Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Total Bilirubin

PURPOSE:
The TBIL method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of total bilirubin in **serum** and **plasma.** Measurements of total bilirubin are used in the diagnosis and treatment of liver, hemolytic, hematological and metabolic disorders, including hepatitis and gallbladder disease.

PRINCIPLE:
There are at least four distinct bilirubin fractions that make up total bilirubin in serum. The direct reacting fractions are mono- and diconjugated bilirubin (β and γ–bilirubin) and the delta fraction (δ bilirubin), which is tightly bound to albumin. Unconjugated bilirubin (α-bilirubin) is water-insoluble and reacts only after addition of an accelerator such as caffeine[1].

The total bilirubin method is a modification of the Doumas reference method.[2] The use of a caffeine/benzoate mixture to solubilize the protein bound unconjugated bilirubin was first reported by Enriques and Silvo.[2] This method is a modification of the Jendrassik and Grof procedure.[3]

Diazotized sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. Bilirubin (unconjugated) in the sample is solubilized by dilution in a mixture of caffeine/benzoate/acetate/EDTA. Upon addition of the diazotized sulfanilic acid, the solubilized bilirubin including conjugated bilirubins (mono and diglucoronides) and the delta form[4] (biliprotein-bilirubin covalently bound to albumin) is converted to diazo-bilirubin, a red chromophore representing the total bilirubin which absorbs at 540 nm and is measured using a bichromatic (540, 700 nm) endpoint technique. A sample blank correction is used.

Solubilized bilirubin + Diazotized sulfanilic acid ———> Red chromophore (absorbs at 540 nm)

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]
1, 4–6	Liquid	Acetate Buffer	
		Caffeine	168 mM
		Sodium Benzoate	337 mM
		Disodium EDTA	2.57 mM
2	Liquid	Sulfanilic acid	25.89 mM

		Hydrochloric acid	132 mM
3	Liquid	Sodium Nitrite	72.5 mM

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

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[5]

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.

STORAGE & STABILITY:
Store at 2 – 8° C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed or unhydrated cartridge wells on the instrument are stable for 30 days.

5 days for wells 1, 4-6

3 days for well 3

15 days for well 2 (diazotized sulfanilic acid formed by the automatic addition of sodium nitrate from well 3)

SPECIMEN REQUIREMENTS:
Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method. Follow the instructions provided with your specimen collection device for use and processing.

Serum and plasma specimens should be separated from cells within 2 hours after venipuncture.

Specimens should be free of particulate matter. To prevent the appearance of fibrin in serum samples, complete clot formation should take place before centrifugation. Clotting time may be increased due to thrombolytic or anticoagulant therapy.

Bilirubin is extremely photosensitive. Care should be taken to protect sample from both daylight and fluorescent light to avoid photodegradation. A 50% decrease in bilirubin within one hour has been reported for samples exposed to direct sunlight. For optimal stability, samples should be stored in darkness at low temperatures.[6]

Separated specimens are stable for 8 hours at room temperature, 7 days at 2-8^{o}C or 6 months frozen at -20 degrees C or colder. Protection from light is required when specimens are stored for more than 8 hours.

Specimen:
- patient preparation: no patient preparation required
- specimen type: serum or heparinized plasma
- handling: see comments above

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 TBIL Flex® reagent cartridge, Cat. No. DF167, is required to perform the TBIL 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.

Test Conditions

- Sample Size: 10 µL
- Reagent 1 Volume: 250 µL
- Reagent 2 Volume: 47 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 bilirubin method:

Assay Range: 0.1 – 25.0 mg/dL [2 – 428 µmol/L]

Reference Material: Primary standards or secondary calibrators such as TBIL/DBIL Calibrator (Cat. No. DC167)

Suggested Calibration Levels: 1.00, 10.00, 25.00 mg/dL [17, 171, 428 µmol/L]

Calibration Scheme: Three levels in triplicate

Calibration Frequency: Every new reagent cartridge lot.

Every 3 months for any one lot.

After major maintenance or service, if indicated by quality control results

As indicate in laboratory quality control procedures.

When required by government regulations

Assigned Coefficients: Standard sample size = 10 µL

C_0 0.00

C_1 0.078

NOTE: Level 1 Calibrator for TBI is not included in the TBI-DBI calibrator carton. Purified Water Diluent or Reagent grade water should be used as the Level 1 calibrator for the TBI method.

Backup Process:
Refer to Brown Clinic Back-up Policy

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

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

REFERENCE RANGE:
Less than or equal to 1.00 mg/dL [17.10 µmol/L]

The reference interval was calculated non-parametrically and represents the central 95% of the population.

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

REPORTING:
report format: mg/dl

LIMITATIONS:
Analytical Measurement Range (AMR): 0.1 - 25.0 mg/dL [2 - 428 µmol/L]

This is the range of analyte values that can be directly measured on the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

Samples with results in excess of 25.0 mg/dL [428 µmol/L] should be repeated on dilution.

Results less than 0.1 mg/dL [2 µmol/L] should be reported as "less than 0.1 mg/dL [2 µmol/L]".

Manual dilutions: Make appropriate dilutions with Purified 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.

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 your Dimension® system manual.

Analytical Specificity

See Known Interfering Substances section for details.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

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: __10/23/2017___
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
04/15/15	Heather Hall	Updated information from new package insert dated 7/25/13
10/18/17	Heather Hall	Updated information to package insert dated 2-26-2016, Modified backup process