Urinary/CSF Protein Calibrator (provided with each reagent kit)
	2. Frequency: a. Every 14 days b. When reagent lot changes c. When QC has shifted d. After major preventive maintenance, or replacement of a critical part
B	**Maintenance**
	Refer to SFOFCD-0408. Refer to SFOWI-1268.

VI. Supplies

All reagents must be dated upon receipt and upon installation. The "on-board" expiration date must also be indicated on installed reagents.	
A	**Reagent**

1. Preparation

Beckman Coulter AU System Urinary/CSF Protein Reagent

Reagent	Ingredient	Concentration	Preparation
R1	Pyrogallol Red Sodium Molybdate Succinic Acid Sodium Benzoate Sodium Oxalate Methanol	47 uM 320 uM 50 mM 3.5 mM 1.0 mM 0.8% w/v	Ready for use

2. Storage & Stability

	Storage	Expiration Date
Unopened	2 - 8°C	Stable until expiration date on label
Opened	In refrigerated compartment of the analyzer	90 days Record open & exp dates on bottle

3. Indications of Deterioration:

a. Discoloration of the reagent, visible signs of microbial growth, turbidity or precipitation in reagent may indicate degradation and warrant discontinuance of use.

4. Precautions:

a. Reagents contain sodium azide as a preservative.

b. Flush with plenty of water when discarding reagents.

B Calibrators

1. Preparation:

a. Calibrator is liquid and is ready for use.

i. Replace stopper and cap immediately after each use to avoid contamination.

2. Storage and Stability

(Warning: Potentially biohazardous material. Calibrator is manufactured from human serum)

Calibrator	Ingredient	Storage	Expiration Date
Urinary/CSF Protein Calibrator	50 mg/dL human serum albumin	2 - 8°C	Opened & unopened: Stable until expiration date on label

C Quality Control Solutions

Refer to SFOFCD-0407.

VII. Quality Control

A	**Stability**
	Refer to SFOFCD-0407
B	**Frequency**

	1. Two levels of QC every 24 hours 2. Each new reagent bottle (even if same Lot #) 3. Each new reagent lot 4. After every calibration 5. After each shipment of the same Lot # 6. After specific maintenance or troubleshooting as detailed in the operators manual or after service/repair.
C	**New QC Lots**
	1. New lots of QC are pre-assayed prior to use as QC.
	2. A minimum 20 data points are collected over several days in order to calculate in- house means and SD's.

VIII. Procedure

A	**Sample Analysis**
	1. If urine is discolored, check pH. If pH is less than 4.6, the urine protein assay is not done. See "Reporting" section.
	2. Centrifuge all urine and CSF samples for 10 minutes prior to analysis.
	3. Refer to SFOWI-1268 (AU680 General Operating Procedures)
B	**Dilutions**

	CSF	Urine
On-board auto dilution	x10	x10
Maximum dilution	x10	x51
Diluent	D.I. Water	D.I. Water

	1. Auto dilution: a. When on-board auto dilution is performed, results are automatically multiplied by the instrument. i.

	2. Manual dilution: ***For Urine only*** a. If the result obtained from auto-dilution still exceeds the AMR, manually dilute the sample with D.I. water using a higher dilution factor than the on-board dilution (x10). i. Refer to SFOFCD-0412 for manual dilution instructions. b. Make two independent dilutions, e.g., x20, and x30 c. Place the manually diluted samples on the Red Rack. d. Manually order sample I.D., the test, sample type, and enter the dilution factor in the "Sample Dilution Rate" field to re-run the assay. e. The system will automatically multiply the result with dilution factor. f. **Confirm the final results from the two dilutions agree within 10%.** i. **Report result from the lower dilution** ii. Consult supervisor if the two dilutions do not agree. iii. If there is any flag/LIH index on the final reportable result, repeat testing is required.
	3. If there is any flag on a diluted result, repeat testing with a higher dilution is required, unless the maximum dilution has been reached.

IX. Limitations, Reportable Range, Calculations, Reference Range, Interpretation and Result Reporting

A	**Limitations**
	1. Urine samples contaminated by hemoglobin will result in falsely elevated values.
	2. Results of studies show that the following substances interfere with this Urinary/CSF Protein procedure by <10%:

SUBSTANCE	Level Tested (mg/dL)
Ammonia	250
Ascorbate	20
Bilirubin	20
Citric Acid	200
Creatinine	300
Cu2+	10
Fe3+	0.055
Gentamycin	2
Glucose	5000
Oxalic Acid	52
Tartaric Acid	200
Tobramycin	3
Uric Acid	300

	3. As with all dye-based methods, analysis of urine samples containing immunoglobulin light chains (i.e. Bence-Jones Protein) may result in the underestimation of protein. Where such samples are suspected it is recommended that the sample be concentrated and further analyzed via electrophoresis. Discrepancies may arise when analyzing total urine protein in samples from patients who have been treated with polypeptide-based plasma substitutes. The polypeptides from the plasma substitute may be excreted into the urine and result in an elevated total urine protein result. Where such samples

	are suspected it is recommended that the sample be concentrated and further analyzed via electrophoresis.
B	**AMR & Reportable Range**
	<u>AMR</u> CSF: 4 - 200 mg/dL Urine: 4 - 200 mg/dL
	<u>Reportable Range</u> CSF: 4 - 2000 mg/dL Urine: 4 - 9800 mg/dL Results outside the linear limits are reported as such.
C	**Calculations**
	All calculations are automatically performed by the Beckman Coulter AU680 Analyzer and RILIS.
	CSF: No calculations are necessary. The results are computed by the instrument.
	Timed urine results and urine protein/creatinine ratio are calculated by the LIS. Urine protein / Urine Creatinine ratio = urine protein random (mg/dL) / urine creatinine random (mg/dL)
D	**Reference Range**
	1. CSF: a. CSF (Newborn - 1 month): 15 - 130 mg/dL b. CSF (1 month – 150 years): 15 - 45 mg/dL
	2. Urine Protein: a. No random urine protein reference ranges have been established. b. Interpretation should be based on protein/creatinine ratio. c. Random protein/creatinine ratio (mg/mg): i. 0-24 months: 1. <0.5 No clinically significant proteinuria ii. 24 months - 150 years: 1. <0.2 No clinically significant proteinuria 2. 0.2-3.5 Clinically significant proteinuria 3. >3.5 Nephrotic range
E	**Critical Values**
	None
F	**Early Notification Values**
	None
E	**Result Reporting & Interpretation**
	1. Confirm ALL flags and indices are properly addressed before reporting any result.

	2. Report CSF Total Protein in mg/dL to one decimal point.
	3. Report random urine protein in mg/dL to one decimal point.
	4. Report random urine protein/creatinine ratio to 2 decimal points
	5. Comments: a. **PHLO (For Uprot):** i. Urine samples with pH less than 4.6: Report as "TND" with comment template **PHLO** (pH TOO LOW FOR ACCURATE ASSAY) b. **Z5 (For Uprot/Ucreat):** i. When random urine protein is < 4 mg/dL and creatinine is <20 mg/dL, report protein:creatinine ratio as "TND" with comment "**Z5**" [Test Not Done: Specimen is Too Dilute (Creatinine less than or equal to 20 mg/dL) for Assessment. If clinically indicated, consider ordering First A.M. microalbumin.]. c. **RBC (For Urine and CSF):** i. If urine or CSF specimen is contaminated with hemoglobin, report result with comment "**RBC**" (Specimen Contaminated with Red Blood Cells; result falsely elevated).

10.0 Corrective Action

A	**QC Out of Acceptable Range**
	1. Review data and LJ charts. If an out of control value appears to be random error, repeat control on new QC aliquot.
	2. Refer to SFOWI-0218 and SFOSOP-0288 for additional QC troubleshooting steps.
	3. Do not use the instrument for patient tests until issues are resolved. Beckman Coulter Tech Support is available at 1-800-854-3633.
B	**Instrument Warning or Error Flag Displayed**
	1. See Reference Manual for troubleshooting steps.
	2. Do not use the instrument for patient tests until issues are resolved. Beckman Coulter Tech Support is available at 1-800-854-3633.

11.0 Associated Documents and Records

SFOFCD-407
SFOFCD-0408
SFOFCD-0412
SFOWI-0218
SFOWI-1268

12.0 References

1. RLWI-2168 (Urine Total Protein and Protein Creatinine Ratio on AU680)
2. Beckman Coulter Instructions for Use: Urinary / CSF Protein, BAOSR6X70 09 (AUGUST 2018)

Associated Documents:

External Documents

Associated Quality System Documents - None

Document Revision History:

Revision: 5	**Date Created:** 08/09/2016 **Date of Last Revision:** 08/13/2019	**Last Approval Date:** 08/13/2019

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Reason for Change:

Revision:	Sec/Para Changed	Change Made:	Date
1	N/A	Initial Issue of Document. This document was a modification of Regional Lab's AU procedures RLWI-2168 rev.6	08/09/2016
2	Edit	Change in Associate Pathologist	10/14/17
3/4	Approver	Changed Lab Director to Dr. Elizabeth Hosfield.	6/17/19
5	Revision Format/Title Adding U-TP	Fixed revision number Changed to new format in preparation for Title 21 Added in-house testing for U-TP.	8/13/19

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