Brown Clinic Northridge

511 14th Ave. NE

Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Direct Bilirubin

PURPOSE:
The DBIL method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of direct (conjugated) bilirubin in **serum** and **plasma**.

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 direct bilirubin method is a modification of the Doumas reference method (a modification of the Jendrassik and Grof procedure).[1, 2]

Diazotized sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. The sample is diluted in 0.05M HCl. A blank reading is taken to eliminate interference from non-bilirubin pigments. Upon addition of the diazotized sulfanilic acid, the conjugated bilirubin is converted to diazo-bilirubin, a red chromophore which absorbs at 540 nm and is measured using a bichromatic (540, 700 nm) endpoint technique. A sample blank correction is used

Conjugated bilirubin + ——————> Red chromophor (absorbs at 540 nm)

Diazotized sulfanilic acid

INSTRUMENT, REAGENTS & SUPPLIES:
Reagents

Wells[a]	Form	Ingredient	Concentration[b]
1-4	Empty[c]		
5	Liquid	Sodium nitrite	72.5mM
6	Liquid	Hydrochloric acid	500mM
7–8	Liquid	Sulfanilic acid	25.89 mM
		Hydrochloric acid	132 mM

a. Wells are numbered consecutively from the wide end of the cartridge.

b. Nominal value per test at manufacture.
c. Diazotized sulfanilic acid will be prepared in these wells automatically by the instrument

Precautions:

Safety data sheets (MSDS/SDS) available on www.siemens.com/healthcare

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.[3]

For *in vitro* diagnostic use

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

REAGENT 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.

Open well stability:

2 days for Wells 1-4 (once the diazotized sulfanilic acid has been prepared)

30 days for wells 5-8

SPECIMEN REQUIREMENTS:

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

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 1 hour has been reported for samples exposed to direct sunlight. For optimal stability, serum samples should be stored in the darkness at low temperatures.

Serum and plasma specimens should be separated 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.

Specimen:

patient preparation: no patient preparation required

specimen type: serum or heparinized plasma

stability of separated samples:

8 hours room temperature

7 days at 2-8 degrees C

6 months frozen at -20 degrees C or colder

Protection from light is required if stored for longer than 8 hours.

Hemolysis may depress DBI results. Follow your laboratory's procedure for reporting results when the sample is hemolyzed.

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:

Materials Provided:

DBI Flex ® reagent cartridge, Cat. NO. DF125

Materials Required but Not Provided:

TBI/DBI Calibrator, Cat. No. DC167

Quality Control Materials

Purified Water Diluent (Cat. No. 710615901) or Reagent Grade Water

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: 25 µL
- Reagent 2 Volume: 50 µL
- Test Temperature: 37°C
- Wavelength: 540, 700 nm
- Type of Measurement: bichromatic endpoint

Backup:

Refer to Brown Clinic Backup Policy

Calibration

The general calibration procedure is described in your Dimension® system manual.

The following information should be considered when calibrating the direct bilirubin method:

Assay Range: 0.05–16.00 mg/dL [0.86–274 µmol/L]

Reference Material: Secondary calibrators such as TBIL/DBIL Calibrator (Cat. No. DC167)

Suggested Calibration Levels: 0.0, 7.00, 17.50 mg/dL [0, 120, 299 µmol/L]

Calibration Scheme: Three levels in triplicate

Calibration Frequency:	Every new reagent cartridge lot
	Every 90 days 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:	C_0 0.06
	C_1 0.075

INTERPRETATION:
The instrument automatically calculates and prints the concentration of direct bilirubin in mg/dL [µmol/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.

REFERENCE RANGE:
0.00–0.30 mg/dL [0–5 µmol/L]
Each laboratory should establish its own reference interval for conjugated bilirubin as performed on the Dimension® system.

REPORTING:
report format: mg/dl

LIMITATIONS:

Results:	>16 mg/dL [274 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.

Samples with results < 0.05 mg/dL should be reported as "less than 0.05 mg/dL"

A HEMOGLOBIN message will appear on the report slip after the TBIL result to indicate the hemoglobin is >100 mg/dL [0.06 mmol/L] and will cause interference in the DBIL result on that sample.

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

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.

REFERENCES:

DBIL Flex® reagent cartridge insert sheet PN 717025.001 Issue Date 2/26/2016

Origination Date: 9-10-07
Date of Implementation: 11-10-10
****

Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____(signature on file)_____ Date: 3/2/2018
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
4/27/15	Heather Hall	Updated information to package insert dated 7/5/2013
10/17/17	Heather Hall	Updated information to package insert dated 2-26-2016 Modified backup method
2/20/2018	Heather Hall	Updated Reagent Concentration to match package insert