Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

# BROWN CLINIC
# LABORATORY PROCEDURE MANUAL

**PROCEDURE:** Total Protein

**PURPOSE:**
The TP method used on the Dimension EXL200 chemistry system is an in vitro diagnostic test intended for the quantitative determination of total protein in **serum** and **heparinized plasma**. Measurements of total protein are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney or bone marrow as well as metabolic or nutritional disorders.

**PRINCIPLE:**
The total protein method is a modification of the biuret reaction first introduced by Kingsley[1] and later modified by Henry[2] and presented as the method of choice for serum by Henry.[3] This method incorporates tartrate as a complexing agent to prevent precipitation of $Cu(OH)_2$. Serum blanking increases method sensitivity and minimizes spectral interference from lipemia.

Cupric ion (Cu++) reacts with the peptide linkages (-C-NH-CH-C-NH-) of protein in a basic solution.

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The blue copper (II) protein complex thus formed is proportional to the total protein concentration in the sample and is measured using a bichromatic (540, 700 nm) endpoint technique.

$$Cu^{++} + \text{Protein} \xrightarrow{OH^-} \text{complex}$$

(absorbs at 540 nm)

**INSTRUMENT, REAGENTS & SUPPLIES:**

| Wells [a] | Form | Ingredient | Concentration [b] |
|---|---|---|---|
| 1–3 [c] | Liquid | Potassium SodiumTartrate | 1.089 g/mL |
| | | NaOH | |
| 4–6 | Liquid | Cupric Sulfate | 0.015 mol/L |

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value per test at manufacture.

**Precautions**: Wells 1-3: Corrosive. Contains sodium hydroxide. Causes severe burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Harmful to aquatic organisms; may cause long-term adverse effects in the aquatic environment.

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[4]

For *in vitro* diagnostic use

Safety Data Sheets (SDS/MSDS) can be found at www.siemens.com/healthcare

**Reagent Preparation:** All reagents are liquid and ready-to-use.

## REAGENT STORAGE & STABILITY:

Store at 2 – 8° C.

**Expiration:** Refer to shelf carton for expiration date of individual unopened reagent cartridges. Sealed cartridge wells on the instrument are stable for 30 days. Once wells 1–6 have been entered by the instrument, they are stable for 5 days.

## SPECIMEN REQUIREMENTS:

Normal procedures for collecting and storing serum may be used for samples to be analyzed by this method.[4]

Follow the instruction provided with your specimen collection device for use and processing.

Complete clot formation should take place before centrifugation.

Specimens should be free of particulate matter.

**Specimen:**

Patient preparation: no preparation required

Specimen type: serum or heparinized plasma

Stability:

Room Temperature: 8 hours

Refrigerated at 2-8$^{o}$ C: 72 hours (3 days)

Frozen at -20$^{o}$ C: 6 months

## CONTROLS:

At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

**PROCEDURE:**
The TP Flex® reagent cartridge, Cat. No. DF73, is required to perform the TP test. This test is performed on the Dimension® clinical chemistry system after the method is calibrated (see Reference Material in Calibration section).

**Test Steps**
Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus dead volume. Precise container filling is not required.

An alternate sample size (10 µL) can be programmed; refer to the Operator's Guide for the use of alternate sample size.

**Test Conditions**

- Sample Size: 15 µL, (10 µL)g
- Reagent 1 Volume: 85 µL
- Reagent 2 Volume: 85 µL
- Diluent Volume: 315 µL
- Test Temperature: 37° C
- Wavelength: 540 and 700 nm
- Type of Measurement: bichromatic endpoint

**Calibration**

The general calibration procedure is described in your Dimension® system Manual (also see Appendix B).

The following information should be considered when calibrating the total protein method:

| | |
|---|---|
| Assay Range: | 2–12 g/dL [20–120 g/L] |
| Reference Material: | Purified human or bovine albumin or secondary calibrators such as Total Protein/Albumin Calibrator (Cat. No. DC31) |
| Suggested Calibration Levels: | 2.0, 6.0, 10.0 g/dL [20, 60, 100 g/L] |
| Calibration Scheme: | Three levels in triplicate |
| Calibration Frequency: | Every new reagent cartridge lot |
| | Every 3 months for any one lot |
| | For each new lot of Flex reagent cartridges |

| | |
|---|---|
| | After major maintenance or service, if indicated by quality control results |
| | As indicated in laboratory quality control procedures |
| | When required by government regulations |
| Assigned Coefficients: | Standard sample size = 15 µL |
| | $C_0$ 3.700; $C_1$ 0.0222 |
| | Alternate sample size = 10 µL |
| | $C_0$ 5.357; $C_1$ 0.031 |

**Backup Process:**
Refer to Brown Clinic Back-up Policy

**INTERPRETATION:**
The instrument automatically calculates and prints the concentration of total protein in g/dL [g/L] using the calculation scheme illustrated in your Dimension® system manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

**EXPECTED VALUES**

6.4–8.2 g/dL [64–82 g/L]

The reference interval was calculated non-parametrically and represents the central 95% of the population, using serum as the sample.

A reference interval for plasma would be 0.3 g/dL higher due to the presence of fibrinogen in the plasma.

Each laboratory should establish its own reference interval for total protein as performed on the Dimension® system.

**REPORTING:**
report format: mg/dl

**LIMITATIONS:**

| | |
|---|---|
| Results | > 12.0 g/dL [120 g/L] |
| Manual dilution | Make appropriate dilution with Reagent Grade Water to obtain result within the assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution. |

Autodilution (AD)Refer to your Dimension® system manual.

Results less than 2.0 g/dL [20 g/L] should be reported as "less than 2.0 g/dL [20 g/L]" instead of the numerical value.

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.

**Analytical Specificity**

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

**Reference: TP Flex® reagent cartridge insert sheet PN 717073.001 Issue Date 2013-09-27**

Origination Date: 9-10-07
Date of Implementation: 11-10-10
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

## REVIEW - REVISION SUMMARY DOCUMENTATION

| Date | By | Revision Summary |
|---|---|---|
| 07/10 | Lori Murray | New format |
| 8-8-12 | Lori Murray | Changed instrumentation to EXL200 |
| 6/19/15 | Heather Hall | Updated diluents required. Updated to package insert dated 9/27/13 |
| 10/18/17 | Heather Hall | Updated information to package insert dated 2/25/2015, modified backup process |