# CHEM.DXC.ASSAY.13.0 BILIRUBIN, TOTAL BY BECKMAN DXC

## INTENDED USE

TBIL reagent, when used in conjunction with the SYNCHRON UniCel® DxC 600 or 800 System and the SYNCHRON® Systems Bilirubin Calibrator, is intended for the quantitative determination of total bilirubin concentration in human serum or plasma.

## PRINCIPLE

TBIL reagent is used to measure the total bilirubin concentration by a timed endpoint diazo method. In the reaction, the bilirubin reacts with diazo reagent in the presence of caffeine, benzoate, and acetate as accelerators to form azobilirubin.

The SYNCHRON Systems automatically proportion the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 35 parts reagent. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of total bilirubin in the sample and is used by the System to calculate and express the total bilirubin concentration.

## DOCUMENT OWNER

Manager, St. Vincent Randolph Hospital Laboratory

## RELATED DOCUMENTS

CHEM.DXC.1.0 *Beckman DxC System Overview*
CHEM.DXC.2.0 *Beckman DxC Assay Calibration*
CHEM.DXC.3.0 *Beckman DxC Assay Quality Control*

## SPECIMEN

A. Patient Preparation: None.

B. Sample Size: 8 µL in addition to the sample container and system dead volumes.
   1. The optimum volume is 0.3 mL in a 0.5 mL sample cup.
   2. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

C. Specimen Type:
   1. Freshly drawn serum or plasma is the preferred specimen.
   2. Lithium, sodium or ammonium heparin are acceptable anticoagulants.

3. Sodium citrate and potassium oxalate/sodium fluoride are **NOT** acceptable anticoagulants.

D. Specimen Preparation:
1. If using serum, allow the sample to clot completely before centrifugation.
2. Within 2 hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube.
3. Keep tubes stoppered at all times.
4. Ensure residual fibrin and cellular matter has been removed prior to analysis.

E. Storage and Stability:
1. Room temperature (+15°C to +30°C): up to 8 hours
2. Refrigerated (+2°C to +8°C): up to 48 hours
3. Frozen (-15°C to -20°C):
   a. Thaw samples only once.
   b. Mix gently by inversion and centrifuge after thawing prior to sample analysis.
4. Bilirubin is photosensitive. Protect samples from light.

**REAGENTS**

All reagents are purchased from Beckman Coulter.

A. TBIL Reagent Cartridge, Ref. No. 442745 – 2 x 300 tests OR Ref. No. 476861 – 2 x 400 tests
1. Preparation:
   a. For Ref. No. **442745** (2 x 300 tests):
      i. Quantitatively transfer 100 µL (0.1 mL) of the contents from the smallest compartment into the center compartment.
      ii. Replace the cartridge caps and gently invert the cartridge several times to ensure adequate mixing. Thorough mixing is necessary for successful calibration.
   b. For Ref. No. **476861** (2 x 400 tests):
      i. Quantitatively transfer 200 µL (0.2 mL) of the contents from the smallest compartment into the center compartment.
      ii. Replace the cartridge caps and gently invert the cartridge several times to ensure adequate mixing. Thorough mixing is necessary for successful calibration.
2. Storage and stability:
   a. Unopened cartridges are stable until the expiration date stated on the cartridge label when stored at room temperature (+15°C to +30°C).
   b. Prepared cartridges are stable for 30 days at +2°C to +8°C unless the expiration date is exceeded.
   c. DO NOT FREEZE.

**EQUIPMENT**

Beckman UniCel DxC 600 or 800 Synchron Clinical System

**CALIBRATION**

The system must have a valid calibration in memory before controls or patient samples can be run.

A. Calibration Material: SYNCHRON® Systems Bilirubin Calibrator, Ref. No. 465915

1. The calibrator is prepared from human serum and stabilized by the use of ethylene glycol. The stabilizing effects of ethylene glycol are threefold:
    a. The high osmolality minimizes bacterial growth.
    b. Its antioxidant property stabilizes oxygen-labile constituents.
    c. The presence of ethylene glycol causes freezing point depression, which allows the calibrator to remain in the liquid state at normal freezer temperatures, i.e., between -15°C and -20°C. The preparation of these stabilized calibration materials in liquid form eliminates errors commonly associated with filling, drying, and the reconstitution of lyophilized calibration products.
2. Storage and Stability:
    a. SYNCHRON® Systems Bilirubin Calibrator is stable until the expiration date printed on the label if stored in the original ampule at -15°C to -20°C.
    b. SYNCHRON® Systems Bilirubin Calibrator is stable at +2°C to +8°C for 24 hours if resealed.
3. Preparation for use:
    a. Remove an ampule from the kit. Gently swirl and tap the contents to the bottom of the ampule.
    b. The neck of the ampule has been “etched” to facilitate opening. Grasp the bottom of the ampule with the thumb and index finger of one hand. Now place the thumb and index finger of the other hand on the top part of the ampule and quickly snap the neck. (To avoid cuts, fingers should be protected). If the neck portion does not snap off readily, rotate the ampule a quarter-turn and repeat the operation,
    c. After opening the ampule, transfer the required amount of calibrator into a sample cup.
    d. During the use period, protect from light and store at +2°C to +8°C.

B. Calibration Frequency:

1. Every 14 days or with each new lot number of reagent.
2. With certain parts replacements or maintenance procedures.
3. When QC results indicate a possible recalibration is required.
4. When there is an environmental change including instrument relocation.

5. When there is an instrument replacement.

C. Procedure: See CHEM.DXC.2.0 *Beckman DxC Assay Calibration.*

**QUALITY CONTROL**

A minimum of two levels of controls spanning the medical decision range are to be run once every 24 hours of assay use. See QC procedure, HBL.GEN.7.0 for specific details.

**PROCEDURE**

A. Testing Procedure:
   1. Load the reagent onto the system.
   2. After reagent load is completed, calibration may be required.
   3. Program samples and controls for analysis.
   4. After loading samples and controls onto the system, follow the protocols for system operations.

B. Sampling, reagent delivery, mixing and processing are automatically performed by the DxC System. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

**CALCULATIONS**

The SYNCHRON® System performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

**RESULTS**

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

**REPORTING RESULTS**

A. Expected values: 0.3 – 1.2 mg/dl

B. Critical values:

1. Per MACL Policy QA.REPORT.1.0 *Critical Values--Reporting of Significant Results*, all critical values must be called and documented.
2. Critical values in mg/dL:

| | |
|---|---|
| <1 day | >12.0 |
| 1 day | >13.0 |
| 2 days | >15.0 |
| 3 days to <15 days | >18.0 |
| 15 days to 2 years | >20.0 mg/dL |

**NOTE:** All hospital registered outpatient bilirubin results on babies <5 days old will be called to the ordering physician regardless of the bilirubin result

C. MACL Sunquest Computer Entry:

1. Manual Entry

| | |
|---|---|
| Function: | MEM |
| Worksheet: | Site specific |
| Test Code: | TBIL |

2. Online Entry

| | |
|---|---|
| Function: | OEM |
| Device: | Site specific |
| Test Code: | TBIL |

D. QLS Computer Entry:

| | | |
|---|---|---|
| 1. | Enter: | 3, 3, 1 |
| 2. | Worksheet: | Enter worksheet from QLS label. |
| 3. | Accession number: | Enter JI number. |

E. If the HIS or LIS system is down, see the appropriate Laboratory Computer Downtime Policy.

**PROCEDURE NOTES**

A. Expected Turnaround Time (TAT): Nursing units are to be notified if the turnaround time is unable to be met per current MACL network turnaround time standards.

B. Backup method: When testing cannot be performed, the testing site's backup policy should be followed.

**LIMITATIONS**

A. AMR:

| **AMR** in mg/dL | **ACTION** | **REPORT** |
|---|---|---|
| 0.1 – 30.0 | Low: None<br>High: Dilute X2 w/known bilirubin | Report low as <0.1<br>Report high as >60.0 |

B. Interferences:
   1. Lipemic samples >+2 should be ultracentrifuged before testing.
   2. The following substances were found to interfere significantly with this procedure:
      a. Hemoglobin at 100 mg/dL
      b. Azide at 5 mg/dL
      c. Citrate at 900 mg/dL
      d. Oxalate at 1000 mg/dL
      e. Gentisic Acid at 5 mg/dL
      f. Acetoacetate at 0.2 ng/mL
   3. The Naproxen metabolite O-desmethylnaproxyn has demonstrated a positive interference with the procedure.

**CLINICAL SIGNIFICANCE**

Bilirubin measurements are used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

**REFERENCES**

A. SYNCHRON® System(s) Chemistry Information Sheet, TBIL/Total Bilirubin, A18553 AL, Beckman Coulter, Inc., Brea, California, April 2013.
B. SYNCHRON® Systems Bilirubin Calibrator, A85955, Beckman Coulter, Inc., Brea, California, April 2010.