Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC,SGAH & WAH	**Date Distributed:**	6/5/2012
Department:	Core	**Due Date:**	6/30/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Protein, Urine and Cerebrospinal Fluid by Dimension® Chemistry Analyzer
GEC.C25, SGAH.C59, WAH.C56 v001

Description of change(s):

Section	Reason
3.1	Deleted other urines except 24hr, revise nursing manual to Lab Service Directory (electronic)
6.7	Add use of TEA for lot to lot runs
9	Add calculation instructions for 24hr urine
10.4	Upper CRR value corrected
10.5	Add reference to section 9, remove QNSR code

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	Signature	Date

Employee signatures are not necessary. Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 001)

Technical SOP

Title	**Protein, Urine and Cerebrospinal Fluid by Dimension® Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 4/12/2011
Owner	Robert SanLuis, Jean Buss Date: 4/12/2011

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Annual Review		
Print Name	**Signature**	**Date**

Form revised 3/31/00

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Urine Total Protein, Random	Dade Dimension® Clinical Chemistry System	UTPR
Urine Total Protein, 24-Hr.		UTP24
CSF Total Protein		CTP

Synonyms/Abbreviations
UTP, Urinary protein, CSFP; Included in Batteries/Packages: CSF Protein is part of CPRO

Department
Chemistry Note: Germantown Emergency Center performs CTP only

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2. ANALYTICAL PRINCIPLE

The UCFP method is an adaptation of pyrogallol red-molybdate method by Y. Fujita, I. Mori and S. Kitano. In the reaction sequence, pyrogallol red combines with sodium molybdate to form a red complex with maximum absorbance at 470 nm. The protein in the sample reacts

with this complex in acid solution to form a bluish-purple colored complex, which absorbs at 600 nm. The absorbance at 600 nm is directly proportional to the concentration of protein in the sample. The analyte concentration is determined by calculation using a logit curve fit on a previously stored calibration curve.

pyrogallol red-molybdate + protein ----------> PR-MO-protein complex
(absorbs at 600 nm)

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations	
Fasting/Special Diets	N/A	
Specimen Collection and/or Timing	Normal procedures for collecting urine and cerebrospinal fluid may be used for samples to be analyzed by this method. URINE: A timed 24-hour collection is preferred. See Laboratory Test Directory (electronic) for collection instructions.	
Special Collection Procedures	CSF:	Cerebrospinal fluid specimens should be collected with care to avoid contamination with plasma proteins.
	24-hr. Urine:	**Inpatients**: See Laboratory Test Directory (electronic) for details. Refrigerate during collection.
		Outpatients: Provide patient with prepared instruction sheet and container.
Other	N/A	

3.2 Specimen Type & Handling

Criteria			
Type -Preferred	Urine: 24-hour specimen CSF: Sterile tube number 1 from lumbar puncture tray		
-Other Acceptable	Urine: Random urine or other timed collections CSF: Tube 3 may be used, if not needed for other testing.		
Collection Container	Timed Urine Collection: 24 hour container, no additives or preservatives. Random urine: Urine collection cup. CSF: Sterile tubes from lumbar puncture tray.		
Volume - Optimum	24 hr. Urine: Total voided in 24 hours	Random urine: 10 mL	CSF: 1.0 mL
- Minimum	24 hr. urine: N/A	Random urine: 5mL	CSF: 0.5 mL

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Criteria		
Transport Container and Temperature	Collection container at room temperature	
Stability & Storage Requirements	Room Temperature:	Urine: 2 hours CSF: test immediately upon receipt
	Refrigerated (2-4°C):	3 days
	Frozen (-20°C) :	Urine: 1 year CSF: 6 months
Timing Considerations	CSF specimens take priority in specimen handling.	
Unacceptable Specimens & Actions to Take	**CSF** samples are unlikely to be recollected; therefore, utilize discretion in rejecting a sample of this type. **Consult your supervisor.** Specimens that are unlabeled, improperly labeled, markedly hemolyzed, or those that do not meet the stated criteria are unacceptable. **Urines:** Request a recollection and credit the test with the appropriate code. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Consult the English text code list for "test not performed" messages from the LIS. Document the request call in the LIS.	
Compromising Physical Characteristics	**CSF:** Blood present in the cerebrospinal fluid invalidates the protein values since it reflects contamination with plasma proteins. **Urine:** Centrifuge urine before analyzing to remove particulates.	
Other Considerations	Measure total 24 hour volume and enter volume into LIS. Prepare, label, and refrigerate an aliquot in a small urine collection cup. Record 24 hour volume on aliquot.	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number	Quantity
UCFP	Siemens, Flex® reagent cartridge, Cat. No. DF26	4 Flex/carton

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Harmful. Contains methanol and sodium hydroxide. **Irritating to eyes and skin.** **Harmful by inhalation, in contact with skin and if swallowed.**

Reagent	**Urinary/Cerebrospinal Fluid Protein**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Once wells 1 – 5, 7, 8 have been entered by the instrument, they are stable for 5 days.
Preparation	Reagents are supplied ready for use. No additional preparation is required.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
UCFP Calibrator	Siemens Dimension®, Cat. No. DC45

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Urinary/Cerebrospinal Fluid Protein Calibrator
Preparation	Allow to equilibrate to room temperature (22-28°C) and swirl to mix before use.
Storage/Stability	• Store at 2-8°C. • The unopened reagents are stable until the expiration date printed on the label. • Once opened, assigned values are stable for 2 months when stoppered and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Urinary/Cerebrospinal Fluid Protein Calibrator
Assay Range	6 – 250 mg/dL

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Suggested calibration level	6.0, 30.0, 60.0, 135.0, 270.0 mg/dL
Frequency	• Every new reagent cartridge lot. • Every 2 months for any one lot. • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Five levels.
Assigned Coefficients	C_0 - 193.89 C_1 1.337.5 C_2 - 2.595 C_3 462.58 C_4 0.5

5.4 Calibration Procedure

1. From Operating Menu press F5:Process Control press F1: Calibration Enter Password press F2: SETUP and RUN
2. Select the test method to be calibrated - if lot number is incorrect Press F1: Other Lot
3. Enter all information on screen
4. Press F8: QC yes/no to change to yes
5. Press F4: Assign cups If additional methods need to be calibrated, select the method.
6. Press F7: Load/run
7. Load cups into assigned position
8. Press F4: RUN

5.5 Tolerance Limits

IF……	**THEN……**
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek Spinal Fluid Control Levels 1 & 2	Bio-Rad Laboratories Catalog # 751 & 752

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Liquichek Spinal Fluid Control Levels 1 & 2
Preparation	Before sampling, allow the control to reach room temperature (20-25°C) and swirl gently to ensure homogeneity.
Storage/Stability	Open controls are stable for 30 days at 2-8°C. Unopened controls are stable until the expiration date at 2-8^{o}C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Dimension® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension® Quick Reference Guide.

6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system, into the Laboratory Information System (LIS), and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed

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Step	Action
	documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Review Patient Data

Technologist must review each result print-out for error messages. Refer to the Dimension® system manual "Error messages" section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.

- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension® Chemistry Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- Plastic serum tubes and serum cups
- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips

8. PROCEDURE

UCFP Flex® reagent cartridge Cat. No. DF26 is required to perform this test.

Urinary/Cerebrospinal Fluid Protein is performed on the Dimension® clinical chemistry system after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

Step	Action
1	For instrument set up and operation: Refer to Startup/Maintenance, Dade Dimension® procedure. Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® system. For details of the automated parameters, see below under "Test conditions."
2	For sites with StreamLab (Lynx): **Qualifying** samples are loaded via the Dimension® Lynx System which automatically routes specimens to the instruments. Refer to the Dimension® Streamlab® Analytical Workcell (Lynx) System manual for instructions.
3	Alternatively, specimens are placed in color-coded Dimension® segments for analysis by the instrument. Refer to the Dade Dimension® Sample Processing procedure. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus 50 µL of dead volume. Precise container filling is not required.

Step	Action
4	For QC placement and frequency, refer to the Dimension® QC Schedule in the Laboratory QC Program.
5	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension® system manual "Error messages" section for troubleshooting.
6	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
7	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Size:	10 µL
Reagent Volume:	350 µL
Diluent Volume:	50 µL
Temperature:	37° C
Wavelength:	600 and 700 nm
Type of Measurement:	Bichromatic endpoint

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Urinary / Cerebrospinal Fluid Protein in mg/dL.

For 24 hour urines, the LIS will calculate the total mg of protein/24hrs if the protein result from the aliquot is within the CRR. If below 6mg/dl, the total mg/24 hrs is manually calculated as follows:

$$\frac{(6\text{mg/dL}) \times (\text{Total Urine Volume})}{100} = \#\ \text{mg/24hrs}$$

A "less than" character should be placed in front of the numerical value when reporting.

For values above the CRR (2500mg/dL), the same calculation is used as above except substitute 2500 for 6 and use the "greater than" character (>) in front of the numerical answer from the calculation.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

mg/dL

10.4 Clinically Reportable Range (CRR)

6.0 – 2500.0 mg/dL

10.5 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated

IF the result is …	THEN…
≤6.0 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: <6.0 mg/dL See Section 9 for Calculation instructions.
≥250.0 mg/dL	**On Board Automated Dilution:** Results ≥250.0 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary. Append the result with code –REP.
>500.0 mg/dL	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 10 **Diluent**: Purified water. Enter dilution factor as a whole number on the "Enter Sample Data" screen. Report the assay with code of –REP.
>2500.0 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: ">2500.0 mg/dL-REP" See Section 9 for Calculation instructions. Bring to the attention of your supervisor prior to releasing result.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

CSF Total Protein:

Age	Female	Male
Adult (>18 years):	15 – 45 mg/dL	15 – 45 mg/dL
Pediatric:		
0 – 14 days	15-153	15-100
15 – 30 days	15-100	15-96
31 days – 2 months	15-93	15-48
3 – 6 months	15-44	15-48
7 – 23 months	15-48	15-50
2 – 7 years	15-45	15-45
8 – 18 years	15-45	15-40

Urine Total Protein Random:
<11.9 mg/dL

Urine Protein, 24 hour:
<149.0 mg/24 hr

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Measurement of the protein content in urine is used in diagnosis and treatment of kidney diseases.
Measurement of the protein content in cerebrospinal fluid is used in the diagnosis and treatment of central nervous system diseases and trauma.
For purposes of diagnosis and treatment, results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to you Dimension Operator's Guide.

A system malfunction may exist if the following 5-test precision is observed:

Concentration	S.D.
32 mg/dL	1.2 mg/dL
142 mg/dL	2.0 mg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

6 – 250 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Within-run	Total
Bio-Rad Urine Control			
Level 1	20.4	0.60	1.51
Level 2	69.8	0.62	1.19
Ciba-Corning			
CSF/LCR Control	26.9	0.58	1.34
UP/CFP Calibrator	142.3	0.88	1.24

14.3 Interfering Substances

Samples containing amikacin, gentamicin, kanamycin, and tobramycin should be avoided since these substances falsely increase UCFP results.
Neomycin sulfate at 15 µg/mL increase UCFP results by 11% and at 7.5 µg/mL the interference is less than 5%.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Dimension® Clinical Chemistry System Operator's Manual
2. Dimension® Calibration/Verification Procedure
3. Dimension® Cal Accept Guidelines
4. Dimension® Calibration summary
5. Sample Processing, Siemens Dimension® procedure
6. Start up and Maintenance, Siemens Dimension® procedure
7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension®
9. Laboratory Safety Manual
10. Material Safety Data Sheets (MSDS)
11. Siemens Dimension® Limits Chart
12. Quest Diagnostics Records Management Procedure
13. Dimension® Error Messages Chart
14. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
15. Hemolysis, Icteria and Lipemia; Interference from (Lab policy)
16. Repeat Testing Requirements (Lab policy)
17. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert UCFP Flex® Reagent Cartridge DF26

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144
2. Package Insert, UCFP Flex® Reagent Cartridge DF26, Siemens Healthcare Diagnostics Inc., 02/06/2009.
3. Package insert, Urinary/Cerebrospinal Fluid Protein Calibrator DC45, Siemens Healthcare Diagnostics Inc., 04/2008.
4. Package insert, Liquichek Spinal Fluid Control, Bio-Rad Laboratories, 03/2010.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes C088.001		
000	5/1/12	3.1	Deleted other urines except 24hr, revise nursing manual to Lab Service Directory (electronic)	J Buss	J Buss, RSL
000	5/1/12	6.7	Add use of TEA for lot to lot runs	L Barrett	J Buss, RSL
000	5/1/12	9	Add calculation instructions for 24hr urine	J Buss	J Buss, RSL
000	5/1/12	10.4	Upper CRR value corrected	J Buss	J Buss, RSL
000	5/1/12	10.5	Add reference to section 9, remove QNSR code	J Buss	J Buss, RSL
000	5/1/12	15	Update to standard wording	L Barrett	J Buss, RSL

19. ADDENDA

None